



INTERFACES DA BIOCIÊNCIA: CÉLULAS, GENES E ALGORITMOS

10th International Meeting of Biosciences and Physiopathology
14º Simpósio de Biociências Aplicadas à Farmácia

ANAIIS 2025





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RESUMO

EXPANDIDO





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INSIGHTS ON THE ANTIFUNGAL EFFECT OF PHOTODYNAMIC THERAPY WITH METHYLENE BLUE IN AN EXPERIMENTAL MODEL OF SKIN WOUNDS

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1. INTRODUCTION

Chronic wounds affect around 20 million people worldwide, and the annual cost of their treatment and management is estimated at over \$31 million (1,2). A wound is characterized as chronic when it does not progress through the normal stages of healing within three months or recurs (3). Many factors contribute to delayed wound healing, including chronic metabolic diseases such as diabetes mellitus, neurological problems, vascular insufficiency, nutritional deficiency, aging, and infections (4,5).

Among the associated factors, wound infections stand out because they directly interfere with epithelial healing and, consequently, patient recovery. *Candida albicans* is among the microorganisms most frequently associated with wound infections (6,7). The complexity of wound management has serious consequences for affected individuals, including reduced quality of life, hospitalizations, long-term treatments, and outcomes such as amputation and death (8).

The impact on healthcare systems resulting from chronic wounds can be reduced through more assertive treatments. In this regard, photodynamic therapy (PDT) emerges as a promising alternative for microbial control of wounds. This approach is based on the activation of a photosensitizer by visible light, activating different oxidative stress pathways capable of inducing the death of target cells. Recent studies have suggested PDT as a viable option for the treatment and healing of wounds, even in the presence of infections (9,10,11). Additionally, it is known that, depending on the photosensitizer, PDT can not only eliminate microorganisms resistant to conventional therapies, but also contribute to tissue recovery and epithelial reorganization (10).

The need to study new therapeutic alternatives for infected wounds, combined with the research group's experience, motivated this study to investigate the antifungal effectiveness of PDT combined with methylene blue in an experimental model of wounds infected with *C. albicans*.

2. MATERIALS AND METHODS

2.1. Ethics statement

All procedures involving animals were approved by the Animal Use Ethics Committee, under protocol number CEUA-UEM (No. 3207020524).

2.2. Microorganism

The reference strain *C. albicans* ATCC 90028 from the Medical Mycology Laboratory of the State University of Maringá was used.



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2.3. Photosensitizer and light sources

The photosensitizer (PS) used was methylene blue (MB) Chemical Dynamics, and the Light Emitting Diode (LED) structures used for the *in vitro* and *in vivo* assays were manufactured by the Center for the Investigation of Light-Matter Interaction, State University of Maringa, Department of Physics.

2.4. *In vitro* photodynamic inactivation

C. albicans ATCC 90028 cells were reactivated in Sabouraud dextrose agar (SDA) medium and incubated for 24 hours at 37°C. The inoculum was then prepared in a 0.85% saline suspension at a concentration of 2×10^6 cells/mL. In 96-well plates, MB was diluted in series to obtain concentrations ranging from 0.488 to 0.0009 μ M. The fungal cells were placed in the same volume together with MB, and the system was incubated in the dark for 30 minutes. After contact of *C. albicans* cells with MB, the system was irradiated using red LED at a fluence of 50.34 J/cm². The fungicidal action of TFD+MB was evaluated by subculture in SDA of each well tested in the system described above. The SDA plates were incubated at 37°C/24h. The Minimum Fungicidal Concentration (MFC) was evaluated by logarithmic counting of colony-forming units per mL (log CFU/mL). Fungicidal action was considered when there was complete inhibition of fungal growth compared to the positive control (*C. albicans* cells without TFD+MB).

2.5. *In vivo* toxicity

The toxicity of topically applied methylene blue was evaluated in terms of metabolic effects. The animals were monitored and, at the end of 14 days, were euthanized for blood collection and metabolic evaluation.

2.5.1 Experimental organization

The animals were divided into experimental groups containing five animals in each group (control and treated). All were anesthetized with a combination of ketamine (80-100mg/kg) and xylazine (5-10mg/kg) in a 1:1 ratio, administered intramuscularly. After anesthesia, two parallel marks were made using a 1 cm² metal delimiter, and the marked skin was excised with scissors. The treated group received 100 μ L of MB 15.63 μ M every 48 hours for 14 days.

2.5.2 Metabolic analysis

After 14 days of experimentation, blood samples were collected from all animals to measure aspartate transaminase (AST), alanine transaminase (ALT), creatinine, and urea levels. The technique was performed according to the manufacturer's instructions, Gold Analisa, Brazil.

2.6. Experimental model of infected wounds

2.6.1 Experimental organization

The animals were divided into two groups (control and treated) with five animals each. All were anesthetized with a combination of ketamine (80-100mg/kg) and xylazine (5-10mg/kg) in a 1:1 ratio, administered intramuscularly. After anesthesia, two parallel marks were made using a 1 cm² metal delimiter, and the marked skin was excised with scissors. Subsequently, a cell suspension containing 2×10^6 yeasts/mL of *C. albicans* was applied to each wound area in both experimental groups.

2.6.2. Treatment of infected wounds

Twenty-four hours after infection, PDT was initiated by applying 100 μ L of 0.782 μ M MB to the animals in the treated group. After application, the animals were kept in the dark for 10 minutes, and then the wounds were irradiated with red LED at the same fluence as in the *in vitro* assays. The animals were euthanized 7 and 10 days after wound incision. Skin sections were removed, weighed, and macerated with



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lysis buffer to quantify colony-forming units (CFU). Logarithmic CFU values were determined after 24 hours of incubation, expressed as CFU/g of skin.

2.7. Statistical analysis

The data were analyzed using Student's t-test, using GraphPad Prism 8.0.2 software, with a significance level of $p < 0.05$.

3. RESULTS

Figure 1A and B shows the results of the *in vitro* assay. It can be observed that PDT+MB had fungicidal efficiency on *C. albicans* cells at concentrations of 0.488 and 0.244 μM . In addition, the concentration of 0.122 μM was able to significantly reduce fungal growth ($p < 0.05$). These results show that the action of PDT+MB may be dose-dependent.

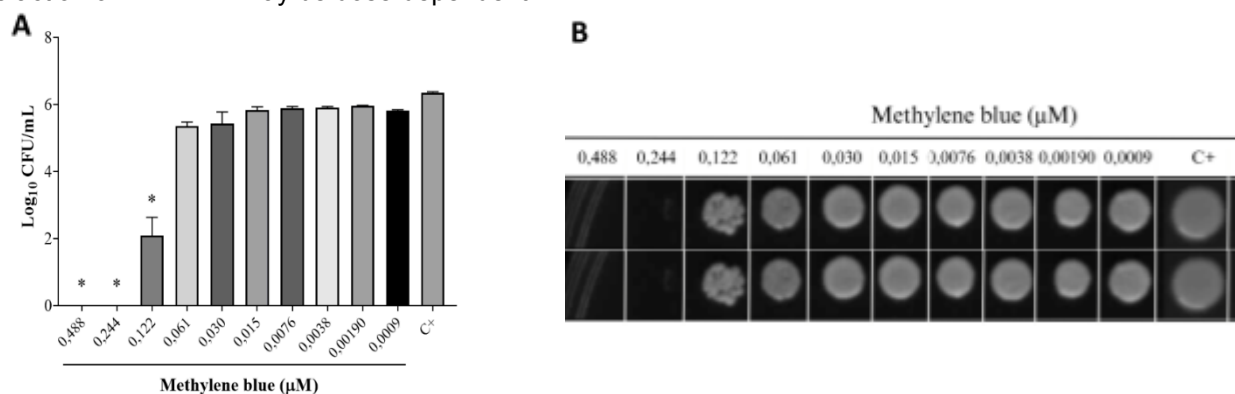
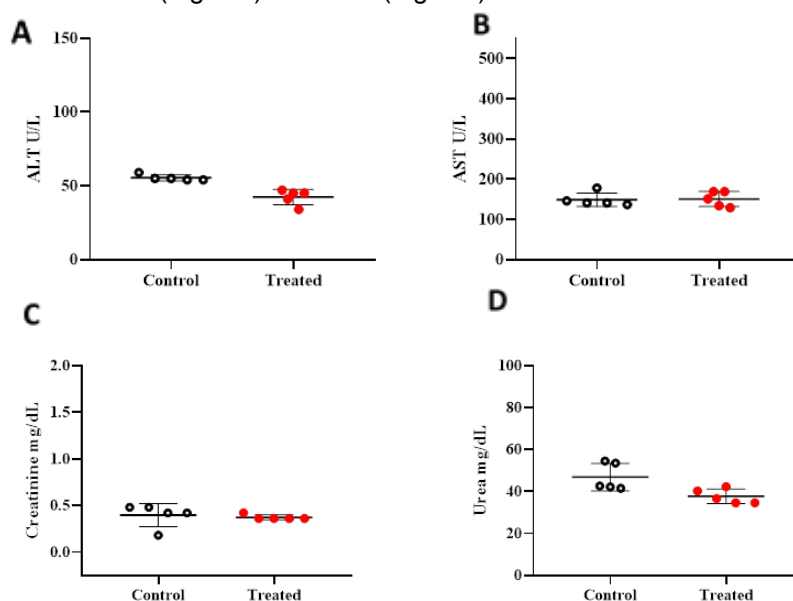


Figure 1. Photoinactivation of *Candida albicans* cells using Methylene Blue. (A) Quantitative evaluation of Colony Forming Units ($\text{log}_{10}\text{CFU/mL}$). (B) Qualitative evaluation of Minimum Fungicidal Concentration (MFC). C+: Fungal cells in the absence of Methylene Blue (MB) and Light. *statistically significant difference compared to C + ($p < 0.05$).

The metabolic evaluation of the topical toxicity of MB is shown in Figure 2. It can be observed that the hepatic biomarkers (Fig. 2A and B) show no hepatic damage, since the levels of ALT and AST enzymes activity are similar to those of the control group ($p > 0.05$). Similarly, MB treatment did not alter renal function, as evidenced by similar creatinine (Fig. 2C) and urea (Fig. 2D) levels between treated and control group.





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Figure 2. Topical toxicity of methylene blue in an experimental wound model. Evaluation of liver damage: A: alanine transaminase (ALT), B: aspartate transaminase (AST). Evaluation of kidney function: C: creatinine, D: urea. Control: Control: animals with wounds and not treated with MB. Treated: animals with wounds and treated with MB.

The efficient *in vitro* results and the absence of toxicity encouraged our research group to investigate the *in vivo* antifungal properties of PDT+MB. After seven days of treatment, there was no significant decrease in fungal load between the control and treated groups, as evidenced by the quantification of colony-forming units (Fig. 3A). However, clinical evaluation of the wounds (Fig. 3B) revealed visual differences, with greater inflammation observed in the control group, while the treated group showed less erythema and signs of scar evolution. On the other hand, treatment of infected wounds with PDT+MB for 10 days showed a significant reduction in fungal load compared to the control ($p < 0.05$). These results are consistent with Figure 3B, which shows wounds with reduced size and signs of inflammation.

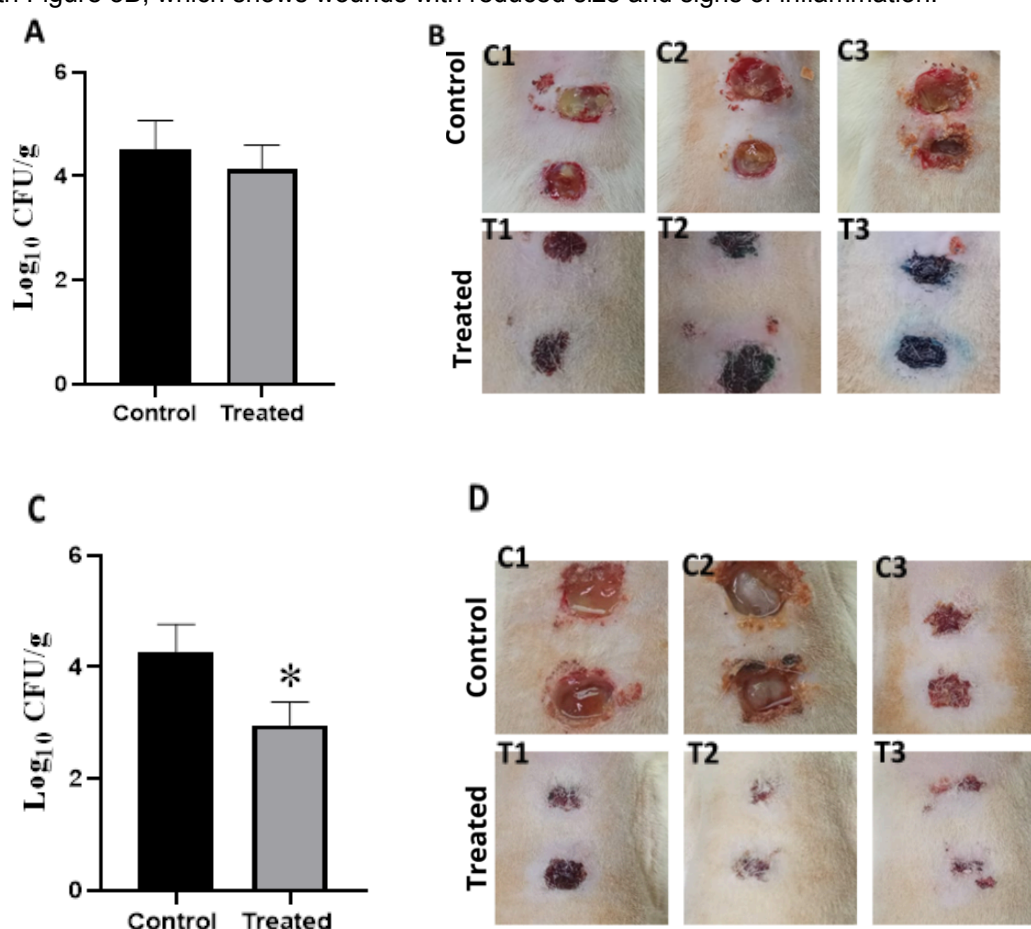


Figure 3. Antifungal activity of photodynamic therapy with methylene blue in an experimental model of wounds infected with *Candida albicans*. A and C) fungal load evaluated seven and 10 days after infection, respectively. B and D) Representative photos of three control animals (C1, C2, C3) and treated animals (T1, T2, T3), evaluated seven and 10 days after infection.

4. DISCUSSION

Infected skin wounds are one of the main pillars for complications in wound healing, increasing the risk of patient mobility and mortality and considerable costs for the health system (1-5). Currently therapy is limited by microbial resistance and low drug availability at the site of infection (2). In recent years, PDT has emerged as a promising tool for primary or adjunctive treatment in wounds with or without infection (9).



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Although bacteria are epidemiologically common in non-healing wounds, fungi also play an important role. Furthermore, the literature associates negative clinical outcomes with the combined presence of fungi and bacteria infecting wounds, creating a multidrug-resistant environment (8). Yeasts of the *Candida* genus, such as *C. albicans*, have been prominent in this context (7,8). PDT's mechanism of action involves generating reactive oxygen species, triggering cell death in the microorganism (9). Although these tests are not part of this study, we believe that the conditions tested were able to photoactivate MB, completely eliminating fungal growth. This conclusion is supported by tests in the absence of light, which showed no antifungal activity of MB (data not shown).

The literature evaluates MB ranging from 1% to 0.01% in most of the articles analyzed (12,13,14). This percentage represents a concentration approximately 10x higher than that used in the present study. The concentration of 0.488 μ M (the first concentration analyzed) corresponds to 0.015% MB. This demonstrates that photoactivation of MB at a fluence of 50.34 J/cm² was able to chemically activate PS at a lower concentration than that found in most literature. It is known that very high concentrations of PSs can reduce light penetration into deep tissue regions, which consequently affects PDT results. Problems related to optical shielding still interfere with the results and pose significant challenges to the clinical applicability of PDT-MB (14).

The *in vitro* results motivated the *in vivo* studies, and the absence of toxicity, it was possible to test the antifungal effectiveness of PDT+MB, in an experimental model of wounds infected by *C. albicans*. The results shown in Figure 3 show that PDT+MB at a concentration of 0.78 μ M (0.025%) significantly reduced fungal burden after 10 days of treatment. In seven days, it was not possible to observe a decrease in the fungal load, but the representative photos (Fig. 3B) show that there are signs of a decrease in wounds and clinical improvement (data under evaluation). In this sense, Fig. 3D complements the fungal load results in Fig. 3B and further highlights the efficacy of PDT+MB in treating the analyzed wounds.

5. CONCLUSION

Few studies have evaluated the kinetics of the infectious process in yeast-infected wounds. Compared to studies with bacteria, our results are promising, with PDT combined with methylene blue (PDT+MB) showing antifungal activity at low concentrations and effectively reducing fungal load. Despite these encouraging findings, a more detailed analysis of wound-related factors is still necessary to fully understand the antifungal potential of this approach. Future perspectives include studying the biofilm structure in the infectious process and the applicability of PDT+MB, together with histological tissue analysis, to assess additional effects that PDT+MB may exert on wound healing and tissue integrity.

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Thematic area: Pathogens of clinical relevance.



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DUFFY ANTIGEN AND LEPROSY: A CASE-CONTROL STUDY IN PARANÁ

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1. INTRODUCTION

1.1 Leprosy

Leprosy is a disease caused by obligate intracellular bacilli, *Mycobacterium leprae* and *Mycobacterium lepromatosis*, both of which have a preference for macrophages and Schwann cells (1,2). Patients affected by the disease may present symptoms such as reduced sensitivity in peripheral nerves, skin lesions, and tingling (3).

There are several ways to classify leprosy, such as the operational classification (World Health Organization - 1982), the Madrid classification (1953), and the Ridley-Jopling classification (1966). The WHO operational classification is divided into paucibacillary (PB) and multibacillary (MB) forms (4). The Madrid classification, on the other hand, consists of the indeterminate, tuberculoid, dimorphic, and virchowian forms (5). The Ridley-Jopling classification combines clinical, bacteriological, and immunological criteria and comprises a broad characterization ranging from polar tuberculoid (TT), with strong immunity, to the intermediate forms borderline-tuberculoid (BT), borderline-borderline (BB), and borderline-lepromatous (BL), to polar lepromatous (LL) and may include the indeterminate form as an initial stage (6).

According to the epidemiological report on Hansen's disease published in 2025 by the Ministry of Health, 22,773 new cases of the disease were recorded in 2023. Among the new cases, 55.4% occurred in men. In terms of age group, 53.6% occurred in people between 30 and 59 years of age. Regarding the clinical form, 82.4% were diagnosed with the multibacillary form (4).

1.2 Diagnosis and treatment

The clinical diagnosis of leprosy is based on the presence of one or more cardinal signs: skin lesions with altered sensation, peripheral nerve involvement with or without thickening, and sensory, motor, or autonomic changes. Additional tests may include skin sensitivity tests, histamine, and pilocarpine. Serological tests, such as PGL-1 detection, molecular tests using PCR, and histopathological examinations can also be performed. (7).

Currently, leprosy is treated with multidrug therapy (MDT) consisting of rifampicin, dapsone, and clofazimine. The regimen depends on the clinical form: PB patients receive six monthly doses of rifampicin and daily dapsone, while MB patients receive twelve monthly doses of rifampicin, clofazimine and dapsone. Along with daily clofazimine and dapsone (7).

Another treatment option, called Rimoxclamin, has been developed by University of São Paulo (USP), in Brazil (8). In the study, it was observed that Rimoxclamin provided faster recovery from neurological damage, early as the third month of treatment. Also, there was a significant reduction in nerve thickening and associated pain, with benefits maintained after the end of treatment. Although this treatment option is still in clinical evaluation phase, its initial results suggest that it may represent an effective alternative with fewer adverse effects compared to traditional PQT (8).

1.3 Duffy



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The Duffy blood group is defined by the glycoprotein DARC (Duffy Antigen Receptor for Chemokines), an atypical receptor with high affinity for pro-inflammatory chemokines such as IL-8, MCP-1, MIP-1, RANTES, and MGSA (9, 10). Beyond ligand binding, DARC regulates immune responses by acting as a reservoir that controls chemokine bioavailability. Its expression on vascular endothelium facilitates transcellular chemokine transport, supporting leukocyte migration and amplifying local inflammation (11).

The DARC glycoprotein is encoded by the *ACKR1* gene, located on chromosome 1q22–q23. Six antigens are currently recognized within this group: Fya, Fyb, Fy3, Fy4, Fy5, and Fy6. The first to be identified were Fya (Fy1) and Fyb (Fy2) in the early 1950s. The distinction between Fya and Fyb results from a single nucleotide polymorphism (SNP) at position 125 of the gene (125G>A), in which guanine is replaced by adenine, leading to an amino acid substitution from glycine to aspartic acid (rs12075) (12, 13). In addition to the Fya/Fyb substitution, a mutation in the promoter region of the *ACKR1* gene, the GATA-1 polymorphism (-67T>C, rs2814778), results in the absence of Fy2 expression on red blood cells, while its expression remains normal in endothelial cells of capillaries, Purkinje cells, renal tubules, and pulmonary alveoli (14). This mutation is highly prevalent in African populations and is strongly associated with resistance to *Plasmodium vivax* malaria (15).

1.4 Objectives

This study aimed to evaluate the frequency of Duffy genotypes and the GATA (-67T>C) mutation in a case–control population from northern and northwestern Paraná.

2. MATERIALS AND METHODS

2.1. Sample selection

Peripheral blood samples were collected from 186 individuals, comprising 92 patients and 94 controls, who were receiving care at the Consórcio Público Intermunicipal de Saúde do Setentrão Paranaense (CISAMUSEP). All participants signed the Informed Consent Form, and clinical and demographic data such as gender, age, ethnicity, and clinical form of leprosy were recorded. Participant selection followed ethical criteria, without discrimination, ensuring confidentiality and protection against risks.

2.2. DNA Extration and genotyping

Genomic DNA was extracted using the Biopur kit. Samples were stored at -20 °C until analysis. DNA amplification was performed by PCR-SSP following the protocol of the Immunogenetics Laboratory at the State University of Maringá (UEM). Reactions were carried out in a final volume of 10 µL with standard reagents and run in a thermal cycler under conditions optimized for each primer pair.

The GATA mutation (-67T>C) was analyzed by PCR-SSP in reactions with a final volume of 20 µL. Amplification products were visualized on 2% agarose gels stained with SYBR Green and examined using a Quantum ST4 transilluminator.

2.3. Statistical analysis

Statistical analyses were performed using OpenEpi, SNPstats, and R, using tests such as chi-square, Fisher's exact test, and logistic regression. Significance was considered for $p < 0.05$ and a 95% confidence interval (CI), and Hardy–Weinberg equilibrium was verified for genotypic frequencies.

2.4. Ethical aspects

This study was approved by the Human Research Ethics Committee of the State University of Maringá under the title “Association of Immune Response Genes in the Immunopathology of Leprosy” (CAAE: 14090713.7.0000.0104; approval number 6,963,269).



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3. RESULTS

The mean age was similar between controls (56.31 ± 17.39) and patients (56.01 ± 14.33) (Table 1). Regarding gender distribution, males accounted for 45% of the controls and 43% of the patients, while females represented 55% and 57% respectively.

With respect to ethnicity, most individuals in both groups self-identified as White, although the proportion was higher among controls (82%) compared to patients (65%). On the other hand, mixed-race individuals were more frequent in the patient group (27%) than in the controls (11%), whereas the proportion of black individuals was comparable between groups (7–8%).

Regarding interpersonal contact, 41% of controls and 27% of patients reported exposure through their spouse. Other types of family contact, such as in-laws, were less frequent in both groups. In addition, 50% of controls reported contact in a nursing home, while this type of exposure was not observed among patients.

Table 1. Characteristics of leprosy patients and controls.

		Controls (n=94)	Patients (n=92)
Age		56.31 ± 17.39	56.01 ± 14.33
Gender	Male	42 (0.45)	40 (0.43)
	Female	52 (0.55)	52 (0.57)
Ethnicity	White	77 (0.82)	60 (0.65)
	Black	7 (0.07)	7 (0.08)
	Mixed-race	10 (0.11)	25 (0.27)
Interpersonal Contact	Spouse	39 (0.41)	
	Son-in-law	5 (0.05)	
	Daughter-in-law		NA
	Brother-in-law	3 (0.03)	
	Sister-in-law		
	Contact in nursing home	47 (0.50)	

NA= not applicable

In table 2, the most frequent genotype was *FY*02FY*02*, observed in 54% of controls and 45% of cases. The genotype *FY*01FY*02* was observed at similar frequencies in controls (46%) and cases (49%). The homozygous *FY*01FY*01* genotype was absent in controls but it was present in 6% of cases. Comparing the alleles, FY02 was predominant in both groups, representing 77% in controls and 69% in cases. The FY01 allele was less frequent overall, but occurred at a higher proportion in cases (31%) compared to controls (23%).

Tabela 2. Genotypic and allelic frequencies of Duffy

Polimorphism	Controls (n=94)	Cases (n=92)	OR (95% CI)
<i>FY*02/FY*02</i>	51 (0.54)	41 (0.45)	1.00
<i>FY*01/FY*02</i>	43 (0.46)	45 (0.49)	1.30 (0.72-2.34)
<i>FY*01/FY*01</i>	0 (0.00)	6 (0.06)	0.08 (0.004 – 1.47)
Allele			
FY02	145 (0.77)	127 (0.69)	1.00
FY01	43 (0.23)	57 (0.31)	1.69 (1.00 – 2.86)

OR= Odds Ratio; CI = Confidence limits.



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In terms of the GATA mutation (Table 3), the homozygous *T/T* genotype was the most common in both groups, occurring in 62% of controls and 69% of cases. The heterozygous *T/C* genotype was present in 33% of controls and 29% of cases, while the homozygous *C/C* genotype appeared at low frequencies, 5% in controls and 2% in cases. At the allelic level, the T allele was predominant in both groups, representing 78% in controls and 83% in cases. The C allele was less frequent overall, observed in 22% of controls and 17% of cases.

Tabela 3. Genotypic and allelic frequencies of GATA

Polimorphism	Controls (n=94)	Cases (n=92)	OR (95% CI)
<i>T/T</i>	58 (0.62)	63 (0.69)	1.00
<i>T/C</i>	31 (0.33)	27 (0.29)	0.80 (0.43-1.50)
<i>C/C</i>	5 (0.05)	2 (0.02)	0.37 (0.07-19.7)
Allele			
T	147 (0.78)	153 (0.83)	1.00
C	41 (0.22)	31 (0.17)	0.73 (0.43-1.22)

OR= Odds Ratio; CI = Confidence limits.

4. DISCUSSION

The sociodemographic profile of the study population revealed balanced distributions of age and sex between cases and controls, ensuring comparability of the groups. The ethnic distribution showed a predominance of white individuals in both groups. However, mixed-race individuals were more frequent among patients, which may reflect broader social and demographic patterns associated with disease distribution.

It is also important to note that the inclusion of interpersonal contact controls is highly relevant for characterising the demographic profile of the study population. Unlike family members, these contacts — such as institutional contacts (50%) and spouses (41.5%) — have no genetic relationship with patients, but maintain close and prolonged interactions with them. This group is essential for assessing the role of environmental exposure in the transmission of *M. leprae* and *M. lepromatosis*.

Regarding the Duffy polymorphism, the *FY02/FY02* genotype was the most common overall, with the *FY01/FY02* heterozygote also frequent, while the *FY01/FY01* homozygote was rare among controls but present among cases. According to Guelsin et al. the *FY*01/FY*02* genotype predominates in populations from São Paulo, whereas the *FY*02/FY*02* genotype is more common in Paraná. This variation likely reflects the historical genetic mixing between the populations of both states (16).

Analysis of allelic distributions showed *FY02* as the most common allele in both groups. However, the *FY01* allele was notably more frequent among cases than controls. Although these results are not definitive, they raise the possibility that *FY*01* may contribute to differential susceptibility, potentially through modulation of chemokine binding and immune cell trafficking.

For the GATA polymorphism, *T/T* genotype was the most common, and *C/C* was rare in both controls and cases. These findings suggest no strong role for this variant in leprosy susceptibility in this sample. A study by Tucunduva et al. (2024) reported that the GATA (-67T>C) allele frequency in a Brazilian population was more prevalent in self-declared non-white individuals compared to individuals who self-declared as white (17).

5. CONCLUSION

In summary, the genotypic frequencies observed in the case–control population are in line with those previously reported in the literature, reinforcing the reliability of our data. As this is a pilot study and there are no previous reports in the literature on the association between Duffy polymorphisms and leprosy, further research with larger cohorts is needed to confirm these findings.



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Thematic area: Etiology and Physiopathology of human and animal diseases.



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DEVELOPMENT OF VACCINE TECHNOLOGIES AGAINST AMERICAN CUTANEOUS LEISHMANIASIS: A SYSTEMATIC REVIEW

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1. INTRODUCTION

Leishmaniasis represents an infectious tropical disease, non-contagious and endemic in several countries, whose etiological agents are parasitic protozoa that infect cells of the mononuclear phagocytic system, belonging to the family Trypanosomatidae and the genus *Leishmania*¹. Considered one of the priority endemics of interest in Brazil, leishmaniasis are a growing public health problem both in the country and in much of the African, American, European, and Asian continents. According to the World Health Organization (WHO), 98 countries have leishmaniasis endemics, and therefore, these diseases are among the six priority diseases for control programs².

They are neglected diseases that, according to the World Health Organization, have an incidence of approximately 1.6 million cases per year worldwide. In Brazil, until the mid-1980s, visceral leishmaniasis (VL) predominantly occurred in rural areas. However, in recent years, there has been a significant shift in this epidemiological profile, attributed to the disease's expansion and urbanization, as evidenced by the increasing number of cases in large cities³. The transmission of leishmaniasis occurs through vector insects called phlebotomines, popularly known as sand flies or birigui. The vectors have easily adapted to the peridomestic environment, seeking shelter inside homes, in addition to exhibiting crepuscular and nocturnal activity, which further contributes to the increase in leishmaniasis cases in urban areas⁴.

The disease can manifest in two primary forms: visceral leishmaniasis (VL), which affects internal organs, and tegumentary leishmaniasis (TL), characterized by skin and mucous membrane affections. The parasite presents in two forms: promastigote (a flagellated form) is present in the vector, and amastigote (an aflagellated form) is obligatorily intracellular, located in the macrophages of reservoir hosts⁵. The main species involved in VL are: *L. (L.) donovani*, which occurs in Asia and Africa; *L. (L.) infantum*, described in Asia, Europe, and Africa, and *L. (L.) chagasi* found in the Americas, including Brazil⁵. In TL, several species are described worldwide, but in Brazil, there are reports of seven species: *L. (V.) braziliensis*; *L. (V.) lainsoni*; *L. (V.) naiffi*; *L. (V.) shawi*; *L. (V.) guyanensis*; *L. (V.) lindenberg*, and *L. (L.) amazonensis*⁵.

Chemotherapy based on pentavalent antimonials often fails, presents toxicity, high costs, long treatment periods, and adverse effects are standard⁷. For these reasons, there is a growing need to develop new prophylactic alternatives, in addition to more effective, safe and accessible drugs for the treatment of TL⁸.

Vaccination remains the most effective strategy for controlling leishmaniasis⁹. The development of a vaccine against leishmaniasis is a global public health priority and an auspicious goal¹⁰. Despite advances in research, which have led to improved laboratory techniques and the creation of various vaccine candidates



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in the preclinical phase, an efficient transition to human trials has not yet been achieved^{11,12}. An ideal vaccine against leishmaniasis must meet various requirements, including being safe, accessible to the population, and capable of stimulating T cell responses (CD4+ and CD8+), while also promoting long-term immunological memory and reducing the need for multiple doses. It should also provide adequate protection against various *Leishmania* species and remain stable at room temperature or 4°C, thereby eliminating the need for storage at -20°C or -80°C¹³. However, achieving lasting immunity and protection against various *Leishmania* species is a challenge, as most candidate vaccines use antigens that confer species-specific protection^{14,15,16}.

In simple terms, a vaccine is composed of two main elements: the antigen, which triggers a specific immune response against the pathogen, and the adjuvant, which activates and directs that response. To effectively combat leishmaniasis, vaccines need specific parasite antigens and molecules that stimulate the immune system^{17,18}. However, the development of vaccines against leishmaniasis remains a complex and challenging task, primarily hindered by the limited knowledge of various parasite strains and the complexity of the immunological responses required for protection¹⁹. Therefore, one of the objectives of this study was to develop a systematic review focusing on advances in the development of vaccine technologies against LT using in vivo studies.

2. MATERIAL AND METHODS

2.1 Search Strategy

The methodology of this systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines²⁰. Moreover, the protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO, CRD42024565575). Between April and May 2024, seven electronic databases: PubMed, Embase, Scopus, Research 4 Life, WOS, Cochrane, and Lilacs/BVS, were used to search for original articles. The design strategy employed in this study is illustrated in the flow diagram shown in **Figure 1**. The first phase of this study involved investigating the characteristics and search strategies of each electronic database. Four researchers conducted an independent search to define the MeSH terms (Medical Subject Headings) in the PubMed database, the terms were validated by two researchers and organized into four blocks: block 1, "Immune System" OR "Immune System Phenomena" OR "immunology" OR "Biomarkers"; block 2, "Vaccines" OR "Vaccine Development" OR "Vaccination"; block 3, "Leishmaniasis" OR "Leishmania" and block 4, "Animals, Laboratory" OR "Animal Experimentation" OR "Models, Animal".

The search in Scopus and Lilacs/BVS was performed using the same MeSH terms, following the same structure as the four blocks from PubMed. In Embase, some MeSH terms were adapted, since the term "Animals, Laboratory" was not found as a subject descriptor. In WOS, the search was performed by topic (TS) due to the larger number of articles retrieved. Moreover, in the Cochrane and Research4Life databases, searches were performed using title, abstract, and keyword terms for improved article retrieval.

2.2. Study Selection

2.2.1. Inclusion Criteria

After removing duplicate articles using EndNote software, titles and abstracts were screened. For subsequent acceptance of the full article, the articles had to meet the following criteria: studies that analyzed technologies in the development of vaccines against American Tegumentary Leishmaniasis in animals, all types, times, frequencies, and dosages of vaccine technology were eligible for inclusion, and studies that presented qualitative and quantitative data from research and promising evidence of vaccines against leishmaniasis. Only original articles written in English that were available were validated. All divergences were resolved through consensus meetings. Throughout the process, the researchers were blinded to each other's analyses.

2.2.2. Exclusion Criteria



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Letters, Guidelines, Editorials, Meta-analyses, Systematic Reviews, Conferences, and Abstracts were excluded. Based on titles and abstracts, articles that did not meet the inclusion criteria were also excluded. Articles on *in vitro* studies, visceral leishmaniasis studies, *in silico* studies, and clinical trials were excluded. Articles whose objective did not address leishmaniasis, evaluated leishmaniasis but did not associate the vaccine, or did not address *in vivo*, were also excluded.

2.3. Data extraction

The data extraction table was organized into a topic structure. The table included the following topics: reference, country, objective, *Leishmania* species (strain), vaccine technology, adjuvant, animal model, treatment groups, sample size, immunization protocol, time of last immunization, inoculation concentration, infection monitoring, lesion size, parasite load, histopathological and biochemical analyses, immune response evaluation, statistical analyses, infection monitoring results, lesion and parasite load reduction results, immune response evaluation results, recovery time, protection level, conclusions, and study limitations. The articles were distributed to researchers and data extraction was conducted, then verified by independent reviewers.

2.4 Bias Analysis

The risk of bias assessment is being performed by four independent reviewers, who evaluate each study using the SYRCLE Risk of Bias (Systematic Review Center for Laboratory Animal Experimentation) tool²¹. The risks of selection, performance, detection, attrition, and other biases are being assessed. Ten questions are being asked of studies accepted for this systematic review: "Yes" indicates a low risk of bias, "No" indicates a high risk of bias, and "Unclear" indicates an uncertain risk of bias. All analyses are being conducted with the researchers blinded, and doubts and disagreements will be resolved through a consensus meeting.

3. RESULTS

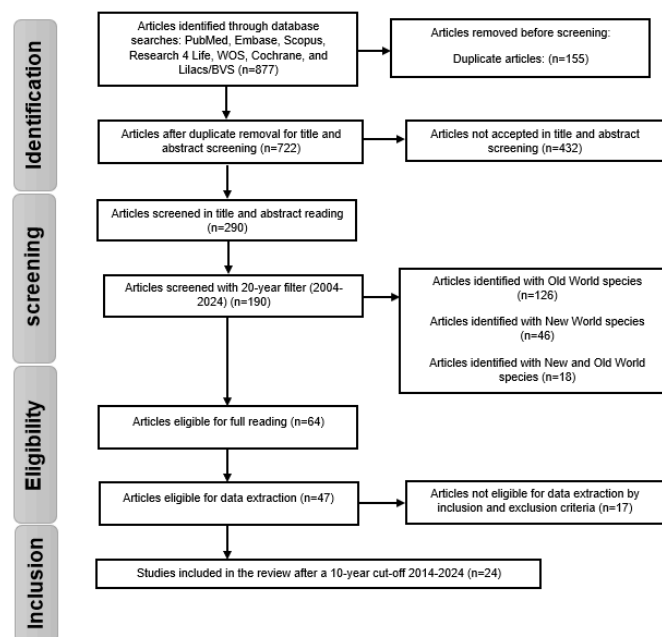


Figure 1. Flow diagram of studies selected for the systematic review. Initially, we identified 877 articles (643 articles in PubMed, 7 in Embase, 70 in Research 4 Life, 44 in Web of Science, 109 in Scopus, 3 in Cochrane, and 1 article in the Lilacs database). At the end of all analyses, a total of 24 articles were included in this systematic review. The search and bibliographic analysis results are summarized in a flowchart (Figure 1), as required by the PRISMA guidelines²⁰. This systematic review selected 24 articles on the most



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recent advances regarding technologies in development for vaccination against American Tegumentary Leishmaniasis in studies with animal models. The articles originated from ten different countries, with the majority from Brazil, followed by the USA, Spain, Argentina, Mexico, Switzerland, Iran, Peru, England, and the Netherlands. The selected articles were published over a decade (2014-2024).

The vaccine technologies tested in vivo included DNA vaccines, recombinant protein, total antigens, attenuated, and mimotopes/phage. The most frequently tested adjuvants included saponin, Montanide ISA, and CpG. The most commonly used *Leishmania* species in the studies was *L. amazonensis*, followed by *L. major*, *L. braziliensis*, *L. mexicana*, and *L. guyanensis*. The most used animal model was BALB/c mice, predominantly the C57BL/6 lineage. Most studies reported partial protection, with significant reduction in lesions and parasite load, but without sterile immunity. Increase in Th1 cytokines (IFN- γ , IL-12, GM-CSF) and reduction in Th2 cytokines (IL-4, IL-10) in vaccinated groups was reported and associated with protection. The cellular immune response, especially mediated by CD4⁺ and CD8⁺ T cells, was evaluated in most studies and showed an increase in IFN- γ production, indicating a protective Th1 response. Frequently an increase in IgG2a antibodies (associated with Th1 response) was mentioned, and relative to IgG1 (associated with Th2 response) in vaccinated groups. Almost all studies demonstrated that the vaccinated groups presented significant reduction in parasite load in infected tissues (footpad, spleen, liver, or lymph nodes), often measured by techniques such as limiting dilution or qPCR, compared to control groups (PBS, empty vector, or adjuvant alone).

4. DISCUSSION

Among the main innate mechanisms to control *Leishmania* infection is the development of a T-helper 1 (Th1) cellular immune response, specific to parasite antigens, triggered by the production of cytokines such as interleukin-2 (IL-2), interleukin-12 (IL-12), granulocyte-macrophage colony-stimulating factor (GM-CSF), and the production of reactive oxygen species (ROS), nitric oxide (NO) production, which is mediated by inducible NO synthase (iNOS), stimulated by cell activation via interferon gamma (IFN- γ)^{16,18,22}. This Th1 response, mediated by CD4⁺ T cells, is essential for controlling primary infection, as cytokines like IFN- γ stimulate phagocytic cells to produce nitric oxide, capable of eliminating internalized parasites^{16,23}. On the other hand, cytokines such as interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-13 (IL-13), interleukin-18 (IL-18), and transforming growth factor beta (TGF- β) promote disease progression, inhibiting the Th1 response and deactivating infected macrophages, favoring infection development¹⁶.

Our results reinforce that protection against cutaneous leishmaniasis depends on a robust Th1 cellular immune response, mediated by IFN- γ and nitric oxide, which activate macrophages to eliminate intracellular parasites. This aligns with the classic knowledge about the immunopathogenesis of leishmaniasis, where Th2 responses (IL-4/IL-10) exacerbate the disease. DNA vaccines like HisAK70 and recombinant protein like rLiHyp1 stood out for promoting cross-protection against multiple species (*L. amazonensis*, *L. major*, *L. braziliensis*). Adjuvants like saponin or CpG were essential, increasing efficacy by up to 70% in studies, suggesting potential for multiantigenic vaccines, useful in endemic regions like Latin America, where the circulation of different species and strains is a major problem.

Recent studies confirm this trend, Rooholamini Z, (2024)²⁴ emphasizes live attenuated vaccines as promising alternatives due to robust immune responses, similar to attenuated vaccines like Ldp27-/- . Ejazi AS, (2025)²⁵ highlights the role of host immunity in vaccine development, reinforcing the importance of an efficient Th1 response. The partial protection observed (e.g., 50-90% reduction in lesions) could reduce morbidity in humans, especially in endemic areas. However, the absence of sterile immunity indicates the need to combine vaccines with emerging immunotherapies, such as immune modulation to reduce drug doses²⁶. Studies in the literature highlight IgG2a/IgG1 ratios ≥ 2 as indicative of Th1 and protection²⁷. In fact, in the study by Devender M, (2024)²⁸ a recombinant vaccine against *L. donovani* using tuzin protein in BALB/c induced significantly higher IgG2a than IgG1, with elevated ratio, correlated to reduction in parasite load and hepatic granulomas similar to the results found in Jesus, M.M study, with the rLiHyp1 adjuvant²⁹.

Articles by Lage, D.P. (2020)³⁰ and Mello, M.F. (2025)³¹ demonstrated that adjuvants such as saponin induced mixed responses, but with a Th1 bias, if optimized. Lage, D.P. (2020)³⁰ tested a ChimeraT/saponin vaccine against *L. infantum*, which elevated IgG2a over IgG1, protecting 6 to 9 logs in the parasite load, in line with the results we found. However, as noted in the study by Mello, M.F. (2025)³¹, some mixed vaccines, such as LaAg + SAP, failed to provide complete protection, inducing an elevation of IgG1 and IgG2a,



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resulting in partial protection against *L. amazonensis*. Robust protection depends on cytokines beyond antibodies³²; our results show that high IgG2a does not lead to sterility³³ and are supported by the work of Gomes DC, (2025)³² here a vaccine against *L. major*, a F1F3 chimera induced high IgG2a/IgG1 ratio post-infection, suggesting Th1, reinforcing that IgG2a is a marker, not the primary effector. Another study emphasizes that, in humans, antibodies may be less predictive than in mice, due to genetic variability³⁴.

5. CONCLUSION

These results demonstrate that different vaccine technologies, from total antigen to DNA, present themselves as potential candidates to compose a vaccine applicable to the complex epidemiological context of leishmaniasis in the Americas.

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EVALUATION OF A NOVEL 1,3,4-OXADIAZOLE DERIVATIVE COMPOUND AGAINST *Paracoccidioides brasiliensis*

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1. INTRODUCTION

Among infectious diseases, those caused by fungal pathogens are often neglected in clinical practice, being overshadowed by bacterial and viral infections. However, fungi are responsible for a wide range of diseases, with some medically relevant species being included in the World Health Organization's (WHO) list of priority pathogens, such as paracoccidioidomycosis (PCM) (1). PCM is a systemic mycosis caused by *Paracoccidioides* species. This infection is endemic to Latin America and Brazil accounts for most cases, with approximately 80% of the total (2). Currently recognized as a public health issue, the annual incidence of PCM in Brazil is estimated at 3 to 4 new cases per 1 million inhabitants, reaching 10 to 30 new cases per million inhabitants in highly endemic regions of the country (3).

The main species responsible for PCM are *Paracoccidioides brasiliensis* and *P. lutzii* (4). However, *P. americana*, *P. restrepiensis*, and *P. venezuelensis* are also capable of causing the disease (5). In the environment, the fungus is found in the soil in its filamentous form, harboring infective fungal propagules that are inhaled during activities involving soil handling, leading to disease transmission. Due to its thermomimetic nature, upon entering the human host at 37 °C, the filamentous form differentiates into the yeast form, in which it persists throughout infection (6). The disease mainly affects rural male workers aged 30–60 years, although cases in women and children are also reported. (7).

PCM presents in two clinical forms: the acute (juvenile) form, which is severe and affects children and adolescents, and the chronic (adult) form, which predominates in men and usually compromises the lungs. Clinical manifestation chronic form typically occurs many years after the initial infection, with pulmonary involvement reported in 90% of cases. Disease progression in this case is generally slow, extending over 4–6 months (8).

The current treatment for PCM is generally effective, but complications remain. Oral itraconazole is recommended, lasting 9–12 months for mild cases and extending longer for moderate ones. As an alternative, sulfamethoxazole–trimethoprim may be used when itraconazole is unavailable or when neurological involvement occurs. In severe cases, intravenous amphotericin B is required, demanding adequate clinical infrastructure (9). Nevertheless, these therapies have notable drawbacks, including long duration leading to non-adherence, inability to prevent respiratory sequelae, high cost, and toxicity to organs such as liver and kidneys (10). Therefore, new therapeutic approaches and antifungal agents are imperative to overcome the current limitations, underscoring the importance of basic research employing *in silico* and *in vitro* strategies (11).

The *in silico* tools enable the selection of molecules targeting a specific site, thereby increasing the likelihood of identifying candidates with potential antifungal activity, while simultaneously reducing costs and avoiding unnecessary efforts. By selecting a pathogen-specific target, it becomes possible to rationally



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design molecules capable of binding to that fungal structure (12). A promising target for this type of investigation is the thioredoxin system, composed primarily of the proteins thioredoxin (Trx) and thioredoxin reductase (TrxR), along with the coenzyme NADPH (nicotinamide adenine dinucleotide phosphate). This system regulates cellular redox balance, playing a crucial antioxidant role in fungal defense under oxidative stress conditions. Moreover, it is also involved in DNA repair and cell death processes (13). When comparing the Trx/TrxR system in fungal and human cells, significant structural and mechanistic differences can be observed in the active site: fungal TrxR harbors cysteine residues at its catalytic center, whereas in humans it contains selenocysteine. This difference provides an opportunity to selectively inhibit the fungal system without affecting human cells, thus avoiding toxicity (14).

Oxadiazole-based compounds include derivatives with antifungal potential, which may target the thioredoxin system. Some studies have already reported promising results against *Candida albicans* and *P. brasiliensis*, highlighting this class as an attractive source in the search for novel antifungal agents (15,16). In addition, Ebselen, an organoselenium compound, has also shown promising antifungal activity (17,18), being able to block the activity of the TrxR enzyme of *Aspergillus fumigatus* (19). In this regard, the chemistry team synthesised a hybrid molecule through the incorporation of the oxadiazole ring into ebselen. Therefore, present study aims to evaluate the activity of this hybrid compound, designated B-O-Ph, against *Paracoccidioides brasiliensis*.

2. MATERIAL AND METHODS

2.1. Fungal Isolates

P. brasiliensis isolates (strain Pb18) were cultured on modified YPD (Yeast Extract Peptone Dextrose Agar) medium and incubated at 35°C for 5-7 days prior to the experiments. The isolates are stored in the Medical Mycology Laboratory at the State University of Maringá (UEM), Brazil.

2.2. Inoculum Preparation

The inoculum was prepared by transferring the fungal growth from the YPD agar slant to another tube containing 5 mL of saline solution (0.85% NaCl). After allowing the suspension to settle for 10 minutes, the supernatant was carefully transferred to a new tube. This suspension was then vortexed and passed through an insulin syringe to disrupt aggregated fungal cells. A 1:10 dilution was prepared and used for cell counting in a Neubauer chamber. Following the count, the inoculum was adjusted to approximately 2×10^4 cells/mL in saline.

2.3. Minimum Inhibitory Concentration (MIC) Assessment

The *in vitro* antifungal activity assay was performed using the broth microdilution method to determine the Minimum Inhibitory Concentration (MIC), in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI; document M27-A3), with modifications. For this experiment, the fungal inoculum was prepared in RPMI 1640 medium and diluted 1:2 in a 96-well plate. The test molecule was serially diluted from 0.5 to 256 µg/mL. The plates were incubated for 7 days at 35°C, after which 20 µL of 0.02% resazurin was added to each well for MIC reading.

2.4. Minimum Fungicidal Concentration (MFC) Assessment

The Minimum Fungicidal Concentration (MFC) was determined by subculturing from each well of the 96-well plate. For this purpose, 5 µL from each well was transferred onto modified YPD agar plates using a replicator guide template. The plates were then incubated at 35°C for 7 days.

2.5. Time-Kill Curve Assay



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An isolate of *P. brasiliensis* was cultured in McVeigh-Morton medium for 10 days under agitation at 150 rpm and 37°C. The inoculum was adjusted, and the tested concentrations of the molecule included sub-MIC, MIC, and 2xMI. An untreated control was also included. The treatment times were 1, 3, 6, 9, 12, and 15 days. At each time interval, the wells corresponding to the concentrations, and the control were serially diluted, plated onto modified YPD agar supplemented with 4% fetal bovine serum and 5% Pb18 culture filtrate, and incubated for at least 14 days at 37°C. Subsequently, the colony-forming units (CFU) were counted.

2.6. In vivo assay

BALB/c mice male mice were infected with Pb18 via the intratracheal route and divided into different groups. After 4 days of infection, the animals received treatment with B-O-Ph (10 mg/mL) or vehicle (DMSO, PBS, and micelle) intravenously. The itraconazole (5 mg/mL) was administered orally. Treatments were administered once daily for 14 days, and the animals were weighed every other day. At the end of this period, the animals were euthanized, and their lungs were collected for fungal burden quantification. The organs were homogenized and plated on modified YPD agar supplemented accordingly, and the plates were incubated at 37 °C for 7 days. All procedures involving animals were carried out according to the ethical principles of animal experimentation and were approved by the Ethics Committee on Animal Use (CEUA) of State University of Maringá (UEM), under protocol number 8316311023.

3. RESULTS

The Minimum Inhibitory Concentrations (MICs) and Minimum Fungicidal Concentrations (MFCs) of the molecule B-O-Ph were determined against three isolates of the genus *Paracoccidioides* spp. Furthermore, amphotericin B was used as a standard drug for comparison in this experiment. The MIC values for each isolate can be found in Table 1.

Table 1: Minimum Inhibitory Concentration (MIC) values of B-O-Ph and Amphotericin B against different isolates of the genus *Paracoccidioides* spp.

Isolates	B-O-Ph		Amphotericin B	
	MIC (µg/mL)	MFC (µg/mL)	MIC (µg/mL)	MFC (µg/mL)
Pb01	0,5	1	1	1
Pb18	4	4	0,25	0,25
BiHUMPb02	2	2	1	1

The molecule B-O-Ph inhibited fungal growth at concentrations of 4 µg/mL and 0.5 µg/mL for the reference isolates Pb18 (*Paracoccidioides brasiliensis*) and Pb01 (*Paracoccidioides lutzii*), respectively. For the clinical isolate BiHUMPb02, the obtained MIC result was 2 µg/mL. For Amphotericin B, the recorded MIC values were 0.25 µg/mL and 1 µg/mL, a result that is consistent with the expected standard for this drug. The MFC values were 4 µg/mL for B-O-Ph and 1 µg/mL for Amphotericin B. These MFC values were identical to (or similar to) the MIC results obtained in the test.

The growth kinetics over time were assessed through a 15-day time-kill curve assay. The concentrations tested were sub-MIC (2 µg/mL), MIC (4 µg/mL), and 2xMIC (8 µg/mL), based on the previously defined MIC value for B-O-Ph. Furthermore, an untreated control was included. For this experiment, the Pb18 isolate was tested, and the results are presented in Figure 1.



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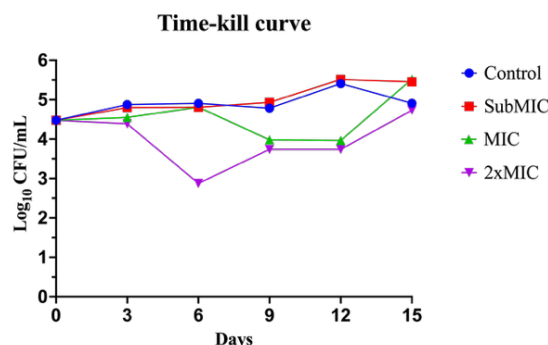


Figure 1: Time-kill curve of molecule B-O-Ph at different concentrations against *Paracoccidioides brasiliensis* (Pb18). The curves plotted from log₁₀ CFU/mL versus time (0-15 day) for B-O-Ph at concentrations 2 (SubMIC), 4 (MIC) and 8 µg/mL (2xMIC), indicating fungistatic profile of new compound. C+ or Control (drug-free control composed of medium plus inoculum).

The new compound demonstrated a fungistatic profile at two concentrations (4 and 8 µg/mL). Furthermore, on the 6th day, a reduction in viable cells was observed, with ≥ 1.5 log₁₀ cells, as compared to the control, at a 2xMIC concentration (**Figure 1**). In the control and sub-MIC samples, the fungal burden was maintained with a slight increase over time. On the other hand, the MIC and 2xMIC conditions showed a reduction in cell viability since the 6th day, suggesting a fungistatic profile. However, at the later points in the curve, an increase in fungal burden was observed, including in groups with higher concentrations. Considering the fungistatic profile and the length of the experiment, it is possible that after 12 days of cultivation, the molecule has been degraded, leading to an increase in fungal burden.

This new molecule does not exhibit cytotoxicity or acute toxicity (data not shown). Therefore, it was possible to evaluate the antifungal activity of B-O-Ph in the experimental murine model of PCM (**Figure 2A**). The groups treated once a day for 15 days with B-O-Ph (10 mg/kg) or ITC (5 mg/kg). The mice presented a weight reduction during the first 4 days due to tracheal infection, indicating the establishment of the disease caused by *P. brasiliensis*. After that, all groups showed a weight gain of just over 5 g. The fungal burden presented in the graph of Figure 2B did not show significant differences among groups; however, a decrease in the B-O-Ph log can be observed, like that seen with itraconazole in this experiment.

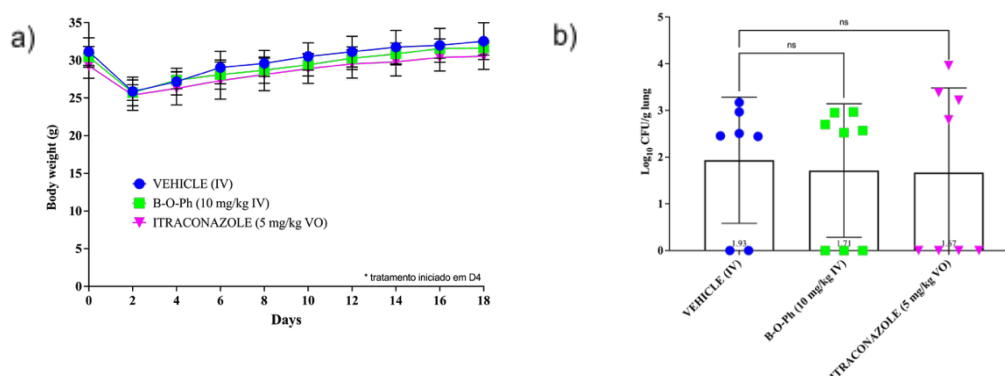


Figure 2: In vivo effect of B-O-Ph on treatment of mice with PCM murine. (A) The average body weight of the animals in each group over the 14 days of treatment and figure (B) Fungal burden in the lung of mice. The mice were infected with reference strain (5×10^6 yeast cells) and treated once a day for 14 days via intravenous injection. Control: treated with PBS and vehicle (DMSO 1% and Pluronic F-127 0.2%); B-O-Ph: treated with B-O-Ph compound (10 mg/kg) and Itraconazole: treated with itraconazole (5 mg/kg, VO). "NS" = not significant, "IV" = intravenously, "VO" = oral route.

The preliminary results indicate that the concentration of 10 mg/kg was not sufficient to control the infection caused by *P. brasiliensis*, despite favorable in vitro results. Therefore, new concentrations should be evaluated to enable the extrapolation of in vitro results to in vivo conditions, facilitating the development of new compounds with promising antifungal activity.



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4. DISCUSSION

Paracoccidioidomycosis (PCM) is a neglected systemic mycosis with limited therapeutic options, which are often associated with prolonged treatment periods and toxicity (20). In this context, the search for new molecules with antifungal potential is increasingly necessary, and 1,3,4-oxadiazole derivatives represent a promising option in this pursuit. In this study, the obtained results demonstrated that the novel oxadiazole-derived molecule, designated B-O-Ph, exhibited promising *in vitro* activity against *Paracoccidioides* spp.

The MIC values obtained for the molecule B-O-Ph (4 µg/mL for Pb18, 2 µg/mL for the clinical isolate BiHUMPb02, and 0.5 µg/mL for Pb01) demonstrate its ability to inhibit fungal growth at a concentration close to that observed for the conventional drug, in this case, Amphotericin B, with an MIC of 1 µg/mL. Similar results have been reported in the literature for other oxadiazole-derived molecules, which exhibited MIC values ranging from 1 µg/mL to 32 µg/mL when tested against *Paracoccidioides brasiliensis* (16). Other derivatives of oxadiazole also showed activity against *Candida albicans*, with MIC values of 8-32 µg/mL, similar to the study cited above (15).

The results obtained from the time-kill curve experiment indicate a fungistatic profile. A reduction in fungal load was observed for the MIC and 2xMIC concentrations after day 6. Other oxadiazole derivatives, lacking Ebselen in their molecular structure, have demonstrated fungicidal activity against *Paracoccidioides brasiliensis* and *Candida albicans* (15,16). Regarding Ebselen, it also exhibited a fungicidal profile in time-kill curve experiments with *C. albicans* and *Cryptococcus neoformans* when tested at a 5xMIC concentration, suggesting that higher concentrations may result in a more pronounced reduction of fungal burden (17). Based on these findings, it is worth considering concentrations greater than 2xMIC in future assays to better assess the potential of B-O-Ph in reducing fungal burden. Although it does not exhibit a fungicidal profile, the combination of a novel molecule with fungistatic activity and existing drugs could benefit patient treatment by reducing the need for high doses and associated adverse effects (21).

The *in vivo* toxicity of B-O-Ph was previously assessed in BALB/c mice, and no adverse effects were observed during the experimental period (unpublished data). Despite this safety profile, treatment of mice infected with *Paracoccidioides brasiliensis* showed no significant reduction in fungal burden when compared with the untreated group or the group treated with itraconazole. In contrast, other oxadiazole derivatives, such as LMM6, have been reported to significantly reduce fungal burden in murine models of fungal infection (15), and Ebselen has also demonstrated strong antifungal activity *in vivo* (17). The lack of efficacy of B-O-Ph in this study may be related to factors such as administration route, compound formulation, or insufficient dosing. The itraconazole results are consistent with previous findings showing its limited capacity to eradicate pulmonary disease in murine PCM models, due to poor absorption and non-linear pharmacokinetics (22). These data support the notion that optimization of dose, delivery method, and treatment duration could improve the *in vivo* efficacy of B-O-Ph in future experiments.

5. CONCLUSION

In conclusion, the results obtained in the present study demonstrate the antifungal activity of the 1,3,4-oxadiazole-derived molecule B-O-Ph against *Paracoccidioides* spp. isolates, revealing a promising but still preliminary antifungal effect that requires further investigation. The MIC and MFC parameters indicated the inhibitory capacity of the molecule, which was further supported by the time-kill curve. With promising *in vitro* findings, B-O-Ph emerges as a strong candidate for further studies to elucidate its mechanism of action and therapeutic potential.

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Palavras-chave: *Paracoccidioides brasiliensis*; new molecules; antifungals.

Área temática: Pathogens of clinical relevance.



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VIABILITY OF HUMAN MILK COLOSTRUM POWDER AS A STRATEGY FOR OPTIMIZED CONSERVATION AND DISTRIBUTION

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1. INTRODUCTION

Breastfeeding is widely recognized as one of the most effective interventions for promoting neonatal health, especially in premature and low-birth-weight newborns. Human milk is considered the ideal food during this phase, as it combines essential nutrients with immunological and bioactive factors that promote the growth and maturation of the immune system (1).

The composition of human milk is unique and adapts throughout the lactation period. In the first few days after birth (up to seven days), colostrum is produced. This thick, yellowish fluid is highly concentrated in proteins, immunoglobulins, regulatory cytokines, antimicrobials, and growth factors (2,3). This strengthens the intestinal barrier, matures the immune system, and protects against infectious and inflammatory processes (4,5), ensuring significant postnatal protection and adaptation (6). After this phase, transitional human milk is produced (between seven and fourteen days), followed by mature human milk (after fifteen days), which provides greater energy support to the infant (7,8).

Despite its importance, the supply of human milk faces limitations, such as low maternal production, diseases, hospitalization, or even maternal death, which may restrict the infant's direct access to this food. In this scenario, human milk banks play a fundamental role in ensuring breastfeeding support (2). To ensure microbiological safety, these services predominantly use pasteurization, although this thermal process may compromise the stability of human milk bioactive compounds (9), and designate rigorous preservation under freezing (8).

In this sense, given the importance of colostrum and human milk for neonatal health, the development of strategies capable of favoring the preservation of bioactive properties of human milk without significant losses becomes essential. In this context, lyophilization stands out as a promising technological alternative to ensure greater stability of human milk bioactive compounds (10). The technique consists of a dehydration process by sublimation at low temperatures and reduced pressure, resulting in stable powdered human milk, easy to handle (11), store, and transport, thus expanding its availability to newborns (12). In addition, lyophilized human milk represents a strategic solution to expand distribution in regions with limited infrastructure, enabling longer storage time without the need for a cold chain (13,14).

However, the effectiveness of lyophilization depends not only on the process itself, but also on storage conditions. Therefore, the choice of suitable packaging is decisive to protect human milk against environmental factors such as oxygen, moisture, and light, which may accelerate enzymatic degradation reactions and oxidation of bioactive molecules (10,15).

In view of this scenario, the present study aims to evaluate the feasibility of human colostrum lyophilization as a promising alternative for human milk banks, considering its applicability in the nutrition of premature and low-birth-weight newborns. The investigation seeks to understand and optimize the



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lyophilization process, aiming to consolidate powdered human colostrum milk as a safe and effective solution for preservation and distribution in different clinical contexts.

2. MATERIALS AND METHODS

2.1. Ethics Committee Approval

The study has an approved link with the Certificate of Presentation of Ethical Appreciation (CAAE) No. 93048818.0.0000.0104 integrated with the research line of the APLE-A-CNPq group.

2.2. Sample Collection

Human milk colostrum samples ($n = 7$) were collected from the Human Milk Bank of the Maringá Regional University Hospital, following pre-defined research criteria, including the selection of human milk that did not meet the quality control standard (identified as dirty). After collection, a transportation protocol was followed, keeping it under a cold chain at a maximum of 4°C, as recommended by the National Health Surveillance Agency (ANVISA) (13). Subsequently, the selection of individual vials was assessed for acidity by section 2.3, analyzing each vial for sampling selection. After sample selection, a pool was created by mixing the entire volume of human milk.

2.3. Titratable Acidity

The acidity content was assessed individually after the collection of human milk samples, as a quality parameter for the research. For this purpose, 10 mL aliquots were collected from each collected vial, and titration was carried out using a sodium hydroxide titrant solution N/9 (Dornic solution), in the presence of 4 drops of the phenolphthalein indicator. For results, it was considered that each 0.1 mL of Dornic solution used to neutralize 10 mL of human milk is equivalent to 1°D (16).

2.4. Freeze Drying

Approximately 1700 mL of pooled human milk was subjected to lyophilization. First, samples were prepared by transferring the volume to rectangular high-density polyethylene containers, weighing the mass of the empty container and the volume of added human milk on a semi-analytical balance (model ATY224, Shimadzu). The samples were then stored at -18°C for 24 hours.

Lyophilization was performed in a freeze-dryer (L108, LioBras) operating at -52°C and a pressure of 11.2 µHg. The processing time was calculated until a moisture content of 4% to 5% was obtained, monitored by an infrared analyzer (model I-Thermo, Bel Engineering), by the established standard for lyophilized human milk (13). The dried samples were then collected in a previously sanitized aluminum tray and ground to a fine, homogeneous powder. They were then individually packaged in silver-colored metal bags, vacuum-sealed, and labeled according to the storage analysis schedule.

2.5. Yield

The mass of the initial and dry human milk colostrum was determined to estimate the yield, taking into account the water loss that occurred during the process. The yield was expressed as a percentage (%), calculated from the ratio of the total solids recovered after freeze-drying to the initial mass of the milk submitted to processing.

2.6. Moisture



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The moisture content of lyophilized human colostrum milk was evaluated using an infrared moisture analyzer (I-Thermo, Bel Engineering, Italy). The analysis was performed in triplicate at $102 \pm 1^\circ\text{C}$ for approximately 3 min until a constant mass was obtained. The results were expressed as a percentage (%).

3. RESULTS

3.1. Sample Selection (Acidity)

The acidity of human milk was evaluated as a quality control parameter, with values ranging from 4 to 6 °Dornic, remaining below the limit of 8 °Dornic established by Brazilian human milk banks (13). After verification, the entire collected volume was homogenized and lyophilized.

3.2. Yield and Moisture Control of Freeze-Dried Human Milk Colostrum

The results obtained after freeze-drying human milk colostrum are presented in Figure 1.

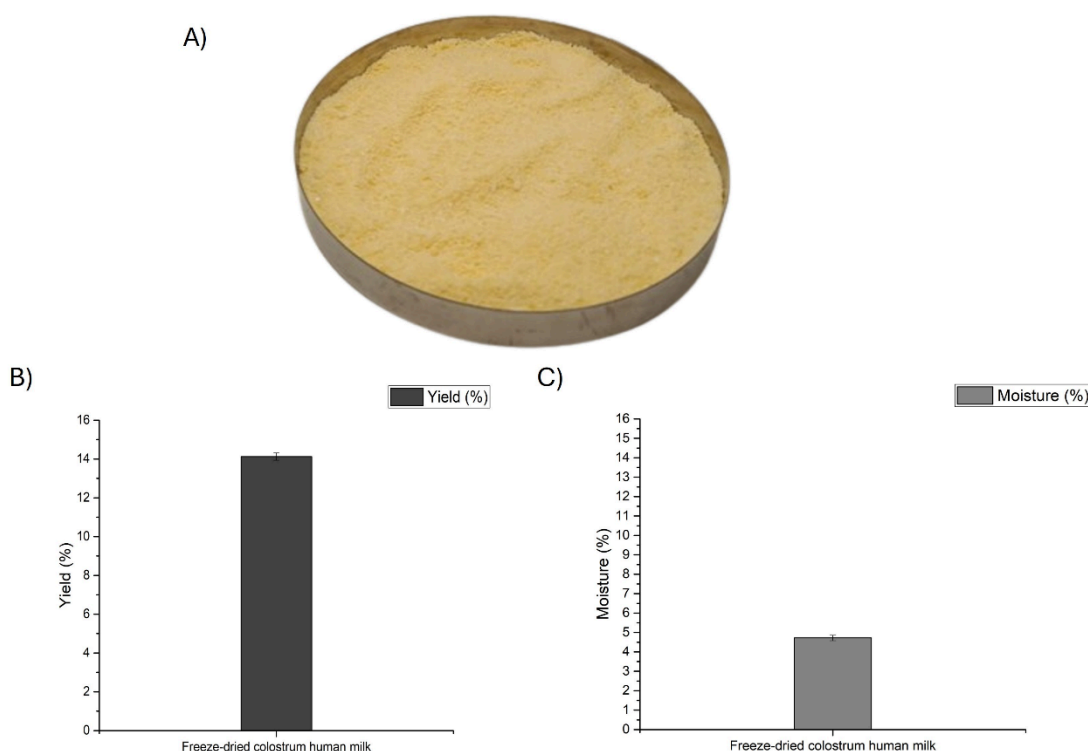


Figure 1. Control of the freeze-drying process of freeze-dried human milk colostrum.

The appearance of the powdered human milk colostrum (Figure 1a) indicates an intense yellow color, characteristic of the lactation phase of the sample. Additionally, the freeze-drying process yielded $14.12 \pm 0.79\%$ (Figure 1b), resulting in a powdered human milk colostrum with a moisture content of $4.72 \pm 0.14\%$ (Figure 1c).

4. DISCUSSION



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The acidity of human milk was found to be of drinking quality and was selected as suitable for processing. Samples exceeding this threshold must be discarded, as they are considered unsuitable for neonatal consumption (13). Acidity is an important indicator of physicochemical stability and preservation of nutrients, immunology, and biochemical components. Alterations in this parameter may lead to metabolic overload, predisposing newborns to acidosis or alkalosis and increasing the risk of necrotizing enterocolitis, particularly in preterm and very low birth weight infants. Increased acidity may be associated with microbiological, lipolytic, and proteolytic changes, reinforcing the need for strict monitoring to ensure the safety and quality of milk intended for infant feeding (19).

Freeze-drying is based on sublimation and subsequent water removal; a drying process consistently associated with high yield and recovery efficiency (10). The yield obtained is particularly relevant for the reconstitution of powdered human milk, as it allows the restoration of its original composition after the addition of water. Previous studies indicate that freeze-drying can reduce the water content of human milk by up to 86.5% (17) thus improving storage stability. Furthermore, freeze-drying has been reported to better preserve the bioactive components of human milk and ensure higher recovery compared to other methods (18), which is especially advantageous for colostrum processing. The yield of powdered human milk is directly dependent on the total solids content of raw human milk. Reported values for soluble solids range from $9.13 \pm 0.23\%$ to $10.36 \pm 0.12\%$ (9,10), while fat content varies between 1.56% and 4.62% (20), both representing the fraction retained after water removal. Alves et al. (10) documented a yield of 11.89% after freeze-drying of human milk. Thus, the total solids composition in human milk largely determines the final powder yield, as higher amounts of dry matter result in higher recovery after drying.

The moisture content is consistent with the literature, confirming the efficiency of the water removal process. Furthermore, the value found is within the range considered appropriate for human milk, which is a final moisture content between 4 and 5% (13). The low moisture content obtained in this study favors the maintenance of the quality of human milk powder, as it reduces its susceptibility to lipid oxidation reactions, enzymatic degradation, microbiological growth, and solubility changes (21).

Furthermore, from a practical perspective, the freeze-drying process offers advantages for nutrient conservation and also addresses issues related to storage and distribution logistics, resulting in reduced volume, ease of transportation and distribution, especially in hard-to-reach areas, and greater flexibility in hospital storage (12).

5. CONCLUSION

The study demonstrated that freeze-drying human milk colostrum under the conditions described is an effective preservation strategy, allowing for volume reduction, good yield, adequate recovery, and ensuring the powder's reconstitution to restore its natural characteristics. The process resulted in moisture content below the limit established by ANVISA, favoring nutrient preservation and greater stability against extrinsic factors. Thus, the removal of water confirms the potential of freeze-dried human milk colostrum as a safe and stable alternative for distribution in human milk banks.

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EPIDEMIOLOGICAL AND CLINICAL PROFILE OF WOMEN WITH CERVICAL CANCER ATTENDED AT A HOSPITAL IN SOUTHERN BRAZIL

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1. INTRODUCTION

Cervical cancer is the fourth most common malignant neoplasm among women worldwide, excluding non-melanoma skin cancer. In 2020, more than 604,000 women were diagnosed with cervical cancer and approximately 342,000 deaths were attributed to the disease (1). In Brazil, excluding non-melanoma skin tumors, cervical cancer ranks as the third most frequent cancer in women. For each year of the 2023–2025 triennium, an estimated 17,010 new cases are expected, corresponding to a crude incidence rate of 15.38 cases per 100,000 women (2).

Regular cytopathological screening (Papanicolaou test) plays an important role in the early detection of precursor cytological abnormalities and cervical cancer, enabling timely intervention and improving the likelihood of successful treatment in its early stages. However, despite the wide availability of the Pap smear screening program through Brazil's Unified Health System (SUS), adherence among women remains suboptimal. Multiple barriers contribute to this low participation, including a lack of perceived need, absence of symptoms, insufficient medical recommendation, embarrassment, limited availability of appointments in public healthcare units, among others (3-6). Therefore, cervical cancer continues to present high incidence and mortality rates, particularly in developing countries such as Brazil (2).

Most cervical cancer cases are causally associated with infection by the human *Papillomavirus* (HPV), with substantial evidence demonstrating that high-risk oncogenic genotypes, particularly HPV types 16 and 18, are present in approximately 70% of cases. In response to this evidence, the International Agency for Research on Cancer (IARC), in collaboration with the World Health Organization (WHO), has recently endorsed the adoption of HPV detection assays as the primary approach for cervical cancer screening (7).

Cervical cancer is histologically classified based on the type of cell in which malignant transformation originates. Squamous cell carcinoma, arising from the squamous epithelium, is the most prevalent subtype, accounting for approximately 70% to 80% of cases. Also referred to as epidermoid carcinoma, this subtype typically develops from the squamous cells lining the external portion of the cervix, known as the ectocervix (8). In contrast, adenocarcinoma represents approximately 10% to 20% of cases and originates from the glandular cells of the endocervical canal. Notably, adenocarcinoma is often more challenging to detect through routine screening methods, such as the Papanicolaou test, which may contribute to delayed diagnosis (8,9).

The therapeutic approach to cervical cancer is primarily guided by the stage of the disease, as established by the International Federation of Gynecology and Obstetrics (FIGO) staging system. This system categorizes tumors into four major stages: Stage I (IA and IB), Stage II (IIA and IIB), Stage III (IIIA and IIIB), and Stage IV (IVA and IVB), with additional subdivisions within each category. Staging is based on a comprehensive assessment of clinical and pathological factors, including tumor size, depth of stromal invasion, lymph node involvement, invasion of adjacent pelvic structures, and the presence of distant metastases (10, 11).

Smaller cervical tumors are generally managed with less invasive surgical procedures, such as conization, trachelectomy, or hysterectomy. In contrast, larger tumors or those with potential for either local or distant metastasis typically require regional lymph node dissection for pathological assessment, with treatment options including primary radiotherapy, surgery, or a combination of both. Tumors exhibiting



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invasive or metastatic characteristics, such as positive lymph nodes or invasion of adjacent organs, often require more aggressive approaches, including total or radical hysterectomy combined with concurrent chemoradiotherapy, or pelvic exenteration in cases of recurrence (10). Cisplatin is considered the most effective chemotherapeutic agent for metastatic control and is commonly used as first-line monotherapy. However, resistance to cisplatin may develop, requiring substitution or combination with other agents, such as paclitaxel, bevacizumab, or topotecan (12). Despite these therapeutic strategies, approximately 30% to 50% of patients fail to achieve an adequate response to treatment (13).

Given the significant relevance of studies addressing this type of cancer, which remains a major public health concern among women, particularly in low- and middle-income countries such as Brazil, this study aimed to assess the epidemiological and clinical profile of women diagnosed with cervical cancer and treated at a hospital in southern Brazil. This analysis seeks to provide data that may be critical for informing more effective public health policies and contributing to the reduction of morbidity and mortality associated with this malignancy in the country.

2. MATERIAL AND METHODS

2.1. Sample collection

This retrospective, descriptive study was conducted through the analysis of medical records of women diagnosed with cervical cancer from 2020 through 2023. Diagnoses were confirmed through biopsy results processed by the Micropar Laboratory (Londrina, Paraná, Brazil), and patients treated at Honpar (Hospital Norte Paranaense), located in Arapongas, Paraná, Brazil.

Patients younger than 18 years of age were excluded from the analysis.

2.2. Collection of epidemiological and clinical data

To obtain clinical and epidemiological data from the study participants, a comprehensive review of the databases of Honpar Hospital was conducted. Both physical and electronic medical records (e-CRF) of patients treated in the oncology department were accessed by an authorized researcher. Key variables, including age, presenting symptoms, date of cervical cancer diagnosis, histopathological biopsy results, tumor stage at diagnosis, and type of treatment administered, among other relevant information, were systematically extracted, organized, and tabulated using Microsoft® Excel for subsequent analysis.

2.3 Disease staging

Disease staging was conducted in accordance with 2018 FIGO guidelines (14) for cervical cancer, which consider local tumor extension, regional lymph node involvement, and the presence of distant metastases.

Staging data were documented in each patient's medical record at the time of diagnosis, based on a comprehensive clinical evaluation, imaging studies (including ultrasonography, computed tomography, and magnetic resonance imaging), as well as relevant laboratory findings.

2.4 Clinical assessment

Clinical evaluations were conducted during scheduled follow-up visits, which both functional status and prognosis were systematically assessed and documented in the patients' medical records.

Functional status was determined using performance status (PS) scales, based on the indices proposed by Zubrod and Karnofsky, as outlined by the Brazilian National Cancer Institute - INCA (15):

- Zubrod 0, Karnofsky 100%–90%: Asymptomatic patient or presenting only minimal symptoms.
- Zubrod 1, Karnofsky 89%–70%: Symptomatic patient, but capable of attending ambulatory visits.
- Zubrod 2, Karnofsky 69%–50%: Patient confined to bed for less than half of the day.
- Zubrod 3, Karnofsky 49%–30%: Patient confined to bed for more than half of the day.



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- Zubrod 4, Karnofsky 29%–10%: Bedridden patient requiring continuous care.
- Karnofsky <9%: Terminal or moribund patient.

The prognosis was established based on clinical evaluation, imaging findings, and laboratory results, and subsequently classified into the following categories:

- Follow-up: defined as ongoing medical surveillance of the disease, with no clinical, radiological, or laboratory evidence of progression;
- Treatment: defined as patients who remain under active therapy due to disease progression or persistent active disease;
- Death: defined as patients who died during the study period.

2.4 Ethical considerations

All ethical and legal principles established by Resolution 466/2012 were strictly observed. This study was approved by the Permanent Committee on Ethics in Research Involving Human Subjects (COPEP) of the State University of Maringá (UEM), under approval number 7.004.109.

3. RESULTS

Between 2020 and 2023, a total of 82 women with biopsy-confirmed cervical cancer were included in the study cohort. The lowest number of confirmed cases was recorded in 2020 ($n = 13$; 15.85%), whereas the highest incidence was observed in 2023 ($n = 29$; 35.36%).

The mean age of the patients was approximately 50 years, with ages ranging from 26 to 86 years. A slight upward trend in age was observed over the study period, with a predominance of patients in the fourth to sixth decades of life. Throughout the study period, most patients self-identified as White ($n = 61$; 74.39%), followed by Brown or mixed-race ($n = 13$; 15.85%), with a smaller proportion identifying as Black ($n = 3$; 3.65%) or Asian ($n = 5$; 6.09%). Regarding marital status, married women ($n = 39$; 47.56%) were the most prevalent groups over the years. In terms of clinical presentation, vaginal bleeding was the predominant symptom observed in all years ($n = 58$; 70.73%), although a small proportion of patients were asymptomatic at diagnosis ($n = 5$; 6.10%). Most symptoms had an evolution period of less than one year ($n = 52$; 63.41%), suggesting relatively early diagnosis in a portion of the patients. The predominant performance status was PS 1 ($n = 47$; 57.31%), representing 69.23% of cases in 2020. In 2021, 2022, and 2023, PS 1 remained the most prevalent; however, a more balanced distribution between PS 1 and PS 2 was observed during these years. Furthermore, cases with poorer functional status, classified as PS 3, emerged in 2023 ($n = 2$; 6.89%).

From a histopathological perspective, squamous cell carcinoma was the predominant subtype throughout the study period ($n = 68$; 82.92%), with a smaller proportion of adenocarcinomas ($n = 9$; 10.97%). In terms of clinical staging, Stage II was the most frequent in 2020 ($n = 11$; 84.61%), 2021 ($n = 14$; 60.86%), and 2022 ($n = 10$; 58.82%). However, in 2023, a notable shift was observed, with 19 of the 29 cases (65.51%) diagnosed at Stage III and one case (3.44%) at Stage IV, indicating a potential delay in diagnosis during that year.

Isolated surgical treatment was the predominant therapeutic approach in 2020 ($n = 9$; 69.23%) and 2021 ($n = 14$; 60.86%). However, from 2022, there was a noticeable shift toward combined treatment modalities, particularly chemoradiotherapy ($n = 4$; 23.54%) and surgery combined with chemoradiotherapy ($n = 6$; 35.29%). By 2023, chemoradiotherapy had become the primary treatment modality ($n = 16$; 55.17%), alongside a notable proportion of cases managed with the combination of surgery and chemoradiotherapy ($n = 5$; 17.24%).

Regarding prognosis, most patients ($n = 56$; 68.29%) remained under follow-up during the four-year evaluation period. However, an increase in mortality was observed over time, rising from one case (7.6%) in 2020 to five cases (21.73%) in 2021, four cases (23.52%) in 2022, and five cases (17.24%) in 2023.

4. DISCUSSION

This study evaluated both epidemiological and clinical profile of 82 women diagnosed with cervical cancer between 2020 and 2023. The mean age of the patients was 49.6 years, with a range between 46.8



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and 52.4 years. These findings align with previous research indicating that the highest incidence of cervical cancer occurs among women in their fourth and fifth decades of life, reflecting the effects of persistent high-risk HPV infection and the progression of precursor lesions (16, 17).

Regarding race/ethnicity, the predominance of White women (74.39%) observed in this study may be attributed to the demographic profile of the population analyzed, as this investigation was conducted in southern Brazil, a region with a higher proportion of White women compared to other regions of the country (18).

From a clinical standpoint, vaginal bleeding emerged as the predominant symptom, corroborating evidence in the literature that identifies it as the most frequent clinical manifestation, particularly during the early and intermediate stages of the disease (14). The occurrence of asymptomatic patients in 2022 and 2023 suggests a limited yet positive impact of opportunistic cytological and colposcopic screening, which, despite being in a transitional phase, continues to be the primary screening strategy employed in Brazil (19). Nevertheless, the marked shift observed in 2023, characterized by a significant increase in cases diagnosed at advanced stages (III and IV), warrants particular attention. This trend may be associated with barriers to accessing healthcare services, a reduction in screening coverage, and diagnostic delays, a scenario widely discussed in the context of the COVID-19 pandemic (20).

From a histopathological perspective, squamous cell carcinoma was the predominant subtype (82.92%), a finding consistent with global literature, which reports this histological type in approximately 80% of cervical cancer cases (16). Although less frequent, adenocarcinoma exhibited proportions similar to those described in previous studies, which associate this subtype with greater diagnostic challenges due to the anatomical location of the endocervical epithelium and the lower sensitivity of cytological screening methods, such as the Papanicolaou test (14).

Regarding functional status, PS 1 remained predominant; however, a progressive increase in patients classified as PS 2 was observed, along with the emergence of cases presenting PS 3 in 2023. This trend may be directly associated with the advancement in clinical staging recorded in the same year, reinforcing the well-documented relationship between delayed diagnosis, deterioration in clinical condition, and the subsequent need for more aggressive therapeutic interventions (21).

The analysis of treatment strategies revealed a significant transition over the study period. While management in 2020 and 2021 was predominantly surgical, from 2022 onward there was a notable increase in the use of combined therapeutic approaches, particularly chemoradiotherapy, which became the primary treatment modality by 2023. This shift is consistent with international recommendations that establish chemoradiotherapy as the gold standard for locally advanced cervical cancer, due to its positive impact on survival outcomes (22). On the other hand, the growing reliance on this modality also reflects a trend toward diagnoses at more advanced stages, which may reduce the potential for curative outcomes (23).

Regarding prognosis, most patients remained under follow-up throughout the study period. Nevertheless, the progressive increase in mortality observed over the years directly reflects the growing proportion of cases diagnosed at more advanced stages, underscoring the critical need for effective prevention and screening strategies aimed at early detection. According to the World Health Organization, the elimination of cervical cancer as a public health problem relies on the simultaneous achievement of key global targets, including widespread HPV vaccination, regular screening programs, and universal access to timely and appropriate treatment (18).

Thus, the findings of this study according with existing literature regarding the age profile, histopathological characteristics, and symptomatology of the patients; however, they also reveal important differences in staging and prognosis patterns, particularly in 2023. These variations may be associated with reduced access to, or decreased utilization of, routine healthcare services during the COVID-19 pandemic (24).

5. CONCLUSION

Based on the data analyzed, an increase in cervical cancer diagnoses was observed during the study period, with a predominance of women in their fifth decade of life and squamous cell carcinoma identified as the most prevalent histological subtype. The higher proportion of advanced-stage diagnoses, the deterioration of clinical conditions, the shift toward combined therapeutic strategies underscore the need to strengthen organized screening programs, address inequalities in healthcare access, and enhance public health policies aimed at improving cervical cancer control.



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PREVALENCE OF HIGH-RISK HUMAN PAPILLOMAVIRUS INFECTION IN WOMEN WITH SYSTEMIC LUPUS ERYTHEMATOSUS FOLLOWED AT THE REGIONAL UNIVERSITY HOSPITAL OF MARINGÁ, PARANÁ, BRAZIL

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1. INTRODUCTION

Cervical cancer is the fourth most commonly diagnosed cancer and the fourth leading cause of cancer-related death in women worldwide, with an estimated 604,000 new cases and 342,000 deaths in 2020 (1). In Brazil, it is the third most frequent cancer in women, excluding non-melanoma skin tumors. Persistent infection with high-risk human papillomavirus (HR-HPV) is the main etiological factor, as most HPV infections are transient, but failure to clear the virus can lead to persistent infection and cervical carcinogenesis (2,3). In Brazil, cervical cancer screening is primarily cytological, using Papanicolaou (Pap) smears. However, HPV-DNA testing has been increasingly adopted due to its higher sensitivity and capacity to detect oncogenic types, particularly relevant for immunocompromised populations such as women with systemic lupus erythematosus (SLE) (4).

SLE is a chronic autoimmune disease characterized by multisystem inflammation and tissue damage resulting from autoantibody production and impaired immune regulation (5). It presents with heterogeneous clinical manifestations and fluctuating periods of activity and remission, potentially involving various organs, mucosal surfaces, and the central nervous system. An estimated 3.4 million individuals are affected worldwide, with a predominance in women. Due to both the autoimmune nature of the disease and the frequent use of immunosuppressive therapies, women with SLE exhibit increased immunological vulnerability, potentially elevating the risk of persistent HR-HPV infections and related complications (6). However, despite this risk, few studies have investigated HR-HPV prevalence and cervical lesions in this population. Investigating these associations is essential to understanding the impact of SLE-related immunosuppression on cervical carcinogenesis and improving prevention strategies in this vulnerable group (7).

The aim of this study is to evaluate the prevalence of HR-HPV infection and cervical lesions in women diagnosed with SLE who are followed at the rheumatology outpatient clinic of the regional University



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Hospital of Maringá (HUM), Paraná, Brazil. This investigation seeks to clarify the potential influence of disease- and treatment-related immunosuppression on cervical carcinogenesis and to support improved strategies for cervical cancer prevention and patient management in this high-risk population.

2. MATERIALS AND METHODS

2.1. Selection of study participants

This is a prospective observational study, in which women diagnosed with SLE, aged between 18 and 75 years, who are followed at the rheumatology outpatient clinic of the HUM, from 10/01/2024 to 08/25/2025, were invited to participate.

Exclusion criteria were: pregnant or postpartum women; women with a history of hysterectomy; vaginal bleeding; no history of sexual activity; recent treatment of any genital tract pathology; ablative or excisional therapy of the cervix in the past 12 months; and those undergoing chemotherapy or radiotherapy for cervical cancer.

2.2. Clinical-epidemiological data and application of the questionnaire

After selecting the participants who met the inclusion criteria, the study's informed consent form (ICF) was applied, informing the participants of its importance and their freedom to participate or not. Subsequently, after signing the ICF, the study questionnaire was administered. The ICF and the study questionnaire were administered by a trained and delegated member of the research team.

For the collection of clinical data from the study participants, a search was carried out in the HUM databases, reviewing physical records and electronic medical records (e-CRF) of the women included in the study by an authorised staff member.

2.3. Pap test

Vaginal, ectocervical, and endocervical samples were collected using Cytobrush-type brushes and placed on glass slides, stored in slide holders with 92.8% alcohol until their use. Subsequently, they were stained by the Papanicolaou method for cytological analysis. The cytological smears were sent to the Clinical Cytology Laboratory of the State University of Maringá (UEM), and all examinations were evaluated and reported according to the Bethesda System 2019. Quality control was performed using SISCOLO (Cytology and Gynecology Quality System), established by the Brazilian Ministry of Health.

Cytological results were considered normal if none of the following alterations were observed: atypical squamous cells of undetermined significance (ASC-US) or cannot exclude high-grade intraepithelial lesion (ASC-H), atypical glandular cells (AGC), low-grade squamous intraepithelial lesion (LSIL), or high-grade squamous intraepithelial lesion (HSIL); cervical intraepithelial squamous cell lesion, squamous cell carcinoma in situ (ISCC), and invasive squamous cell carcinoma (CC).

2.4. Molecular detection and typing of HR-HPV

Vaginal, ectocervical, and endocervical samples were collected using Cytobrush-type brushes and transferred to a vial containing preservative solution (ThinPrep® PreservCyt™ Solution, Hologic, USA) for subsequent molecular analysis.

For the detection and typing of HR-HPV, the Anyplex™ II HPV HR Detection system (Seegene, South Korea) was used, which detects and genotypes 14 types of HR-HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), as well as an Internal Control (IC). In PCR, efficiency can be reduced by inhibitors that may be present in clinical samples. The Anyplex™ II HPV HR Detection system uses a housekeeping gene as an endogenous IC, which can ensure DNA purification, PCR reaction verification, and clarification of the cellular adequacy of each sample. The IC is co-amplified with target nucleic acids within clinical samples.

2.5. Ethical aspects



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The ethical and legal principles of Resolution 466-2012 were respected. This project was approved by the Permanent Committee on Ethics in Research Involving Human Beings of the State University of Maringá (COPEP), opinion No. 7.152.136, and by the Commission for the Regulation of Academic Activities (COREA) of the HUM, process No. 053/2024.

3. RESULTS

Up to August 25, 2025, 73 patients diagnosed with SLE and under follow-up at HUM were considered eligible and were included in the study. The age of the patients ranged from 18 to 68 years, with a mean of 42.3 years (SD = 11.6; range 18–68 years). Regarding race, there was a predominance of White women (n = 30, 41.1%), followed by Brown (n = 26, 35.6%), Black (n = 13, 17.8%), and Asian (n = 4, 5.5%). There were no records of indigenous patients.

Among the 73 women included, 10 (13.7%) tested positive for HR-HPV. Considering both single and co-infections, 8 genotype detections were identified in these 10 women. Single-type infections accounted for 9 cases (90.0%), whereas one case (10.0%) was a co-infection with HPV 16 and HPV 59. Among single-type infections, eight distinct genotypes were identified: HPV 52 in 2 cases (22.2%) and HPV 16, 18, 31, 35, 56, 58, and 59 in 1 case each (11.1%).

Table 1. HPV type distribution in SLE patients with single HR-HPV infections

HPV TYPE	POSITIVE PATIENTS (n)	POSITIVE PATIENTS (%)
HPV 52	2	22.2
HPV 59	1	11.1
HPV 16	1	11.1
HPV 58	1	11.1
HPV 56	1	11.1
HPV 35	1	11.1
HPV 31	1	11.1
HPV 18	1	11.1
TOTAL	9	100

Cytological analysis was performed on 69 patients included in the study, among whom 5 (7.3%) demonstrated cytological abnormalities, all of which were associated with HR-HPV positivity. Specifically, 2 patients (2.9%) were diagnosed with ASC-US, 1 patient (1.4%) with ASC-H, and 2 patients (2.9%) with LSIL.

4. DISCUSSION

This study aimed to assess the prevalence of HR-HPV infection and cervical cytological abnormalities in women diagnosed with (SLE) who are followed at the rheumatology outpatient clinic of the Regional University Hospital of Maringá (HUM), Paraná, Brazil. A high prevalence of HR-HPV was identified in this population (13.7%), considerably higher than that reported among women at standard risk in the same geographic region. Additionally, a notable frequency of cytological abnormalities was observed, affecting 7.3% of patients.

The racial analysis revealed that most HR-HPV infections occurred in brown women (50%), followed by white women (40%) and Asian women (10%). Although the sample size is limited for robust racial



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comparisons, these findings suggest no clear predominance of HR-HPV among racial groups, supporting the literature indicating that individual immunological and behavioral factors may be more determinant than race itself (8,9).

An epidemiological study conducted in the same region, in the state of Paraná, Brazil, reported an HPV infection rate of 6.7%, considering high- and low-risk types in the general women population (8). In contrast, our study focused exclusively on HR-HPV in women with SLE, with a prevalence of 13.7%. This difference may reflect the greater susceptibility of immunocompromised women with SLE to persistent infection with oncogenic HPV types.

In this study, the prevalence of HR-HPV infection among women with SLE followed at HUM was 13.7%. This finding aligns with previous research indicating increased susceptibility of immunocompromised patients, including those with systemic autoimmune diseases, to HPV infection compared to the general population. In adult women with SLE, the prevalence ranges from 5% to 12%, while in the general population it is approximately (6,7%) (7,8).

The HR-HPV genotype profile in SLE patients revealed that HPV 52 was the most prevalent genotype, identified in two patients (22.2%), whereas HPV 16, 18, 31, 35, 56, 58, and 59 were each detected in one patient (11.1%). Although HPV 16 is widely recognized as the most prevalent genotype associated with squamous intraepithelial lesions and cervical cancer cases (10), the higher frequency of HPV 52 in this cohort is noteworthy, as recent studies highlight its important role in the development of squamous intraepithelial lesions, particularly in immunosuppressed populations (11). In this study, one case (10.0%) involved a co-infection with HPV 16 and HPV 59. The identification of this co-infection is consistent with the literature, since, although less frequent, co-infections are relevant as they may contribute to viral persistence and increase the risk of lesion progression (11).

Cytological analysis revealed abnormalities in 7.3% of patients, all of which were associated with HR-HPV infection. The observed alterations included ASC-US (2.9%), ASC-H (1.4%), and LSIL (2.9%). These findings reinforce the association between HR-HPV infection and cellular changes, confirming the virus's role in the development of cervical lesions, including in women with SLE (12). It is important to note that, although the prevalence of cytological abnormalities is not high, continuous surveillance is essential, as patients with SLE may have an increased risk of progression to high-grade lesions due to chronic immunosuppression and the use of immunomodulatory therapies (11).

Furthermore, our study highlights the importance of cervical cancer screening programs tailored for women with SLE, including the molecular detection and typing for HR-HPV, since early detection enables timely interventions and prevention of progression to invasive carcinoma (12). Strategies such as HPV vaccination and regular screening can be particularly beneficial for this high-risk group (13).

Future perspectives for this study include increasing the number of patients and a detailed analysis of the medications used by each participant, including immunosuppressive therapies and data on behavioral factors. This approach will allow us to evaluate how different treatment may influence susceptibility to HR-HPV infection and the occurrence of cytological abnormalities, providing a more comprehensive understanding of the impact of SLE clinical management on patients' cervical health.

5. CONCLUSION

The findings of this study demonstrate a high prevalence of HR-HPV infection and a relevant occurrence of cervical cytological abnormalities among women with SLE, reinforcing their increased susceptibility to HPV-related complications. These results underscore the importance of incorporating routine screening, vaccination, and specialized gynecological care into the clinical management of women with SLE. Strengthening these preventive strategies is essential to reduce morbidity, enable early intervention, and improve health outcomes in this vulnerable population.

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Keywords: *human papillomavirus*; cervical lesions; systemic lupus erythematosus.

Thematic area: Etiopathophysiology of human and animal disease.



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TEMPORAL ANALYSIS OF DATA COMPLETENESS IN ACUTE RESPIRATORY INFECTION (SARI) SURVEILLANCE IN PARANÁ STATE IN THE CONTEXT OF THE COVID-19 PANDEMIC

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1. INTRODUCTION

The ability of respiratory viruses such as Influenza, SARS-CoV-2, and respiratory syncytial virus to cause severe infections is linked to their virulence, immune evasion capabilities, tropism for respiratory tract cells, and the induction of exacerbated inflammatory responses (1, 2). Exacerbated inflammatory responses can lead to complications such as pneumonia and severe acute respiratory failure, among others, which are associated with prolonged hospitalization (often in Intensive Care Units – ICU, due to the need for ventilatory support) and higher mortality rates, particularly among patients with comorbidities (3).

Epidemiological surveillance of respiratory viruses allows for a better understanding of the epidemiological profile and, consequently, the planning of public health actions. In Brazil, this surveillance has been carried out since 2009 through the Respiratory Virus Surveillance Network, which comprises a structured and hierarchical set of healthcare services that operate in an integrated manner to monitor, identify, and characterize respiratory viruses such as Influenza, SARS-CoV-2, and other pathogens of public health importance, coordinated by the Ministry of Health (4).

The National Epidemiological Surveillance Network consists of Sentinel Units for Influenza-like Illness (located in Primary Healthcare Units and Emergency Care Units), which are strategically selected to report cases and collect respiratory samples for laboratory analysis from patients presenting with influenza-like symptoms. For the notification of Severe Acute Respiratory Infection (SARI), the network includes the participation of hospitals responsible for completing SARI notification forms (providing clinical, epidemiological, and laboratory information) in the SIVEP-Gripe system for hospitalized cases and deaths. These hospitals also collect clinical samples for viral identification, conducted by laboratories equipped to perform molecular and serological testing (4).

The state of Paraná has the highest number of Sentinel Units, including those distributed throughout the interior of the state, operating in a manner that enables sentinel surveillance to detect less prevalent viruses (5). Given these characteristics within the Epidemiological Surveillance Network, it is considered relevant to assess the completeness of SARI notifications to enhance and strengthen Brazil's surveillance system. The completeness of these notification forms is essential for providing reliable data for accurate epidemiological analyses, identifying risk groups, and monitoring disease progression. Incomplete data hinder the evaluation of factors such as comorbidities, vaccination status, clinical progression, and outcomes, thereby impairing the health system's response and the development of effective public policies (5).

Thus, this study aims to assess the degree of incompleteness of SARI data recorded in the SIVEP-GRIPE system across the four health macro-regions of Paraná state, Southern Brazil, by comparing the pre-pandemic, pandemic, and post-pandemic periods of COVID-19.



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2. MATERIALS AND METHODS

This retrospective observational study analyzed data from the SIVEP-Gripe database (<https://opendatasus.saude.gov.br/dataset/bd-srag-2021>, maintained by the Brazilian Ministry of Health). The system compiles notifications of hospitalized SARI cases from public and private healthcare facilities across Brazil. SARI was defined as the presence of cough or sore throat associated with dyspnea, oxygen saturation <95%, or respiratory distress in patients who required hospitalization or died from these symptoms, even if not hospitalized (4).

The analysis included cases of patients aged 18 years or older hospitalized in the state of Paraná. Data were grouped into three time periods: pre-pandemic (2019), pandemic (2020–2022), and post-pandemic (2023–2024). Paraná is the fifth most populous Brazilian state, with over 11.4 million residents and an HDI of 0.769 (6,7), and is divided into four health macro-regions, all of which contain sentinel hospitals for SARI surveillance.

Variables analyzed were extracted from the SIVEP-Gripe notification form, prioritizing *essential variables*, fields considered key for epidemiological surveillance, and available in the dataset, and *mandatory variables*, which are required by the system for submission. Mandatory variables included sex, age, self-reported race/skin color, and pregnancy status. These were used to characterize the sociodemographic profile of hospitalized patients. Among the essential variables, two domains were considered: (a) signs and symptoms: fever, cough, sore throat, dyspnea, respiratory distress, oxygen saturation <95%, diarrhea, vomiting, abdominal pain, fatigue, anosmia, ageusia, and other symptoms; and (b) risk factors/comorbidities: presence of comorbidities such as puerperium, chronic cardiovascular disease, chronic hematologic disease, Down syndrome, chronic liver disease, asthma, diabetes mellitus, neurological disorders, chronic pulmonary disease, immunodeficiency or immunosuppression, chronic kidney disease, obesity, and other reported conditions.

Data was extracted on August 8, 2025, and processed using Power Query in Microsoft Excel 365 (8). Completeness was calculated based on the SIVEP-Gripe Data Dictionary (version dated February 10, 2023), excluding fields marked as blank, ignored, or missing. The degree of incompleteness was classified according to Romero & Cunha (9) as: excellent (<5%), good (5–10%), fair (10–20%), poor (20–50%), and very poor (>50%).

This study complied with Brazilian data protection regulations (LGPD – Law No. 13,709/2018) and international ethical standards. Data confidentiality was preserved through anonymization, and the study was approved by the Research Ethics Committee of the State University of Maringá (COPEP/UEM), under approval number CAAE 50719221.9.0000.0104.

3. RESULTS

Table 1 presents the general characteristics of patients with reported SARI cases in the state of Paraná from 2019 to 2024. During this period, a total of 262,059 SARI cases were recorded. Overall, the majority of cases occurred among males (53.0%), individuals self-identified as white (73.3%), those aged over 60 years (53.2%), with elementary education (16.7%), and residents of urban/peri-urban areas (84.2%).

When each period was analyzed separately, SARI cases were consistently more frequent among individuals who self-identified as white, had elementary education, and lived in urban or peri-urban areas. In contrast, sex and age distributions varied across periods: a) pre-pandemic period: the cases were more common among females (52.3%) and in individuals under 60 years of age (57.1%); b) pandemic period: males (53.9%) and those aged 60 and over (51.7%) predominated; and c) post-pandemic period: the proportion of female cases increased again (53.1%), and cases among individuals aged 60 years or older further increased, exceeding the proportions observed during the pandemic period, reaching 66.6%.

Of the total number of women with SARI (123,049) reported in the state of Paraná from 2019 to 2024, 4,146 cases (3.4%) occurred among pregnant women. This subgroup accounted for 7.8% of reported cases in the pre-pandemic period, 2.9% during the pandemic, and 6.0% in the post-pandemic period.

Table 1. General characteristics of adult SARI patients (2019–2024) across the COVID-19 pre-pandemic, pandemic, and post-pandemic periods, Paraná State, based on variables from the notification form, including mandatory fields (sex, age, pregnancy status, race/skin color) and



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essential but non-mandatory fields (education level and area of residence)

Period	Pre-pandemic (2019)		Pandemic (2020-2022)		Post-pandemic (2023-2024)	
Variable	N	%	N	%	N	%
Sex						
Male	1,402	47.7	124,198	53.9	13,396	46.9
Female	1,539	52.3	106,341	46.1	15,179	53.1
Unknown	0	0.0	4	0.0	0	0.0
Total	2,941	100.0	230,543	100.0	28,575	100.0
Age (years)						
< 60	1,678	57.1	111,372	48.3	9,548	33.4
≥ 60	1,263	42.9	119,171	51.7	19,027	66.6
Total	2,941	100.0	230,543	100.0	28,575	100.0
Residence area						
Urban and peri-urban	2,739	93.1	194,721	84.5	23,151	81.9
Rural	151	5.1	9,922	4.3	1,864	6.5
Unknown	51	1.8	259	11.2	356	12.5
Total	2,941	100.0	230,543	100.0	28,575	100.0
Race/color						
White	2,265	77.0	161,265	70.0	20,815	72.8
Non-white*	490	16.7	32,800	14.2	5,731	20.1
Unknown	186	6.3	36,478	15.8	2,029	7.1
Total	2,941	100.0	230,543	100.0	28,757	100.0
Education						
No education/illiterate	109	3.7	5,491	2.4	720	2.5
Elementary cycle	936	31.8	37,740	16.4	5,133	17.9
High School	532	18.1	24,257	10.5	2,424	8.5
Higher Education	199	6.8	10,775	4.7	790	2.8
Unknown	1,165	39.6	152,280	66.0	19,508	68.3
Total	2,941	100.0	230,543	100.0	28,575	100.0
Pregnancy						
Yes	120	7.8	3,119	2.9	907	6.0
No	1,054	68.5	8,481	79.7	11,700	77.1
Not Applicable	309	20.1	12,012	11.3	1,921	12.6
Unknown	56	3.6	64	6.0	651	4.3
Total	1,539	100.0	106,341	100.0	15,179	100.0

*Non-white: includes individuals self-identified as Black, Asian or Indigenous;

**Not applicable: females outside reproductive age or younger than 9 years.

3.1. Completeness of clinical conditions variables in the SIVEP-Gripe database

Table 2. Classification of incompleteness of clinical condition variables in adult SARI cases by health macro-region and COVID-19 period, Paraná State, Brazil



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Period	Pre-pandemic (2019)				Pandemic (2020-2022)				Post-pandemic (2023-2024)			
Variable/ Macroregion	E	W	NW	N	E	W	NW	N	E	W	NW	N
Symptoms												
Fever	E	E	E	E	P	E	G	G	P	G	G	F
Cough	E	E	E	E	F	E	G	G	F	G	G	G
Sore throat	E	E	E	E	P	E	G	F	P	F	F	P
Dyspnea	E	E	E	E	P	E	E	G	F	E	E	G
Respiratory discomfort	E	E	E	E	P	E	G	G	P	G	G	F
Oxygen saturation < 95%	E	E	E	E	P	E	G	G	P	G	F	F
Diarrhea	E	E	E	E	P	E	F	F	P	F	G	P
Vomiting	E	E	E	E	P	E	F	F	P	F	F	P
Abdominal pain	-	-	-	-	P	G	F	F	P	F	F	P
Fatigue	-	-	-	-	P	G	F	F	P	F	G	P
Anosmia	-	-	-	-	P	G	F	P	VP	F	F	P
Disgeusia	-	-	-	-	p	G	F	P	VP	F	F	P
Risk Factors/Comorbidities												
Postpartum	P	P	P	P	VP	F	P	P	VP	P	P	P
Chronic Cardiovascular Disease	P	P	P	P	VP	F	P	P	VP	F	P	P
Chronic Hematologic Disease	P	P	P	P	VP	F	P	P	VP	P	P	VP
Down Syndrome	P	P	P	P	VP	F	P	P	VP	P	P	VP
Chronic Liver Disease	P	P	P	P	VP	F	P	P	VP	P	P	P
Asthma	P	P	P	P	VP	F	P	P	VP	P	P	VP
Diabetes mellitus	P	P	P	P	VP	F	P	P	VP	P	P	VP
Chronic Neurological Disease	P	P	P	P	VP	F	P	P	VP	P	P	VP
Chronic Lung Disease	P	P	P	P	VP	F	P	P	VP	P	P	P
Immunodeficiency/Immunosuppression	P	P	P	P	VP	F	P	P	VP	P	P	VP
Chronic Kidney Disease	P	P	P	P	VP	F	P	P	VP	P	P	P
Obesity	P	P	P	P	VP	F	P	P	VP	P	P	P
Others	P	P	P	P	VP	F	P	P	VP	P	P	P

E = excellent (<5%), G = good (5–10%), F = fair (10–20%), P = poor (20–50%), VP = very poor (>50%) (9); (–) Variable not included in the SARI notification form; Macroregion: E= Eastern, W= Western, NW= Northwest, N= Northern

Table 2 shows the incompleteness of clinical condition variables in adult SARI cases by health macro-region and COVID-19 period in Paraná State, as follows: (a) Signs and symptoms: Of the 12 signs and symptoms recorded in the SIVEP-Gripe system, 8 (fever, cough, sore throat, dyspnea, respiratory distress, oxygen saturation <95%, diarrhea, and vomiting) were included before the pandemic. Completeness of these variables was rated excellent across all four health macro-regions of Paraná in the pre-pandemic period. However, data quality declined during the pandemic, with only the Western macro-region maintaining excellent completeness. This region's data quality further deteriorated to fair or poor in the post-pandemic period. The Eastern macro-region consistently showed the poorest completeness, with most variables classified as poor since the pandemic onset.

Regarding the additional variables introduced into the notification form during the pandemic (abdominal pain, fatigue, anosmia, and ageusia), the Western macro-region again demonstrated the highest data completeness during the pandemic, with a classification of good. However, this declined to fair in the



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post-pandemic period. The Eastern macro-region consistently exhibited the lowest completeness, with most variables classified as poor or very poor in both periods.

(b) Risk factors/comorbidities: In the pre-pandemic period, all variables were classified as having poor completeness. During the pandemic, only the Western macro-region improved to a fair classification, which was not sustained in the post-pandemic period. The other three macro-regions consistently exhibited poor or very poor completeness for all variables across all three periods.

4. DISCUSSION

This study aimed to assess the degree of incompleteness in SARI data recorded in the SIVEP-GRIPE system across the four health macro-regions of Paraná State, Southern Brazil, by comparing the pre-pandemic, pandemic, and post-pandemic periods of COVID-19. We found that the incompleteness of variables related to signs and symptoms progressively increased during the pandemic and remained at similar levels in the post-pandemic period. For variables related to risk factors/comorbidities, incompleteness was classified as poor or very poor across all three periods analyzed.

During the pandemic, SARI predominantly affected older males in Paraná, likely due to gender-specific vulnerabilities and the impact of SARS-CoV-2 variants (2). In the post-pandemic period, the rise in cases among females and the elderly suggests a return to seasonal virus patterns, such as RSV, which disproportionately affect these groups (1).

A decline in the completeness of signs and symptoms variables was observed starting from the pandemic period and persisted into the post-pandemic phase across most health macro-regions. This issue was even more pronounced for variables related to risk factors and comorbidities, which were consistently classified as having poor or very poor completeness across all four macro-regions and throughout all three periods analyzed. It is well established that information on risk factors/comorbidities is crucial for understanding severe outcomes such as prolonged ICU stays and mortality (3). Furthermore, database incompleteness is known to introduce bias, particularly when it involves essential but non-mandatory variables that are crucial for case investigation and the calculation of epidemiological or operational indicators (10). Consequently, these findings raise significant concerns as they impede accurate epidemiological profiling and may undermine the effectiveness of surveillance and public health responses. Therefore, our results emphasize the urgent need for targeted interventions aimed at improving data quality within the SIVEP-Gripe system, particularly by revising the collection and operationalization of variables with consistently low completeness. Such improvements are vital to enhance the system's sensitivity, responsiveness, and capacity for timely and effective monitoring.

Based on these findings, it is evident that although the state of Paraná has the highest number of Sentinel Units, including those distributed across the interior of the state, the quality of data entered into the SIVEP-Gripe system still requires significant improvement. It is recommended that epidemiological surveillance services implement routine evaluations of the most critical variables with low completion rates, in order to eliminate data incompleteness and strengthen the quality of SIVEP-Gripe records. Additionally, improvements in work processes are suggested, particularly through regular updates and ongoing training of the professionals responsible for completing notification forms.

5. CONCLUSION

This study highlights significant limitations in the completeness of SARI data recorded in the SIVEP-Gripe system across the health macro-regions of Paraná, particularly for variables related to signs and symptoms and, more critically, risk factors and comorbidities. The persistence of poor data quality across all phases of the COVID-19 pandemic compromises the reliability of epidemiological analyses and the effectiveness of surveillance actions. These findings reinforce the urgent need to strengthen data collection practices and review the operational structure of key variables within the system. Improving completeness is essential to support timely, evidence-based public health decisions, particularly in the context of emerging respiratory threats.

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Keywords: severe acute respiratory infection; epidemiological surveillance; incompleteness data.

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SURVIVAL AND BIOFILM FORMATION IN *Acinetobacter baumannii*: RELATIONSHIP WITH POLYMYXIN B RESISTANCE

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1. INTRODUCTION

Acinetobacter baumannii is a notorious opportunistic pathogen that causes serious nosocomial infections. Due to its high genomic plasticity this pathogen has the ability to rapidly develop resistance to a variety of antibiotics available today and has remarkable ability to adhere and persist in hospital environments. Isolates resistant to carbapenems and polymyxins (CRAB-PMB-R) have raised significant concerns among global health organizations, including the World Health Organization (WHO), which since 2017 has classified *A. baumannii* as a critical priority pathogen for the development of new antimicrobials (1,2).

In addition to its multidrug resistance, *A. baumannii* is mainly known to be part of the microbiota of hospitalized patients and a frequent contaminant in hospital environments, persisting for long periods on abiotic surfaces. Consequently, and due to its ability to form biofilms, invasive devices such as urinary catheters, central venous catheters, and ventilators become significant sources of *A. baumannii* infections, especially in immunocompromised patients admitted to intensive care units (ICUs) (3). The survival mechanisms of this bacteria remain controversial, particularly those potentially associated with multidrug resistance (4). This is because resistance in *A. baumannii* has often been linked to reduced fitness and virulence (5,6,7).

Several proteomic studies have shown that protein aggregation is the primary mechanism responsible for *A. baumannii* survival of under adverse conditions (8,9). The role of biofilm formation in the survival of this bacteria has already been explored, indicating that strong biofilm-producing isolates tend to survive longer on abiotic surfaces (10). Boll et al. (2015) suggested that resistance to cationic polypeptides such as polymyxins favors survival under abiotic conditions (11). On the other hand, Sharma et al. (2020) reported longer survival times in polymyxin B-susceptible *A. baumannii* isolates (5). Our research group demonstrated results similar to Boll et al (2015), showing that polymyxin-resistant isolates were able to survive longer on various abiotic surfaces (4).

Considering that a larger knowledge gap exists pertaining to how polymyxin resistance mechanisms might be linked to survival and biofilm formation of *A. baumannii* on abiotic surfaces, the objective of this study was to evaluate and compare the survival capacity and biofilm production of carbapenem- and polymyxin-sensitive - CSAB-PMB-S and carbapenem- and polymyxin-resistant – CRAB-PMB-R.

2. METHODS

2.1. Bacterial strains

Two clinical isolates and a standard ATCC were selected: Ac56 (CSAB-PMB-S: *A. baumannii* sensitive to both carbapenems and polymyxins) and Ac576 (CRAB-PMB-R: *A. baumannii* resistant to both carbapenems and polymyxins) and ATCC 19606 (sensitive to all antimicrobials). The clinical isolates belong



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to the same cluster based on Enterobacterial Repetitive Intergenic Polymerase Chain Reaction (ERIC-PCR), but exhibit distinct antimicrobial resistance profiles. All strains were preserved in the Laboratory of Medical Microbiology (LMM) at the State University of Maringá. Identification and antimicrobial susceptibility profiles of bacterial isolates were performed by the automated system PhoenixTM (BD Diagnostic Systems, Sparks, MD). The ATCC 19606 was used as a control to compare with the isolates.

2.2. Preparation of bacterial suspensions

The isolates preserved at – 20°C in Trypticase Soy Broth (Difco Laboratories, Detroit, MI) with 30% glycerol were recovered using the streak plate technique in *Mueller Hinton Agar* (Becton, Dickinson and Company) and incubated at 35°C for 20 hours. Bacterial suspensions were prepared in 0.9% sterile saline solutions (NaCl 0.9%) and standardized to the 0.5 McFarland [bacterial suspension containing 1.5×10^8 colony forming unit per milliliter – CFU/mL]. The bacterial suspensions obtained were used for antimicrobial susceptibility testing, survival and biofilm assays in this study.

2.3. Determination of MICs: broth microdilution method

The minimal inhibitory concentration (MIC) of polymyxin B (PMB) for *A. baumannii* was determined in duplicate using a broth microdilution method as previously described by Clinical and Laboratory Standards Institute (CLSI) M07-A11 (12). The inoculum was prepared from an overnight culture plate and standardized to the 0.5 McFarland as cited. The test was performed with Cation-adjusted Mueller-Hinton Broth (Ca-MHB; Becton, Dickinson and Company). The results were analyzed according to CLSI PMB breakpoints established in M100-S33 (13).

2.4. Screening of biofilm related genes

Polymerase chain reaction (PCR) technique was used to screen the following biofilm formation genes: *csu*, *algC* and *bap*.

2.5. Preparation of stainless-steel coupons and artificial contamination: test surface to survival and biofilm formation assay

The test surfaces were placed into flasks with distilled water and sterilized in autoclave for 15 mins at 121°C. Bacterial suspensions at 0.5 McFarland were made from overnight culture plates as cited. Aliquots of 10µL of the suspensions were applied on the stainless-steel coupons. After the inoculum dried, the coupons were aseptically transferred to petri dishes and kept at room temperature in biosafety cabinet for the survival assay and to 24-well plates to the biofilm formation assay. During desiccation, temperature and humidity were measured every day.

2.5.1 Surface survival in stainless-steel coupons

Survival of two *A. baumannii* clinical isolates CSAB-PMB-S and CRAB-PMB-R, and the standard strain ATCC 19606 was evaluated in stainless-Steel Coupons measuring 10x20mm, according to Lodi et. al. 2019, with some modifications (4).

The isolates that survived to desiccation were recovered immediately (time zero) and after every 72hr in duplicate by transferring aseptically the stainless-steel coupons to tubes that contained 5mL of Brain Heart Infusion broth (Becton, Dickinson and Company). These tubes were incubated at 35°C for at maximum 5 days or until growth. Turbidity of the tubes was considered to be positive if the characteristics of the developed organisms were compatible to *A. baumannii* through Gram staining and/or streaked plates on MacConkey agar. The presence of bacteria adhered to the stainless-steel coupons was also evaluated in soft agar 0.4% incubated for 24hr at 35°C.

2.5.2 Biofilm formation



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Coupons of stainless-steel contaminated with *A. baumannii* strains were aseptically introduced for the formation of biofilm in 24-well plate. For the formation of biofilm in absence of PMB (considered a positive control), bacteria was inoculated with plain *Brain Heart Infusion* broth (BHI; Becton, Dickinson and Company), meanwhile for the biofilm with PMB, bacteria were inoculated in BHI with PMB at sub-MIC (1/2 MIC) and MIC. As a negative control, plain BHI was applied to the wells without any bacteria. The plate was incubated at 35°C for 72hrs.

2.5.3 Quantification of biofilm

The removal of the biofilm formed in each stainless steel was performed by rinse technique on times 24hr, 48hr and 72hr. First, each coupon of stainless-steel artificially contaminated was carefully rinsed in a tube containing 5mL of 0.9% sterile saline solution (NaCl 0.9%), to remove the planktonic cells that were not adhered. Subsequently, the surfaces were transferred to other tubes containing sterile saline solution and vortexed for 2 minutes. After the removal of the biofilm, each tube was serial diluted until 10^{-7} and 20µL of the obtained dilutions were plated by drop method in *Mueller Hinton Agar* (Becton, Dickinson and Company) plate. All plates were incubated at 35°C for 24 hours for counting the colonies in CFU/mL. The experiment was performed in duplicate and for the calculations was used the equation: $CFU/mL = average\ colonies \times 50 \times dilution\ factor$. All data and results analyzed were expressed in Log_{10} and interpolated in graphics using Microsoft® Excel® 365 (Microsoft Office 365 MSO).

2.6 Scanning electron microscopy (SEM)

SEM was performed for all desiccation samples. Stainless steel coupons with a maximum size of 1 x 1 cm were sterilized with distilled water for 15 mins at 121°C. All desiccation samples were contaminated in the same day and were directly individually fixed in glutaraldehyde 2.5% in sodium cacodylate buffer 0.2 M (pH 7.2 - 7.4) every 72 hours (same time as desiccated bacteria recovery). The fragments were stored at 4°C in the fridge until the preparation for critical point. The samples were dehydrated with an increasing concentration of anhydrous alcohol (PA): 50%, 70%, 80%, 90%, 95% and 100%, for 15 min in each concentration, and repeated three times in the pure alcohol step. After this, the samples were transferred to the automatic critical point (Leica; EM CPD300) and were fixed to aluminum stubs with carbon tape to be coated with a layer of gold (20 nm) using a metallizer (Bal-TEC SCD 050). The analysis and photos were conducted using scanning electron microscope Shimadzu; SS-550 (Shimadzu; JPN) in COMCAP at State University of Maringá.

3. RESULTS

All isolates (clinical and ATCC) were positive to all the biofilm genes screened: *bap*, *algC* and *locus csu A/B-E*, related to adhesion, maturation, and extracellular matrix. All other results including MIC values for PMB and clinical data about the isolates are presented in Table 1.

Table 1. Phenotypic and molecular characteristics of clinical isolates

Strains	Hospital	Sample	MIC PMB (µg/mL)	Biofilm genes					
				bap	algC	csuA- csuB	csuB- csuC	csuC- csuD	csuD- csuE
Ac56	A/1996	Tracheal secretion	1 (S)	X	X	X	X	X	X
Ac576	A/2011	Tracheal secretion	32 (R)	X	X	X	X	X	X
ATCC 19606	USA/1948	urine	1 (S)	X	X	X	X	X	X

PMB: Polymyxin; MIC: minimal inhibitory concentration. Breakpoints: Polymyxin B – S ≤2; >2 R



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For the survival assay, the average temperature during the experiments was 24°C, and the relative humidity was 67%. All isolates survived for at least 5 days on the tested surface. Among the isolates evaluated, Ac576 and the ATCC 19606 strain showed the longest survival (9 days), whereas Ac56 survived for only 6 days.

All *A. baumannii* isolates formed biofilms at 24, 48, and 72 hours. The highest biofilm formation was observed at 48 hours, both in the absence (C+) and presence of PMB (at ½ MIC and MIC concentrations). Among the clinical isolates, Ac576 maintained high biofilm levels in both conditions, especially at 72 hours. Overall, our data showed that at 24 hours, both isolates had nearly the same CFU/mL (Table 2), with an increase at 48 hours for both, although Ac576 showed slightly lower biofilm formation. At 72 hours, biofilm levels decreased for both isolates, but Ac576 exhibited slightly higher biofilm formation than Ac56. All results are presented in Figure 1 and Table 2.

In the presence of PMB at MIC concentration, both isolates maintained relatively stable biofilm levels between 24 and 48 hours. However, at 72 hours, Ac56 showed a significant decrease, reaching log values below 1, while Ac576 experienced a smaller reduction. The ATCC 19606 strain (known for high biofilm formation) also formed substantial biofilms in both C+ and PMB at ½ MIC, with a notable decrease at 72 hours, similar to Ac56.

Table 2. Averages of biofilm formation in Log₁₀ CFU/mL

	C+			1/2 MIC PMB (µg/mL)			MIC PMB (µg/mL)		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Ac 56	6.07	6.18	6.11	4.65	6.05	3.47	4.07	4.86	0.740363
Ac 576	6.12	6.54	5.62	4.47	5.1	4.72	4.47	5.47	2.439333
ATCC	7.07	7.11	6.24	6.47	6.39	5.96	4.74	2.54	1.60206

C+: Positive Control; MIC: minimal inhibitory concentration; ½ MIC : sub- minimal inhibitory concentration

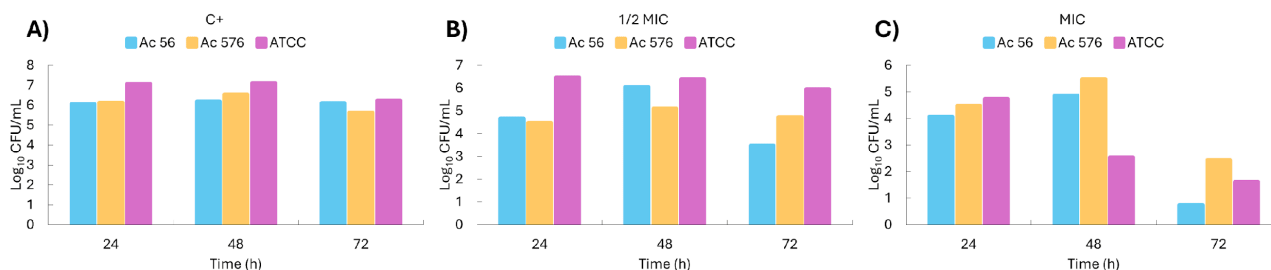
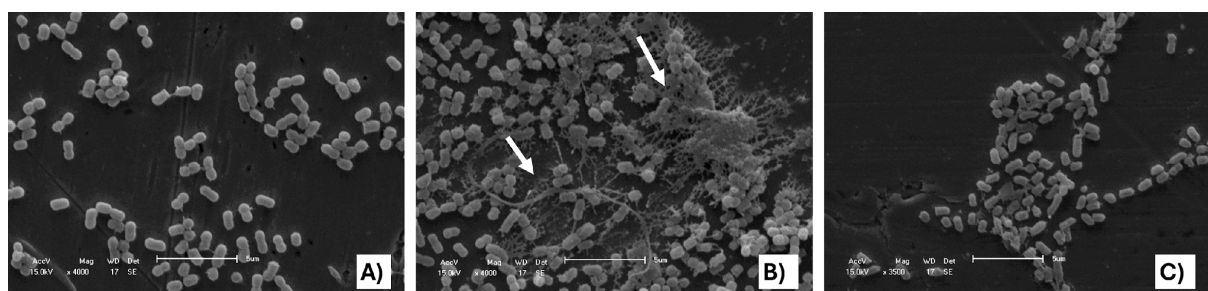


Figure 1. Effect of polymyxin B at sub-MIC and MIC on biofilm formation of *A. baumannii*. Colorful bars indicate the Log₁₀ biofilm cells in *A. baumannii*. Bar graph data are shown as the average. Summarized results showing the Log₁₀ biofilm cells in strains Ac56, Ac576 and ATCC 19606 cultured in BHI broth without polymyxin (A); and with polymyxin at sub-MIC (B) and MIC (C).

The SEM for the desiccated cells for the biofilm of the three isolates showed that Ac576 formed a thick layer of biofilm matrix surrounding the cells suggesting an adaptation to desiccation. For the sensitive isolates, Ac56 and ATCC SEM revealed typically rod-shaped cells (Figure 2).





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Figure 2. Photographs obtained by scanning electron microscopy of *A. baumannii* biofilms formed on the stainless-steel surface. A) Overview of the Ac56 *A. baumannii* biofilm (4,000x). B) Overview of the Ac576 *A. baumannii* biofilm. White arrows show thick layer of a polysaccharide matrix (4,000x). C) Overview of the ATCC 19606 *A. baumannii* biofilm (3,500x).

4. DISCUSSION

The ability of *A. baumannii* to form biofilms and survive desiccation has been investigated by several researchers aiming to understand its remarkable persistence in hospital environments, where it is responsible for significant outbreaks of healthcare-associated infections (4,9,11). Although the association between resistance and virulence is of critical importance, it remains controversial.

The results obtained in this study reveal a correlation between polymyxin B resistance, biofilm formation, and the survival of *A. baumannii*. The isolate Ac576, with a MIC of 32 µg/mL (PMB-R), demonstrated not only greater resistance to PMB but also the ability to maintain a more robust biofilm under antibiotic pressure and survive for up to 9 days. In contrast, Ac56, with a MIC of 1 µg/mL (sensitive), showed a marked reduction in biofilm formation at MIC and survived only 6 days.

All of our isolates tested positive for the target genes, including the sensitive strain (ATCC). The presence of these genes confirms the ability to form biofilms even in the presence of PMB at MIC concentrations. Biofilms are a key virulence factor, contributing to bacterial adhesion to both abiotic and biotic surfaces, such as medical devices and host cells, respectively. The polysaccharide matrix (PM) provides resistance to antimicrobials and tolerance to desiccation, antibiotics, and oxidative stress. This enhances the survival of *A. baumannii* in hospital settings, facilitating colonization and infection of patients (14). Scanning electron microscopy (SEM) images revealed that isolate Ac576 (Figure 2B) produces this polysaccharide matrix, which may partially explain its greater survival compared to other isolates. This PMB-resistant isolate has previously been used in research conducted in our laboratory and had shown to survive for 113 days on mattress fabric (4).

Resistance in *A. baumannii* has often been associated with reduced fitness and virulence. However, the data from this study—showing biofilm formation even in the presence of polymyxin B (MIC and ½ MIC) and prolonged survival of Ac576—suggest otherwise. According to Boll et al. (2015) and Choudhary et al. (2022), possible modifications in LPS not only confer resistance to polymyxins but also strengthen biofilm structure, promoting greater tolerance to desiccation and persistence on abiotic surfaces (10,11). On the other hand, Sharma (2020) reported longer survival times in polymyxin B-sensitive *A. baumannii* strains (5). Additionally, other mechanisms such as protein aggregation have been reported as contributing to *A. baumannii* survival, as noted by Gayoso et al. (2014) (9).

Our findings support the hypothesis proposed by Boll et al. (2015). Therefore, further studies evaluating the genotype and searching for mutations in genes such as *lpx* and the *pmrAB* operon—which are associated with polymyxin resistance—are necessary to assess the relationship between resistance-related mutations and their influence on *A. baumannii* survival. This remains a key objective of our ongoing research.

5. CONCLUSION

The data presented suggest that polymyxin B resistance in these isolates favors the survival of *A. baumannii*. Furthermore, biofilm formation was not blocked by the presence of polymyxin in the culture medium at MIC or sub-MIC levels; rather, at the next MIC level, biofilm survival was even promoted after 72 hours. These observations indicate that polymyxin B therapy for *A. baumannii* infections should be administered with great caution. Pharmacokinetic and pharmacodynamic parameters should be considered to ensure that the dosage does not inadvertently stimulate biofilm production, even in isolates that are sensitive to this drug.

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CAPES.



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Keywords: *Acinetobacter baumannii*; polymyxin; survival

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PRECLINICAL STUDIES ON THE ANTITUBERCULAR ACTIVITY OF THE THIOSEMICARBAZONE DERIVATE *HIT21*

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1. INTRODUCTION

Tuberculosis (TB) continues to represent one of the foremost global public health challenges, despite substantial progress in diagnostic technologies and therapeutic approaches. TB has re-emerged as the leading cause of mortality attributable to a single infectious agent worldwide, surpassing COVID-19 and accounting for approximately 1.25 million deaths (1). The global burden of the disease remains considerable, with an estimated 10.8 million incident cases in 2023 (1). Brazil mirrors this concerning scenario, ranking among the 30 high-burden countries and reporting persistently elevated incidence rates with profound socioeconomic repercussions (1, 2). At the national level, incidence reached 39.8 cases per 100.000 population in 2023 (3) underscoring the pressing need to strengthen strategies for prevention, timely diagnosis, and effective treatment.

The etiological agent, *Mycobacterium tuberculosis* (MTB), is an acid-fast bacillus characterized by slow replication and a remarkable capacity for intracellular persistence (4). These characteristics hinder both infection control and the development of novel therapeutic options. Transmission occurs predominantly through the airborne route, following the inhalation of aerosolized droplets expelled by individuals with active pulmonary TB during coughing, sneezing, or even speaking. Current estimates indicate that nearly one-quarter of the global population harbors *M. tuberculosis* infection (1). Although most of these individuals remain in a latent state, without clinical manifestations or risk of transmission, approximately 10% progress to active disease over their lifetime, particularly those with immunosuppression, malnutrition, HIV coinfection, or other comorbidities (1, 4, 5).

The treatment of TB is based on a standardized regimen with four first-line drugs involving isoniazid (INH), rifampicin (RIF), pyrazinamide (RHZ) and ethambutol (EMB), administered during the intensive phase, followed by INH and RIF in the continuation phase. Although effective, this regimen requires a prolonged duration of at least six months, which contributes to high rates of treatment discontinuation and poor adherence (8). Adverse effects, drug interactions, and the hepatotoxicity of certain agents further contribute to premature treatment interruption. This scenario promotes the emergence of resistant *M. tuberculosis* strains, including multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB), which pose a major public health threat as they necessitate longer, more toxic, and less effective regimens (9). In 2023, more than 175,000 individuals worldwide were diagnosed with MDR-TB or rifampicin-resistant TB (RR-TB) (1).

In this context, the development of novel drugs capable of shortening treatment duration, minimizing adverse effects, and overcoming bacterial resistance has become an urgent priority. Research efforts have been undertaken to identify innovative compounds, repurpose existing drugs, and evaluate more effective



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therapeutic combinations. The investigation of new molecules is essential not only to optimize clinical outcomes but also to support the global targets for TB elimination proposed by the World Health Organization (WHO) (1). In this context, the aim of this study was to conduct preclinical investigations of the thiosemicarbazone derivative (*Hit21*), a molecule previously selected from earlier studies due to its relevant MIC, as a promising candidate against *M. tuberculosis* infections.

2. MATERIAL AND METHODS

2.1. Bacterial inoculum

The reference strain *M. tuberculosis* H₃₇Rv (ATCC 27294), pan-susceptible, was obtained from the mycobacteria collection of the Medical Bacteriology Laboratory at the State University of Maringá (UEM). The bacterial inocula were cultured in Middlebrook 7H9 medium (Difco Laboratories, Detroit, USA), supplemented with OADC (BBL/Becton-Dickinson, Sparks, MD, USA), and incubated for 15 to 20 days at 35–37°C.

Additionally, nontuberculous mycobacteria (*Mycobacterium abscessus* and *M. fortuitum*) were included for comparative analysis. For selectivity assessment, representative Gram-negative and Gram-positive bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, were also tested. All strains were cultured under standard conditions recommended for each species.

2.2. Compound *Hit21*

The novel thiosemicarbazone molecule (*Hit21*) was provided by Prof. Dr. Fábio Vandresen from the Chemistry Laboratory of the Federal University of Technology State of Paraná (UTFPR), Londrina campus. The molecule was diluted in 1 mL of DMSO and stored frozen at a concentration of 10,000 µg/mL.

2.3. Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined by the Resazurin Microtiter Assay (REMA) using the standard strain *M. tuberculosis* H₃₇Rv and specific strains resistant to RIF and/or INH and/or ethambutol. The REMA method was performed following Palomino et al. 2007 (10), using 96-well plates in which the compound of interest was serially diluted in Middlebrook 7H9 medium supplemented with OADC. A standardized bacterial inoculum adjusted to McFarland scale No. 1 and diluted 1:20 in Middlebrook 7H9 supplemented with OADC was then added. The MIC was defined as the lowest drug concentration capable of inhibiting bacterial growth by 90% or more after 7 days under optimal incubation conditions.

2.4. Acute Toxicity Assessment in *Tenebrio molitor* larvae

The acute toxicity in *Tenebrio molitor* larvae was defined as the highest concentration of the *Hit21* that could be used without causing acute toxicity, serving as an initial *in vivo* evaluation. *Hit21* was initially prepared at 10,000 µg/mL in 1 mL of DMSO and then diluted in Middlebrook 7H9 supplemented with OADC to achieve final concentrations of 500, 1000, and 2000 µg/mL. Control groups were included, in which larvae were exposed to 500, 1000, and 2000 µg/mL of pure DMSO. All larvae were incubated at 28°C with their respective rearing media and monitored daily for 10 days or until the death of all larvae, observing signs of toxicity (12).

2.5. Cytotoxicity assessment in J774A1 and VERO cells

For the determination of the *in vitro* cytotoxicity of the *Hit21* molecule, murine macrophage cell line J774A1 and African green monkey kidney epithelial cell line VERO, obtained from the cell bank of the Medical Bacteriology Laboratory at the State University of Maringá, were used. The cells were cultured in



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DMEM (Dulbecco's Modified Eagle Medium) supplemented with fetal bovine serum (FBS) at 37°C in a 5% CO₂ atmosphere. Cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. The cells were exposed in triplicate to the test compound *Hit21* and to a DMSO control solution at different concentrations obtained by serial dilution in 96-well plates, as described by Scodro et al. (2013) (13). The 50% cytotoxic concentration (CC₅₀) and the selectivity index (SI) were subsequently determined (14,15).

2.6 Evaluation of drug combination activity

The combinatorial activity between drugs was assessed using the checkerboard method against the reference strain *M. tuberculosis* H₃₇Rv (ATCC 27294). The Resazurin Drugs Combination Microtiter Assay (REDCA) (11) was employed to evaluate the interaction of the *Hit21* with first-line anti-TB drugs, including EMB, RIF, and INH. Assays were performed in triplicate, testing the compound of interest *Hit21* in combination with each compound individually. The Fractional Inhibitory Concentration Index (FICI) was calculated according to the formula: $FICI = (MIC\ A+B / MIC\ A) + (MIC\ B+A / MIC\ B)$ where $FICI \leq 0.5$ indicates a synergistic effect, $FICI > 0.5-4$ denotes indifferent effect, and $FICI > 4$ indicates an antagonistic effect.

3. RESULTS

The novel thiosemicarbazone derivative *Hit21* exhibited relevant antimicrobial activity against the reference strain *M. tuberculosis* H₃₇Rv, with a MIC of 0.48 µg/mL determined by the REMA assay. For nontuberculous mycobacteria, such as *Mycobacterium abscessus* and *M. fortuitum*, MIC values ranged from 7.8 to 15.62 µg/mL. In contrast, for Gram-negative and Gram-positive bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, the MIC was >1000 µg/mL, indicating high selectivity for mycobacteria, particularly MTB, as shown in Table 1.

Table 1. Minimum Inhibitory Concentration (MIC) of *Hit21* compound against various bacteria

	MIC (µg/mL)					
	H ₃₇ RV	<i>M. abscessus</i>	<i>M. fortuitum</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
<i>Hit21</i>	0.48	7.81	15.62	>1000µg/mL	>1000µg/mL	>1000µg/mL

MIC: Minimum Inhibitory Concentration; *M. abscessus*: *Mycobacterium abscessus*; *M. fortuitum*: *Mycobacterium fortuitum*; *E. coli*: *Escherichia coli*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*.

In the acute toxicity assay using *Tenebrio molitor* larvae, the number of deaths in larvae exposed to *Hit21* was lower than that observed in the DMSO control group. Survival remained above 80%, confirming the non-toxic profile of the compound, as shown in Figure 1.

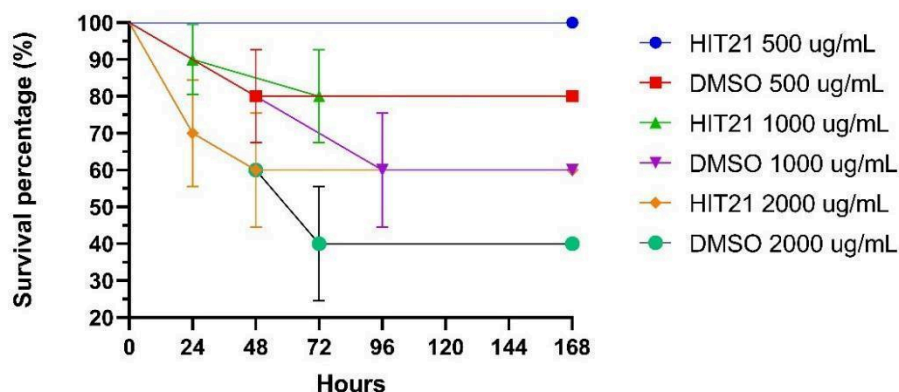


Figure 1. Acute toxicity analysis of the *Hit21* compound against *Tenebrio molitor* larvae.



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Mortality: Number of *Tenebrio molitor* larvae dead at the end of the experiment after exposure to the tested sample (*Hit21*).

For VERO cells, the mean 50% cytotoxic concentration (CC_{50}) was 496.4 $\mu\text{g/mL}$, whereas for the murine macrophage cell line J774A.1 it was 263.8 $\mu\text{g/mL}$. Considering that the MIC against the reference strain *M. tuberculosis* H₃₇Rv was 0.48 $\mu\text{g/mL}$, the selectivity index ($SI = CC_{50}/MIC$) was estimated at 1034.4 for VERO cells and 527.6 for J774A.1 cells.

The two-dimensional checkerboard assay was performed by combining compound *Hit21* with INH, RIF, and EMB, yielding FICI values of 0.375, 0.281, and 1.13, respectively. Accordingly, the combinations of *Hit21* with INH and RIF were classified as synergistic, while the combination with ethambutol was classified as indifferent.

4. DISCUSSION

The results of this study demonstrate the strong potential of the novel thiosemicarbazone molecule (*Hit21*) as a candidate for the development of a new anti-TB drug. The potent antimicrobial activity against the reference *M. tuberculosis* strain confirms the efficacy of the substance at significantly concentrations, being comparable to that of first-line drugs such as RIF, INH and BEM (16). This finding is of great importance, considering the increasing need for new classes of anti-TB agents due to the emergence and spread of strains resistant to first-line drugs (1, 9). The MIC obtained for *Hit21* is comparable to or even superior to some anti-TB drugs under development or already in use, which underscores its promising activity profile (17, 18).

The maintenance of activity against non-tuberculous mycobacteria (NTM), such as *M. abscessus* and *M. fortuitum*, reinforces its broad spectrum of action within the genus *Mycobacterium*. Although the main focus is TB, activity against NTMs is an additional benefit, since these infections are increasingly recognized as a public health issue, especially in immunocompromised patients (19). The clear selectivity for mycobacteria, demonstrated by MIC values against Gram-positive and Gram-negative bacteria higher than 1000 $\mu\text{g/mL}$, is a highly desirable feature. This specificity minimizes action on the commensal microbiota, reducing the likelihood of gastrointestinal side effects and lowering the risk of resistance development in other bacteria, a common drawback of broad-spectrum antibiotics (20).

Another relevant aspect is the observed safety profile. The acute toxicity analysis in *Tenebrio molitor* larvae showed no significant deleterious effect, with survival rates above 80%. This preliminary *in vivo* model is a positive indication of low systemic toxicity (21). The *in vitro* cytotoxicity assays corroborate these results, with CC_{50} values of 496.4 $\mu\text{g/mL}$ for VERO cells and 263.8 $\mu\text{g/mL}$ for the murine J774A.1 cell line. The selectivity index (SI), calculated as the ratio between CC_{50} and MIC ($SI > 500$ for both cell lines), demonstrates a wide therapeutic margin and a favorable safety profile. Considering that toxicity is one of the main barriers in the development of new anti-TB drugs, often leading to the interruption of clinical trials (22), these findings represent an important advantage for *Hit21*, suggesting strong potential for further *in vivo* studies.

The evaluation of combinatorial action revealed clinically relevant interactions. The synergy observed with INH (FICI = 0.375) and RIF (FICI = 0.281) is particularly significant. Drug combinations are the basis of TB treatment to prevent the emergence of resistance and increase efficacy (23). Synergy allows dose reduction, which may minimize adverse effects, improve treatment adherence, and potentially shorten therapy duration, crucial aspects for successful disease control (24). Moreover, the absence of antagonism with ethambutol (FICI = 1.13, classified as indifferent) further supports the feasibility of incorporating *Hit21* into existing or future combination regimens without compromising treatment efficacy.

The data from this study highlight three key points: the high antimycobacterial potency of the molecule, its favorable preliminary *in vitro* and *in vivo* safety profile related to the toxicity assays performed; and the potential for use in combination regimens with classical drugs, showing synergistic effects with INH and RIF. These findings are particularly relevant in the current scenario, where resistance to conventional regimens, toxicity, and treatment abandonment represent critical barriers to TB control (1, 9, 22).

5. CONCLUSION



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The *Hit21* compound emerges as a promising candidate among new antimycobacterial agents, demonstrating potency, selectivity, preliminary safety, and synergy with first-line anti-TB drugs. Further investigations using preclinical models and pharmacokinetic evaluations will be critical to confirm its potential as a therapeutic alternative against both drug-susceptible and drug-resistant TB. The continuation of studies, including tests in animal infection models and elucidation of its mechanism of action, will be crucial to advance *Hit21* into the next phases of clinical development, with the ultimate goal of delivering a novel therapeutic option in the fight against this devastating disease.

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IDENTIFICATION OF 10 HLA ALLELES BY NEXT GENERATION SEQUENCING BY IMUNOGEN-UEM: 5 NEW AND 5 CONFIRMATORY

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1. INTRODUÇÃO

The HLA (Human Leukocyte Antigen) system is fundamental to the functioning of the immune system. Its molecules are responsible for presenting antigens to T lymphocytes, allowing recognition between self and non-self¹. This presentation capacity is crucial for the body to be able to respond to infectious agents, mutated cells, or even organ transplants, directly influencing immune activation and tolerance². In addition, the high rate of HLA polymorphism ensures a wide diversity of responses. However, it is also associated with susceptibility to various diseases and even adverse reactions to medications^{3,4}. This balance between protection and predisposition makes HLA a key player in clinical areas such as transplants, pharmacogenomics, and personalized medicine^{5,6}.

Structurally, class I HLA molecules are expressed in all nucleated cells and are formed by a heavy chain (α), composed of three domains, associated with β 2-microglobulin. Their main function is to present peptides of endogenous origin to TCD8+ cells, which are essential in controlling viral infections and recognizing tumor cells^{1,7}.

Class II molecules are composed of two polypeptide chains, α and β , and are restricted to antigen-presenting cells. They are responsible for presenting exogenous peptides to TCD4+ cells, coordinating the activation of adaptive immunity and antibody production^{2,8}. The structural and functional distinction between classes I and II ensures that the immune system is able to detect and respond to a wide variety of threats^{4,9}.

The HLA region is located on chromosome 6p21 and comprises six classical HLA genes and approximately 132 protein-coding genes, with more than 224 genes reported¹⁰. Currently, 28,983 class I HLA alleles and 13,210 class II HLA alleles are described, demonstrating the polymorphism of the gene¹¹. The variability of the HLA gene is important for diversity among individuals and species permanence¹².

HLA typing is performed by aligning the individual's sequence with reference databases. When mutations do not correspond to described alleles, they are reported as new and need confirmation by at least two independent PCR reactions, according to IMGT standards¹¹.

Mutations can lead to changes in protein structure, and some do not alter amino acids due to synonymous mutations¹³. To alter the amino acid reading codons, the mutation must occur in exons, which encode critical functional regions, impacting the three-dimensional structure and function of the protein¹⁴.

Several methodologies can be applied for HLA genotyping, such as serological methodologies (CDC), molecular biology techniques such as Restriction Fragment Length Polymorphism (RFLP) Polymerase Chain Reaction (PCR), Sequence Specific Primer (SSP) PCR, PCR with Oligonucleotide Specific Probes (SSO), Sanger Sequencing, and Next Generation Sequencing (NGS)¹⁵.

Next Generation Sequencing (NGS), with platforms such as Ion Torrent and Illumina, ensures high-resolution typing and has accelerated the discovery of new alleles. However, the limitations of short reads in haplotype phasing led to the development of technologies such as Oxford Nanopore (ONT). ONT technology sequences long native DNA molecules through a protein nanopore, measuring changes in the ionic current. This approach unequivocally resolves phasing ambiguities^{15,16}.



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Given the growing number of alleles discovered by new technologies, an update to the official nomenclature was necessary to ensure global standardization. As of April 2010, a system was implemented that uses two dots as a separator between the fields of an allele name¹⁷.

The first field designates the allele group, generally corresponding to serology; the second field distinguishes alleles that alter the amino acid sequence of the protein; the third field indicates synonymous mutations in the exons; and the fourth field indicates polymorphisms in non-coding regions. The use of this nomenclature is essential for understanding high-resolution readings, as reflected in Brazil by Ministry of Health Ordinance N^o.685, dated June 16, 2021, which established the mandatory use of high-resolution HLA typing for the registration of new donors in REDOME (National Registry of Voluntary Bone Marrow Donors)¹⁷.

The present study aimed to report the identification of five previously unreported HLA alleles and the confirmation of five rare alleles previously described only once, using REDOME-registered donors analyzed at the Immunogenetics Laboratory of the State University of Maringá.

2. MATERIAL E MÉTODOS

Voluntary bone marrow donors registered in the National Registry of Voluntary Bone Marrow Donors (REDOME) signed the Informed Consent Form. Genomic DNA was isolated from peripheral white blood cells using the QIAamp® DNA Blood Mini Kit (Qiagen – Hilden, Germany), according to the manufacturer's instructions. HLA typing was performed using next-generation sequencing on the iSeq 100 platform (Illumina – San Diego, United States) using the Alltype™ FASTPlex™ NGS 11 Loci kit (One Lambda Thermo Fisher – Los Angeles, United States). Sequencing data were analyzed using TypeStream Visual® 3.1.0 software (One Lambda Thermo Fisher – Los Angeles, United States).

For confirmation methodologies, the alleles *DRB1*03:01:04*, *DRB1*11:04:03*, *DRB1*13:80* and *DRB1*16:22* were resequenced by Illumina, following the same methodology. For the *HLA-DQB1*02:211* and *HLA-A*03:01:133* alleles, the confirmation methodology used was Sanger sequencing by the Sanger Sequencing Kit (SeCore™ kits - One Lambda), and the analysis software used was uTYPE 7.3. For the validation of the *HLA-DRB3*03:69* allele, the data were submitted to a new analysis using a separate software tool, NGSengine® (GenDx, Utrecht, Netherlands). For the *HLA-DRB1*11:341* allele, the sample was reanalyzed using the IonChef/S5 platform (Ion Torrent), and the sequencing data were evaluated with TypeStream Visual 3.1.0 software.

Finally, the *HLA-B*35:20:03* and *HLA-DRB1*11:333* allele underwent a second analysis with NanoTYPE 24/11 (Omixon) on MinION equipment with MinKNOW software from the Oxford Nanopore Technology (ONT) platform to validate the new allele designation, and NanoTYPER RUO version 2.0.0 was used as the analytical software. Another confirmation strategy was employed for this alleles, which was again performed using the Alltype™ FASTPlex™ NGS 11 Loci kit (One Lambda Thermo Fisher – Los Angeles, United States). However, this time, the analysis was performed on the IonChef/S5 platform (Ion Torrent) and used TypeStream Visual® 3.1.0 software (One Lambda Thermo Fisher – Los Angeles, United States), with version 3.55 of the IPD-IMGT/HLA database.

3. RESULTADOS

The new *HLA-DQB1*02:211* allele (GenBank accession number OP821761, IPD-IMGT/HLA submission number HWS10064383) differs from *DQB1*02:02:01:02* at position 314 in exon 2, where C-A (codon 73.2 GCG-GAG) results in the change of the amino acid from alanine to glutamate. The donor's complete HLA typing was as follows: *A*02:14*, *24:02:01*; *B*39:01:01*, *41:01:01*; *C*07:01:01*, *12:03:01*; *DRB1*07:01:01*, *16:01:01*; *DRB4*01:01P*; *DRB5*02:02:01*; *DQA1*01:02:02*, *03:03:01*; *DQB1*02:211*, *05:02:01*; *DPA1*01:03*, *03:01*; *DPB1*02:01:02*, *105:01:01*¹⁸.

The new *HLA-DRB3*03:69* allele (GenBank accession number OR888444, IPD-IMGT/HLA submission number HWS10068499) differs from *DRB3*03:01:01:03* at position 409 in exon 3, where C-G (codon 108.1 CCC-GCC) results in the change of the amino acid from proline to alanine. The donor's complete HLA typing was as follows: *A*02:01:01*, *32:01:01*; *B*35:01:01*, *45:01:01*; *C*04:01:01*, *16:01:01*; *DRB1*13:01:01*, *13:02:01*; *DRB3*01:01*; *03:69*; *DQA1*01:02:01*, *01:03:01*; *DQB1*06:03:01*, *06:09:01*; *DPA1*01:03:01*, *02:01:01*; *DPB1*02:01:19*, *05:01:01*¹⁹.

The new *HLA-B*35:20:03* allele (GenBank accession number PQ009210, IPD-IMGT/HLA submission number HWS10091671) differs from *B*35:20:01* at position 615 in exon 4, where C-A (codon 205.3 GCC-GCA) does not result in any change in coding. The donor's complete HLA typing was as follows: *A*31:01:02*, *34:02:01*; *B*35:20:03*, *58:02:01*; *C*04:01:01*, *06:02:01*; *DRB1*09:01:02*, *16:02:01*;



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DRB4*01:01:01; DRB5*02:02:01; DQA1*03:03:01, 05:05:01; DQB1*02:02:01, 03:01:01; DPA1*01:03:01, 02:02:02; DPB1*01:01:01, 04:02:01²⁰.

The new HLA-DRB1*11:341 allele (GenBank accession number PQ473687, IPD-IMGT/HLA submission number HWS10093411) differs from DRB1*11:01:01:01 at position 5741 of intron 2, where A · T, and at position 574 of exon 4, where C · A (codon 192.2 TCT · TAT), leading to an amino acid change from serine to tyrosine. The donor's complete HLA typing was as follows: A*11:01:01, 23:01:01; B*15:17:01, 51:01:01; C*05:01:01, 15:02:01; DRB1*11:341, 15:03:01; DRB3*02:02P, DRB5*01:01P; DQA1*01:02:01, 05:05:01; DQB1*03:01P, 06:02:01; DPA1*01:03:01, 01:03:01; DPB1*02:01:02, DPB1*04:01:01²¹.

The new HLA-A*03:01:133 allele (GenBank accession number: PQ672531, IPD-IMGT/HLA, submission number: HWS10093853) differs from A*03:01:01:01 by a single nucleotide substitution at position 624 in exon 4, where C · T (codon 184.3 CCC · CCT), which does not result in an amino acid change. The donor's complete HLA typing was as follows: A*03:01:133, 26:01:01; B*07:02:01, 35:01:01; C*04:01:01, 07:02:01; DRB1*01:01:01, 11:01:01; DRB3*02:02P; DQA1*01:01:01, 05:05:01; DQB1*03:01P, 05:01:01; DPA1*01:03:01, 01:03:01; DPB1*04:01P, 06:01P²².

The confirmatory allele DRB1*03:01:04 (Genbank accession number PV646494, IPD-IMGT/HLA submission number HWS10100930), the complete typing of the donor was as follows: A*01:01:01, 02:01:01; B*07:02:01, 08:01:01; C*07:01:01, 07:02:01; DRB1*03:01:04, 08:02:01; DRB3*01:01P; DQA1*04:01:01, 05:01:01; DQB1*02:01:01, 04:02:01; DPA1*01:03:01, 02:01:02; DPB1*01:01P, 04:02P²³.

The confirmatory allele DRB1*11:04:03 (Genbank accession number PV646493, IPD-IMGT/HLA submission number HWS10100929), the complete typing of the donor was as follows: A*03:01:01, 24:02:01; B*07:02:01, 15:01:01; C*03:03:01, 07:02:01; DRB1*11:04:03, 15:01:01; DRB3*02:02P; DRB5*01:01P; DQA1*01:02:01, 05:05:01; DQB1*03:01P, 06:02:01; DPA1*01:03:01, 01:03:01; DPB1*04:01P, 04:02P²³.

The confirmatory allele DRB1*11:333 (Genbank accession number PQ009209, IPD-IMGT/HLA HWS10091669 submission number), the complete typing of the donor was as follows: A*02:01:01, 23:01:01; B* 49:01:01, 57:03:01; C*07:01:01, 18:02:01; DRB1*11:333, 13:01:01; DRB3*02:02P, 03:01:01; DQA1*01:02:01, 05:05:01; DQB1*03:01P, 05:01:01; DPA1*01:03:01, 01:03:01; DPB1*04:02P, 03:01P²³.

The confirmatory allele DRB1*13:80 (Genbank accession number PV646492, IPD-IMGT/HLA submission number HWS10100928), the complete typing of the donor was as follows: A*11:01:01, 26:01:01; B*38:01:01, 44:02:01; C*05:01:01, 12:03:01; DRB1*11:03:01, 13:80; DRB3*01:01, 02:02P; DQA1*01:03:01, 05:05:01; DQB1*03:01P, 06:03:01; DPA1*01:03:01, 01:03:01; DPB1*04:02:01, 04:02:01²³.

The confirmatory allele DRB1*16:22 (Genbank accession number PV610712, IPD-IMGT/HLA submission number HWS10100909), the complete typing of the donor was as follows: A*24:02:01, 31:01:02; B*39:13:01, 49:01:01; C*07:01:01, 07:02:01; DRB1*07:01:01, 16:22; DRB4*01:01; DRB5*02:02:01; DQA1*02:01:01, 05:05:01; DQB1*02:02:01, 03:01:01; DPA1*02:01, 02:02; DPB1*05:01:01, 11:01:01²³.

4. DISCUSSÃO

Detailed analysis of the variants identified in this study reveals different patterns of single nucleotide polymorphisms (SNPs) and their potential structural and functional impacts, considering both the location of the alteration (in exons considered key for peptide binding and recognition by the TCR) and the nature of the substitution (synonymous or non-synonymous).

The HLA-DQB1*02:211 allele presented an amino acid substitution located in a key exon directly involved in the formation of the peptide binding groove²⁴. Changes in this region can modify the three-dimensional conformation of the binding site, altering the repertoire of presented peptides and, consequently, modulating the immune response^{25,26}. SNPs of this nature are particularly relevant from an immunogenetic point of view, as they can influence both transplant compatibility and susceptibility or resistance to HLA-mediated diseases^{27,28}.

The HLA-DRB3*03:69 allele also presented an amino acid substitution, but located in a non-key exon, i.e., outside the critical peptide binding regions²⁹. Although changes in this position are less likely to directly alter antigen specificity, they can impact the stability of the molecule or interactions with other subunits of the HLA complex²⁹. Similarly, HLA-DRB1*11:341 showed a non-synonymous substitution in a non-key exon and an additional alteration in an intron. The change in the non-key exon may affect secondary structural properties or protein folding dynamics, while the intronic alteration, although not translating amino acids, may influence messenger RNA processing, modulating splicing and molecule expression^{30,31}.

The HLA-B*35:20:03 and HLA-A*03:01:133 alleles did not show amino acid changes, with substitutions located in non-key exons. These synonymous variants, although they do not directly alter the



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protein sequence, may influence translation efficiency or mRNA stability, in addition to being potentially associated with indirect effects via binding imbalance with other functional variants^{32,33}.

Thus, the identified SNPs show that even when there are no changes in central functional regions or in the protein sequence, there are potential implications for molecular biology and clinical immunogenetics. The combined analysis of the location of the alteration, its nature (synonymous or not), and its structural context in HLA is fundamental to interpreting the functional significance of each new variant identified^{13,14}.

Regarding confirmatory alleles, when comparing the HLA typing of cells in the database with the respective confirmation samples, there is a significant increase in the resolution and scope of typing, with the inclusion of previously absent loci, such as DQA1, DPA1, and DPB1. These confirmations, carried out in Brazil in 2024 and 2025, contributed to much more complete genotypic profiles compared to the original versions described in Australia (2007), Italy (2005), the US (2007), and Brazil (2013, 2024)¹¹.

Regarding the typing previously recorded in the database, the presence of a possible common haplotype is noted when comparing the confirmatory typing with the original data¹¹. This reinforces the hypothesis that the confirmed rare alleles are inherited en bloc with certain sets of alleles, forming haplotypes that are conserved in certain populations³³.

In contrast, the final sample, which carried the DRB1*16:22 allele, exhibited a distinct haplotype pattern, diverging from the pattern observed in the other samples. Nevertheless, the importance of this discovery lies in its validation at the national level, underscoring the growing importance of Brazilian immunogenetics in the identification and confirmation of new HLA alleles with clinical and population implications.

5. CONCLUSION

The new HLA alleles have been officially named by the WHO Nomenclature Committee, ensuring international standardization. Their identification and confirmation of rare variants increase the representativeness of the Brazilian population in genomic databases such as GenBank and IPD-IMGT/HLA, reducing historical underrepresentation. This inclusion directly impacts transplant medicine, promoting greater compatibility between donors and recipients and increasing the safety of procedures. In addition to the immediate clinical benefit, the findings strengthen knowledge about human immunogenetic diversity, providing support for personalized therapies and research into autoimmune, infectious, and neoplastic diseases related to different HLA profiles.

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INVESTIGATING THE PHARMACOLOGICAL POTENTIAL OF β -LAPACHONE AND AMPHOTERICIN B IN TARGETING *Leishmania* (*Leishmania*) *amazonensis*

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1. INTRODUCTION

Leishmaniasis are parasitic tropical diseases endemic in several countries, caused by protozoa *Leishmania* spp., which display marked tropism for cells of the mononuclear phagocyte system. Transmission to the vertebrate host occurs through the bite of infected female sandflies of the genus *Lutzomyia* that inoculate metacyclic promastigote forms during the blood meal (1, 2). The biological cycle of these parasites alternates between two developmental stages: the promastigote, a flagellated and fusiform form adapted to the vector's intestinal environment, and the amastigote, an intracellular, rounded, and aflagellated form (3).

Depending on the clinical presentation, leishmaniasis are classified as visceral (VL) or cutaneous leishmaniasis (CL). CL is typified by a distinctive lesion, often featuring raised borders and a central ulceration, which may progress to mucosal involvement. This clinical manifestation can be attributed to more than ten parasite species, including *Leishmania* (*Leishmania*) *amazonensis* (LLa) (2, 3). LLa is also implicated in the diffuse form of the disease, which is characterized by widely disseminated macules and diffuse cutaneous infiltrations (1, 2). Leishmaniasis predominantly affects populations in Africa, Asia, and Latin America and is associated with malnutrition, population displacement and inadequate housing conditions. In 2022, 99 countries were considered endemic for leishmaniasis, among these, Brazil exhibited endemicity for both VL and CL (4, 2).

The primary protocol for leishmaniasis management is based on the administration of Glucantime®. In cases of severe clinical manifestations or specific contraindications, alternative agents such as amphotericin B (AmB) are used (6). Nevertheless, the current pharmacological approach is associated with notable limitations, including significant rates of treatment failure and high systemic toxicity (7). As a therapeutic alternative, plant-derived compounds are gaining attention in the search for novel strategies for leishmaniasis treatment (7). In this sense, β -Lapachone (β -lap) is an *ortho*-naphthoquinone, which can be primarily obtained through the semisynthesis of lapachol, a compound extracted from the purple lapacho tree (*Tabebuia avellanedae*). β -Lap constitutes a low weight molecule that exhibits a broad spectrum of bioactivities, including leishmanicidal activity (12).

Furthermore, combination of drugs with distinct mechanisms of action has been employed to prevent resistance in the treatment of several diseases. Both in vitro and in vivo studies have demonstrated that anti-*Leishmania* agents currently used in clinical practice can exhibit increased efficacy when administered in combination with other bioactive compounds (9). Accordingly, the present study aimed to investigate the pharmacological potential of β -lap in combination with AmB for the in vitro treatment of LLa, alongside an assessment of their cytotoxic effects on host cells.

2. MATERIALS AND METHODS

2.1. Reverse docking and molecular docking study



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The molecular structures of β -lap was retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) and analyzed using the canonical Simplified Molecular Input Line Entry System (SMILES) at SwissADME[®]. A reverse molecular docking study was conducted using the online SEA (Similarity Ensemble Approach) Search Server (<https://sea.bkslab.org/>). Additionally, the molecular structure exhibiting the highest similarity to β -lapachone was identified in the National Library of Medicine (NLM) structure database (<https://www.ncbi.nlm.nih.gov/structure>). The 3D structure of β -lap was converted to the appropriate format using Chimera (version 1.17.3) by removing water molecules and adding hydrogen atoms. The docking simulations were conducted using PyRx (version 1.2). Ligand flexibility was allowed, while the receptor was kept rigid. Grid boxes were defined around the active site of the target molecules with dimensions to encompass the binding pocket. The binding affinities were evaluated based on predicted binding energies (kcal/mol), with more negative values indicating stronger interactions.

2.2. Assessment of the anti-*Leishmania* effect and cytotoxicity on J774A.1 macrophages of β -Lap and AmB combination using the checkerboard assay

To evaluate the combined effect of β -lap and AmB, drugs were arranged in 96-well plates: β -lap was first added to the first row and serially diluted down the vertical axis, while AmB was added to the last column and serially diluted across the horizontal axis. Subsequently, *Lla* (4×10^6 parasites/100 μ L) were added to all wells. A decreasing concentration curve of *Leishmania* was included as a control for parasite viability. Plates were incubated for 24 hours at 25°C and parasite viability was determined using the XTT reduction assay. The interaction between β -lap and AmB was assessed using SynergyFinder[®] (<https://synergyfinder.org/>) and the Zero Interaction Potency (ZIP) model. Regarding the J774A.1 macrophage culture, the cell suspension was adjusted to 1×10^6 cells/mL, and 100 μ L of this suspension was distributed into 96-well culture plates. Plates were incubated for 24 hours at 37°C in a 5% CO₂ atmosphere. After incubation, drugs were added as described above and the plates were incubated at 37°C in 5% CO₂ for 24 hours. Cellular viability was determined using the XTT reduction assay and untreated cells were used as control.

2.3. Determination of membrane permeability in J774A.1 macrophages

Cell suspensions (5×10^5 cells/mL) were added in 24-well plates with 300 μ L per well and incubated for 24h at 37°C in a humidified atmosphere containing 5% CO₂. Subsequently, combinations of β -lap and AmB were added at concentrations corresponding to CC₅₀, CC₇₅, and 2xIC₅₀, at a fixed ratio of 4:5 (AmB: β -lap, μ g/mL). In addition, β -lap and AmB were also tested individually at their respective CC₅₀ concentrations. Following 4h of incubation, propidium iodide (PI, 100 μ g/mL) was added to each well to assess cell membrane permeability. Plates were then analyzed using EVOS[™] inverted microscope (Thermo Fisher Scientific).

2.4. Determination of Reactive Oxygen Species (ROS) in *Lla* promastigotes

Parasites (4×10^7 mL) were treated with β -lap and AmB at the IC₅₀ (inhibition concentration of 50%), IC₇₅, and 2xIC₅₀ at a fixed ratio 4:5 (AmB- β -lap, μ g/mL) for 24 hours at 25°C in a BOD incubator. After incubation, ROS release was assessed using the cell-permeable probe 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA) (Sigma-Aldrich[®]). H₂DCFDA is a non-fluorescent probe that can be oxidized and converted into the intracellular compound 2',7'-dichlorofluorescein (DCF) in the presence of intracellular ROS. Its fluorescence can be detected by flow cytometry using a 488 nm laser for excitation and a 530 nm detector for emission. A total of 10,000 events were acquired using a BD FACSCalibur[™] flow cytometer. Quadrant marker limits in dot plots and histograms were established based on negative controls with the probe, ensuring accurate discrimination between positive and negative populations.

3. RESULTS

3.1. Molecular docking, reverse docking and pharmacological properties predictions



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In SwissADME[®] platform, β -lap physicochemical and pharmacological characteristics were analysed, as described in Table 1. In silico pharmacokinetic profiling of β -lap revealed features consistent with favorable membrane permeability. The topological polar surface area (TPSA) was calculated as 43.37 Å², indicative of low surface polarity and a capacity for efficient passive diffusion across biological membranes. Lipophilicity, estimated by the partition coefficient Log P_{o/w} (iLOGP) was 2.23, placing β -lap within the optimal range for drug-like molecules, balancing aqueous solubility with lipid permeability.

Predicted solubility values obtained from the ESOL and Ali models yielded Log S scores of -2.97 and -2.74, respectively, both classified as soluble, suggesting that, despite its moderate lipophilicity, β -lap retains a solubility profile compatible with oral absorption. Consistently, predictive models indicated high gastrointestinal absorption. Finally, the predicted skin permeation coefficient (Log K_p) was -6.22 cm/s, indicative of low transdermal diffusion. This result suggests that β -lap, similar to AmB, is more amenable to oral or parenteral formulations rather than systemic topical delivery.

Box 1. Physical-chemical properties of β -lapachone obtained from SwissADME[®]

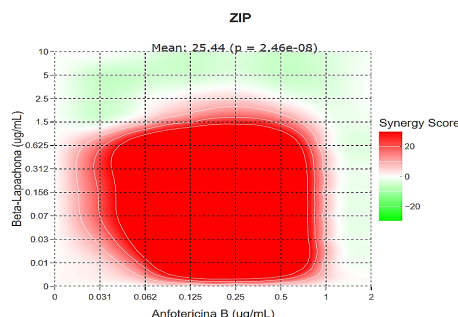
Parameters	β -lapachone
TPSA	43.37 Å ²
Log P _{o/w} (iLOGP)	2.23
Log S (ESOL)	-2.97
Class	Soluble
Log S (Ali)	-2.74
Class	Soluble
Gastrointestinal absorption	High
Log K _p (skin permeation)	-6.22 cm/s

TPSA: topological polar surface area; Log S: log of solubility; Log P: arithmetic mean of n-octanol-water partition coefficient values for lipophilicity; LogK_p: log of skin permeability

The reverse docking showed a MaxTC of 1.00 with a p value of 4.885⁻¹⁵ for the NAD(P)H dehydrogenase [quinone] 1 molecule (NQO1). The Uniprot search for *Leishmania* enzymes similar to NQO1 suggested the enzyme Quinonoid Dihydropteridine Reductase (QDR) from *L. donovani* with a sequence identity of 16% (PDB: 8B5U). The QDR structure was used in docking simulations with the PyRx program where the average score for the substrate was -6.6 and for the ligand (β -lap) was -7.2. These results suggest that β -lap exhibits a higher binding affinity to the macromolecule compared to the reference ligand, indicating that QDR may represent a potential molecular target.

3.2. Anti-*Leishmania* effect of β -lap and AmB combination

After 24 hours of incubation, the combination of β -lap and AmB demonstrated a synergistic effect against *LLa* with ZIP synergy score of 25.44 (Fig. 1). At a fixed ratio of 4:5 (AmB: β -lap, µg/mL), the combination markedly reduced the IC₅₀ values to 0.258 ± 0.1 µg/mL for *LLa*. Compared with the isolated compounds, this corresponded to IC₅₀ reductions of approximately 84% (vs. β -lap) and 72% (vs. AmB).





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Figure 1. 2D Synergy Map: Results of Pharmacological Interaction Analysis. Synergy map of the combination between Amphotericin B (X-axis) and β -Lapachone (Y-axis) generated using the ZIP model. The color scale represents the *Synergy Score*, where positive values (red) indicate synergistic effects and negative values (green) indicate antagonism. Predominantly synergistic regions were observed, with a mean synergy score of 25.44 ($p = 2.46 \times 10^{-8}$), suggesting a strongly synergistic pharmacological interaction between the tested compounds.

3.3. Cytotoxicity on J774A.1 macrophages and membrane permeability

Regarding the CC_{50} , when the drugs were tested individually, the values obtained were 1.8 μ g/mL for β -lap and 1 μ g/mL for AmB, corresponding to therapeutic selectivity indices (SI) of 1.64 and 1.11, respectively. The drug combination at a 4:5 ratio exhibited a CC_{50} of 1.83 ± 0.06 μ g/mL, corresponding to an 83% reduction in cytotoxicity compared with AmB alone and essentially matching the cytotoxicity observed for β -lapachone individually. The SI of the drug combination was 7.09, reflecting a substantial enhancement in therapeutic selectivity. For membrane permeability assessment, qualitative inspection revealed that the combination of β -lap and AmB, even at higher concentrations such as CC_{75} and $2 \times CC_{50}$, exhibited membrane permeability comparable to that observed with the individual drugs at their respective CC_{50} values.

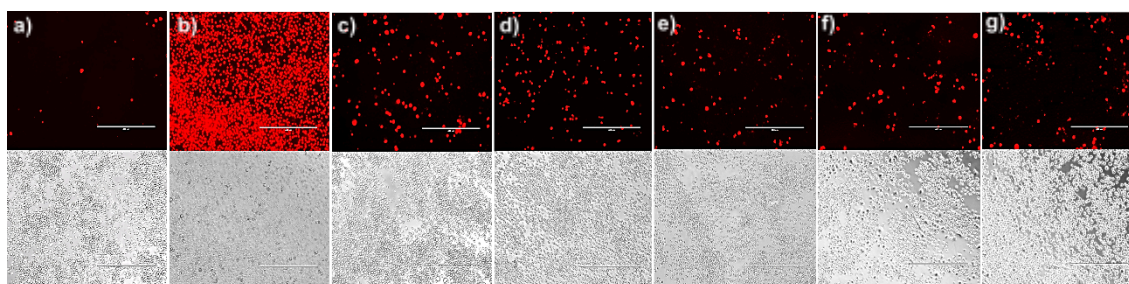


Figure 4. Membrane permeability in J774A.1 macrophages assessed by propidium iodide staining. Representative images show: a) negative control b) positive control (H_2O_2 -treated cells) c) β -lapachone at CC_{50} , d) Amphotericin B at CC_{50} , and the fixed-ratio (4:5) drug combination at e) CC_{50} , f) CC_{75} , and g) $2 \times CC_{50}$ concentrations.

3.4. Determination of Reactive Oxygen Species (ROS) in LLa promastigotes

AmB-treated samples exhibited a slight leftward shift in fluorescence compared to the negative control, reinforcing that parasite death induced by AmB is not associated with oxidative stress. In contrast, β -lapachone treatment resulted in a pronounced rightward displacement, indicative of increased ROS generation by the promastigotes. Similarly, the 4:5 AmB- β -lap combination at IC_{50} , IC_{75} , and $2 \times IC_{50}$ induced a moderate yet consistent elevation in ROS production.

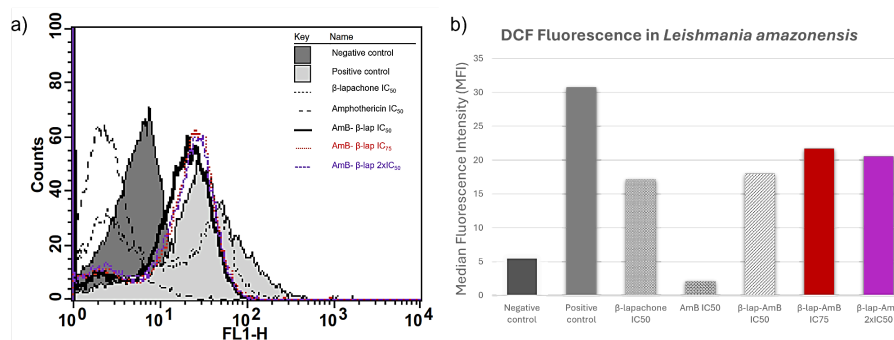


Figure 5. Oxidative stress analysis in *Leishmania amazonensis* using the 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) probe. a) Fluorescence histograms (FL1-H) of untreated cells (negative control), H_2O_2 -treated cells (positive control), and cells under different treatment



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conditions with β -lapachone and Amphotericin B. b) Median fluorescence intensity (MFI) for the same conditions, showing increased intracellular ROS in response to the treatments.

4. DISCUSSION

Leishmaniasis constitute one of the most prevalent tropical diseases caused by protozoan parasites. According to the Epidemiological Report on Leishmaniasis in the Americas (December 2023), Brazil recorded 12,878 cases in 2022, with an upward trend, particularly in northern states such as Pará and Amazonas. In this context, the development of novel anti-*Leishmania* compounds that are effective, less toxic, and accessible to vulnerable populations becomes imperative, highlighting the therapeutic potential of plant-derived substances as alternative interventions (1, 3, 7).

Combination therapies have gained prominence for overcoming limitations associated with current anti-*Leishmania* drugs. Pharmacological interactions occur when the concomitant administration of two or more drugs modifies the therapeutic effects of the individual agents. These interactions may be additive, when the combined effect equals the sum of individual effects; synergistic, when the combined effect exceeds the sum; or antagonistic, when the combined effect is inferior to that of each drug alone (9). In this study, in vitro evaluation of the β -lap and AmB combination revealed synergistic effects across multiple concentration ratios. Notably, the 4:5 ratio exhibited not only synergy but also reduced cytotoxicity relative to other combinations, as evidenced by a decreased equivalent dose of both compounds required to reach IC₅₀ and a selectivity index averaging 5.36-fold higher. These results indicate a substantial expansion of the therapeutic window and demonstrate that the drug combination exerts potent antiparasitic activity while reducing host cell cytotoxicity.

Naphthoquinones represent a structural subclass of quinones, a class of compounds categorized as secondary plant metabolites. The α -naphthoquinone β -lapachone is a molecule that exhibits a broad spectrum of bioactive properties, including leishmanicidal activity against both promastigote and amastigote forms, as demonstrated in previous studies. Evidence suggests that β -lapachone primarily exerts its effect by inducing oxidative stress through excessive ROS production, resulting in mitochondrial damage, redox imbalance, and parasite cell death (10). Accordingly, in silico analyses identified quinonoid dihydropteridine reductase (QDR) as a potential target of β -lapachone. QDR is essential for pteridine metabolism in *Leishmania*, regenerating tetrahydrobiopterin (H₄B) from its oxidized form, as the parasite is unable to endogenously synthesize pteridines. Its inhibition leads to the accumulation of the oxidized form, compromising metabolic processes and ROS neutralization, thereby increasing oxidative stress. These findings are consistent with flow cytometry results demonstrating that β -lapachone, as well as the 4:5 (AmB- β -lap) combination, induce ROS release.

Amphotericin B is widely used as a first-line treatment for pregnant patients and as a second-line therapy when pentavalent antimonials are ineffective or contraindicated. Also, despite nephrotoxicity, AmB shows lower therapeutic failure rates than antimonials (2). Its action against *Leishmania* is well established, involving ergosterol binding, membrane disruption, ion leakage, and parasite death. Notably, investigations into the cellular mechanisms underlying AmB-induced death in *Leishmania* have shown that its effect is not mediated by oxidative stress pathways. This was evidenced by the flow cytometry analyses, which failed to detect any increase in ROS production following AmB treatment. Taken together, the combination of β -Lap with AmB demonstrated synergistic antiparasitic activity, suggesting enhanced efficacy over monotherapies. This combination thus emerges as a promising strategy, though further studies are required to validate its potential for cutaneous leishmaniasis.

5. CONCLUSION

The interaction of β -lap with AmB in *Leishmania* promastigotes demonstrated a synergistic effect, resulting in a reduced IC₅₀ compared to the compounds administered individually. The drug combination at a 4:5 ratio also exhibited lower cytotoxicity and a selectivity index of 7.39, indicating a seven-fold higher specificity for the parasite compared to host cells. Therefore, combination therapy represents a promising approach for the treatment of leishmaniasis, enabling dose reduction of the individual drugs while minimizing toxicity.

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LACK OF ASSOCIATION BETWEEN INTERLEUKIN-22 GENE POLYMORPHISMS AND ANKYLOSING SPONDYLITIS: A CASE-CONTROL STUDY

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1. INTRODUCTION

Ankylosing spondylitis (AS) makes part of a group of diseases called spondylarthritis, which also include reactive arthritis, psoriatic arthritis, enteropathic arthritis and unclassified spondyloarthropathy, being AS with greater focus and recurrence (1). It is an autoimmune inflammatory systemic and multifactorial disease (sex, age, family history, and environmental factors) that affects nearly 0.1 – 1.4% of population and presents a progressive profile. The major clinical manifestations are vertebral spine involvement and sacroiliac joints, while laboratory findings are negativity for rheumatoid factor, enthesitis inflammation and genetic association with *HLA-B*27*. Despite many studies about this disease, its etiology and physiopathology still have not been completely understood, which reflects the difficulties and challenges of diagnosis, management and treatment of patients (2–4).

Diverse studies proposed theories about the role of *HLA-B27* in the development of AS. Among them is the misfolding hypothesis, which submits that *HLA-B*27* specific alleles code peptide sequences that fold slower, resulting in defective proteins that accumulate in the endoplasmic reticulum, leading to cell stress and autophagy by interleukin 23 (IL-23) (5–7); and the molecular mimicry hypothesis, which defends that peptides derived from bacteria present in the intestinal mucosa have cross-reaction with *HLA-B*27* molecules, and it might generate autoreactive Th17 cells (8,9). In fact, it has been well established that Th17 cells and innate lymphoid cells type 3 (ILC3) play a central role in AS pathogenesis (10,11). Davydova and collaborators reported higher production of interleukin 17 (IL-17) and IL-23 in spondylarthritis (12). In addition, Ikeuch et al. showed higher levels of interleukin 22 (IL-22) in autoimmune joint diseases, like rheumatoid arthritis. The authors suggest this cytokine production in the synovial fluid might induce fibroblast and macrophage proliferation (13). As well, higher plasmatic levels of IL-22 were presented in AS patients (14). Those findings emphasize a probable involvement of the IL-22 in AS.

IL-22 is in the interleukin 10 (IL-10) cytokine family. Mainly produced by ILC3s, subsets of lymphocytes (Th1, Th17 and Th22), natural killer cells (NK), mucosal-associated invariant T cells (MAIT), macrophages and dendritic cells, it prevents epithelial cell apoptosis in response to repair, consequently important in immunological maintenance and diseases that affect the tegument, lung and gastrointestinal tract. However, there are data of high expression in fibrotic disease and chronic inflammation, which demonstrate the possible duality of IL-22 and the need for its role understanding according to the context (15,16). The rate of expression could be changed depending of single nucleotide polymorphisms (SNPs) in non-coding regions. In this respect, two polymorphisms present in *IL22* gene stand out because they have already been associated with autoimmune disease; they are rs2227473 (C>T) and rs2227513 (T>C), which are located respectively in the promoter region and first intron. The T allele of rs2227473 SNP was associated with IL-22 higher expression (17), as was the C allele of rs2227513 SNP associated with IL-22 higher levels (18).



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Within this scope, the aim of this study was to evaluate the influence of rs2227473 (C>T) and rs2227513 (T>C) polymorphisms in the *IL22* gene in AS development.



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2. MATERIAL AND METHODS

2.1. Cases and controls

Adult patients with AS were assessed (n=103), both sexes, living in several cities from the northwest of the state of Paraná, diagnosed in private rheumatologic clinics and submitted to the Immunogenetics Laboratory at the Basic and Health Science of State University of Maringá (Imunogen). The diagnosis was made following assessment criteria from Assessment of SpondyloArthritis International Society (ASAS) (19). The control group (n=98) was composed of adult volunteer individuals, without any kind of rheumatologic and autoimmune disease and without family history of rheumatic disease.

This research was approved by the Permanent Ethics Committee from State University of Maringá (CAAE 27723114.0.0000.0104), and all participants signed the Written Free and Informed Consent.

2.2. Sample collection and nucleic acid extraction

Peripheral blood samples up to 4 mL were collected from cases and controls, following all biosecurity recommendations and using blood collection tubes containing EDTA anticoagulant (20). The collected samples were divided into aliquots and stored at -20°C until genomic DNA extraction, which was performed using DNA BIOPUR® commercial extraction kit (Biomatrix, Curitiba, Paraná, Brazil) according to the manufacturer's recommendations (21). The purity and concentration of the extracted DNA were evaluated by spectrophotometry using NanoDrop™ 2000 equipment (ThermoFisher Scientific, Waltham, Massachusetts, EUA).

2.3. PCR-SSP genotyping

Polymorphism genotyping was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP), which is a reliable, fast and low-cost technique. Primers, both for the polymorphisms of interest and for the human growth hormone – used as internal control of the reaction – were created from available sequences in the Genbank® database, and PCR reactions were standardized in the Immunogenetics Laboratory of UEM. Amplified genotypes and alleles in the PCR-SSP were visualized by a UV photographic documenter after running on a 2% electrophoresis gel, stained with SYBR™ Safe DNA Gel Stain (ThermoFisher Scientific, Carlsbad, California, EUA).

2.4. Statistical analysis

Association statistical analysis, including Hardy–Weinberg equilibrium (HWE), and calculation of odds ratio (OR) with a 95% confidence interval (CI), were performed using SNPStats software (22) and SPSS Statistics, version 26.0 (IBM, Armonk, NY, USA), by means of the χ^2 test and binary logistic regression. The binary logistic regression model included the covariates: sex, age and ethnic. R software, version 4.5.1 (R Studio, Vienna, Austria), was used for textual demographic data analysis and complementary statistical test application. The significance level adopted was 5%.

3. RESULTS

Among the participants, 56% of controls and 63% of patients were female, while 44% and 37%, respectively, were male ($p = 0,3$). Although in a univariate analysis it did not show statistical significance with the disease, sex was considered as a covariable in the next analyses, due to being a previously described factor influencing AS (23). Mean age was similar between groups, with 45,8 years ($\pm 15,4$) in controls and 46,2 years ($\pm 15,3$) in patients ($p = 0,8$). In relation to ethnicity, most individuals self-declared as White (77%), Brown (15%), Black (3%), Asian (3%) and Indigenous (2%).

Table 1. Frequency and distribution of genotypic alleles for rs2227473 associated with AS.



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rs2227473(C>T)	Controls n (%) n=91	Patients n (%) n=103	OR (95%CI)	p-value
Codominant				
C/C	35 (43.8%)	45 (56.3%)	1.00	
C/T	54 (52.4%)	49 (47.6%)	0.68 (0.35-1.33)	0.26
T/T	2 (18.2%)	9 (81.8%)	4.55 (0.53-39.22)	0.17
Dominant				
C/C	35 (43.8%)	45 (56.3%)	1.00	
C/T-T/T	56 (49.1%)	58 (50.9%)	0.78 (0.40-1.51)	0.46
Recessive				
T/T	2 (18.2%)	9 (81.8%)	1.00	
C/C – C/T	89 (48.6%)	94 (51.4%)	0.18 (0.02-1.46)	0.11
Over dominant				
C/T	54 (52.4%)	49 (47.6%)	1.00	
C/C – T/T	37 (40.7%)	54 (59.3%)	1.69 (0.88-3.23)	0.12
Alleles				
C	124 (68.1%)	139 (67.5%)	1.03 (0.59-1.78)	0.92
T	58 (31.9%)	67 (32.5%)		

Table 2. Frequency and distribution of genotypic alleles for rs2227513 associated with AS.

rs2227513(T>C)	Controls n (%) n=92	Patients n (%) n=77	OR (95%CI)	p-value
Codominant				
T/T	68 (55.7%)	54 (44.3%)	1.00	
T/C	23 (52.3%)	21 (47.7%)	0.93 (0.42-2.04)	0.85
C/C	1 (33.3%)	2 (66.7%)	7.39 (0.35-154.91)	0.19
Dominant				
T/T	68 (55.7%)	54 (44.3%)	1.00	
T/C-C/C	24 (51.1%)	23 (48.9%)	1.05 (0.49-2.26)	0.91
Recessive				
C/C	1 (33.3%)	2 (66.7%)	1.00	
T/T – T/C	91 (54.8%)	75 (45.2%)	0.13 (0.01-2.77)	0.19
Over dominant				
T/C	23 (52.3%)	21 (47.7%)	1.00	
T/T + C/C	69 (55.2%)	56 (44.8%)	1.12 (0.51-2.45)	0.78
Alleles				
T	159 (86.4%)	129 (83.8%)	1.18 (0.59-2.38)	0.64
C	25 (13.6%)	25 (16.2%)		

No statistically significant associations were observed between the polymorphisms rs2227473(C>T), rs2227513(T>C) and AS in any of the assessed genetic models. Although the T/T genotype (rs2227473) and C/C genotype (rs2227513) were more frequent in patients, these differences did not reach statistical significance.



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4. DISCUSSION

Although the contribution of genetic factors to AS has been largely recognized, gaps remain regarding the role of SNPs, especially with regard to markers not belonging to the HLA-B27 complex. In the present study, we described for the first time, in a southeast Brazilian population, the frequencies of rs2227473(C>T) and rs2227513(T>C) polymorphisms. A mild relevance of association of the T allele from rs2227473 and the C allele from rs2227513 with the disease was noted; however, these findings did not reach statistical significance. The absence of association might be related to limited size sample, which reduces the power to detect effects of lesser magnitude.

When considering the analyzed rs2227473 polymorphism, previous investigations showed its possible modulation of IL-22 levels associated with the T allele, particularly in intestinal tuberculosis patients, although those observations were conducted in a Chinese population (17). In addition, that same SNP was implicated in cerebral malaria predisposition and in pulmonary tuberculosis in several populations, conditions in which inflammatory process plays a central role in pathogenesis (23,24). By contrast, Wang et al. did not find an association with several cancer cases in a case-control study (25). Combined with others, these reports highlight the relevance of this polymorphism in different infectious contexts and increase its participation in common inflammatory pathways across several diseases. However, the absence of significance in our study may suggest that these associations depend on the presence of an infectious triggering agent, which does not occur directly in AS.

Fundamentally, analogous results in autoimmune diseases have already been reported in different populations. Jinping Yin and collaborators identified a significant association among the rs2227513 polymorphism, IL-22 levels and fibroblasts proliferation in patients with rheumatoid arthritis (26). Similarly, Rong Wang et al. reported an association of this same SNP with IL-22 expression in patients with systemic lupus erythematosus and renal dysfunction as prognostic (27). This polymorphism is located in the first intron of the *IL22* gene and could influence the modulation of gene expression, as the nucleotide change T>C weakens protein bonds during the splicing process. This mechanism was reported in a study that evaluated the mutation's impact on human immunodeficiency virus (HIV) infectious (18). Given the context, previous studies have described a heterozygous genotype association of that SNP with susceptibility to visceral leishmaniasis (28). Even though leishmaniasis and HIV infections are diseases related to pathogens and not autoimmune conditions, these findings enhance the polymorphism's participation in common inflammatory pathway, indicating IL-22 as mediator in several pathological contexts.

Therefore, the gathered convergences support the biological plausibility of our findings. Nevertheless, the reduced sample size and the absence of adjustments for genetic factors, such as *HLA-B*27*, could have limited the detection of stronger associations. In this way, future perspectives of this study include the continuation of case-control genotyping in order to extend sample size, balance the groups, and increase the statistical power of genetic association analyses. Furthermore, additional analyses incorporating *HLA-B*27*, a genetic factor well established in the literature, will be relevant to exclude possible confounding effects and allow a more comprehensive understanding of both the *IL22* polymorphism gene and its relation to the disease.

Even so, higher frequencies of the T allele of rs2227473 and C allele of rs2227513 were noted in cases, but without statistical significance. These results, while not confirming a definitive association, contribute to expanding knowledge about genetic variability related to AS in our population.

5. CONCLUSION

In this study, the analyzed polymorphisms in *IL22* gene showed no significant association with susceptibility to AS. However, given the previously reported role of IL-22 in the modulation of inflammatory responses in different conditions, the continuation of research becomes relevant. Future studies, with larger participant numbers and including additional factors as *HLA-B*27*, could more broadly highlight the impact of IL-22 on the disease pathophysiology.

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COMPARATIVE ANALYSIS OF THE ACCURACY OF HIGH-RESOLUTION HLA ALLELE INFERENCE STRATEGIES IN THE PARANÁ POPULATION

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1. INTRODUCTION

In the human species, the Major Histocompatibility Complex (MHC) is known as the Human Leukocyte Antigen (HLA) system. It is composed of cell surface molecules that are essential for both the activation of adaptive immunity and the maintenance of innate immune response homeostasis. The extensive polymorphism in HLA genes directly influences the organism's response to tumors, infections, vaccines, the development of hypersensitivities, and, crucially, the success of transplants (1, 2).

Transplantation between genetically different individuals induces immunological responses against the graft, primarily due to HLA gene incompatibilities, however, with advancements in typing technologies and immunosuppressive drugs, the risk of acute rejection within one year post-transplant is less than 15% (3). For solid organ transplants, HLA typing is typically performed at low/medium resolution, but recent research on the molecular mismatch burden has shown that high-resolution typing is more precise and aids in supporting clinical decisions and transplant research (4). Low- to medium-resolution methodologies define the serological type by identifying the allelic group of HLA molecules with similar serological reactivity, whereas genomic sequencing techniques provide high-resolution HLA typing results that define the specific HLA protein, or allele (5).

Currently, Next-Generation Sequencing (NGS) has established itself as the gold standard for high-resolution HLA typing, however, its implementation faces practical and technical obstacles, including high cost, long processing time, and cis-trans ambiguities in short-read sequencing methodologies. Despite these challenges, NGS technology has enabled significant advances in the HLA typing process, particularly through the description of new alleles in different populations, thereby increasing the representation of genetic diversity in global databases (6).

Given the high cost of NGS and the time constraints for obtaining results in HLA typing for deceased organ donors, the imputation, or statistical inference, of high-resolution HLA alleles from low/medium-resolution data emerges as a viable alternative. This imputation is performed using computational tools that make predictions through expectation-maximization algorithms, representing a significant technological advancement, particularly for the analysis of large databases. However, its accuracy is highly dependent on the quality, size, and population representativeness of the reference panels used (7).

Therefore, the objective of this study was to perform a comparative analysis of the accuracy of different high-resolution HLA prediction strategies. This involved comparing the performance of a statistical inference platform developed by the National Marrow Donor Program (NMDP), known as HaploStats (8), which utilizes an expectation-maximization algorithm, against a simple inference method based on population HLA allele frequencies.

2. MATERIALS AND METHODS



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2.1. Sample selection

This study used the HLA typing results from individuals registered in the Brazilian Volunteer Bone Marrow Donor Registry (REDOME) who were called for confirmatory testing (phase III) between January 2015 and January 2025 in the northwestern region of Paraná. All donors registered in REDOME sign an informed consent form for HLA typing, and all personal information provided at the time of registration is kept confidential, in accordance with the Privacy Policy available on the redome.inca.gov.br website (9). The study was initiated using the HLA results from 56 donors for whom the corresponding REDOME code was available.

2.2. Data imputation strategies

Initially, the low-, medium-, and high-resolution HLA typing data from the selected volunteer bone marrow donors were organized in Excel spreadsheets. For the specific allele imputation, the NMDP codes (10) were entered into the HaploStats platform, which employs an expectation-maximization algorithm to infer the most probable allele based on a haplotype reference database. A second imputation was performed by direct decoding, which involved selecting the first allele from the list of possibilities generated by the NMDP code, as this conventionally represents the most frequent allele in the NMDP database population. The high-resolution data were used as the gold standard to compare against the imputation results to verify their accuracy.

Subsequently, the imputation results were analyzed according to four distinct strategies: 1) Allele imputation based on allele frequency after direct decoding of the NMDP code (<https://hml.nmdp.org/MacUI/#/>) without using HaploStats; 2) Allele imputation in the HaploStats software using NMDP codes, considering all correct and incorrect results with the Caucasian reference population; 3) Allele imputation in the HaploStats software using NMDP codes, excluding donors with alleles that were not represented in the Caucasian population; 4) Allele imputation in the HaploStats software using NMDP codes, excluding donors with alleles that were not represented in any population present in the software.

2.3. Comparative analysis

The accuracy of each of the four imputation strategies was calculated and compared. For strategies 1 and 2, the analysis included the entire sample, corresponding to 56 genotypes. For strategies 3 and 4, adjusted accuracy analyses were performed on subsets of the sample. In strategy 3, the exclusion criterion removed donors who had at least one allele not represented in the Caucasian reference panel. The objective was to evaluate the performance of HaploStats on genotypes fully represented in this database, which resulted in a final sample of 30 genotypes. Following the same logic, strategy 4 evaluated the software's accuracy on genotypes that were fully represented in the platform's global database. After applying this exclusion criterion, the final sample for this analysis consisted of 33 genotypes.

2.4. Characterization of imputation errors

The alleles incorrectly imputed by the software using prediction strategy 2 were organized into a characterization table. This classification was based on the allelic frequency of these alleles within the study population (N=56) and on their designation as common, intermediate, or well-documented according to two catalogs: the international Common, Intermediate and Well-Documented (CIWD) HLA Alleles in World Populations, version 3.0.0 (11), and the CIWD Brazil catalog (12). This data was subsequently used to assess the frequency and documentation status of these alleles in the northwestern region of Paraná.

3. RESULTS

The study sample consisted of 56 volunteer bone marrow donors registered in REDOME from the northwestern region of Paraná. The demographic profile of the sample included 33 male (59%) and 23 female (41%) individuals, with ages ranging from 18 to 46 years (mean 24.7 years). The predominant ethnicity was White, accounting for 62% of the participants (N=35).



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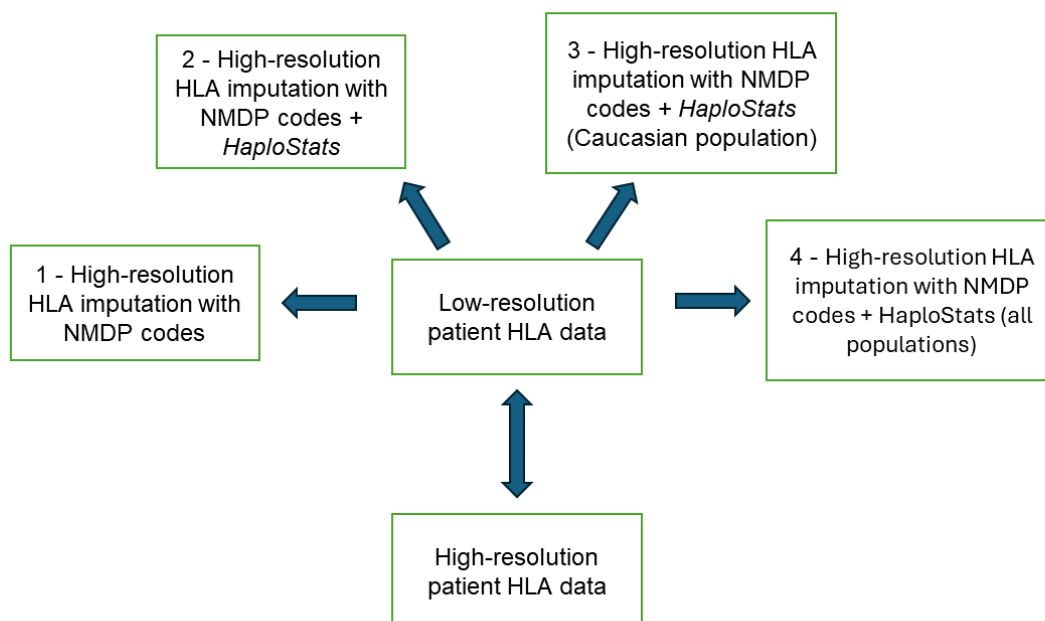


Figure 1. Schematic representation of the different imputation strategies performed.

In the comparative accuracy analysis of the total sample (N=56), strategy 1 resulted in an accuracy of 94.30% (528 correct and 32 incorrect imputations). Strategy 2 achieved an accuracy of 94.11% (527 correct and 33 incorrect imputations). A qualitative analysis of the 33 errors generated by HaploStats showed that 11 occurred in Class I HLA alleles and 19 in Class II. According to the CIWD Brazil catalog, 14 of the erroneously imputed alleles are classified as common in the Brazilian population. One example is the DQB1*02:02 allele, which had a frequency of 0.32 in the sample and was classified as common, yet it had seven incorrect and two correct imputations.



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Table 1. Classification of alleles with imputation errors according to their allelic frequencies in the international and Brazilian CIWD catalogs.

Alleles	AF	CIWD Internacional	CIWD Brazil
DRB1*14:54	0,18	C	C
DRB1*04:11	0,14	C	C
DRB1*04:05	0,07	C	C
DQB1*06:05	0,04	WD	NR
DQB1*04:02	0,29	C	C
DQB1*03:19	0,07	C	C
DQB1*03:02	0,29	C	C
DQB1*02:02	0,32	C	C
C*02:10	0,11	C	C
B*40:129	0,04	WD	C
B*39:13	0,04	WD	C
B*39:10	0,04	I	C
B*35:11	0,07	I	C
B*35:03	0,07	C	C
B*15:05	0,04	I	C
A*31:15	0,04	I	C

AF: Allelic frequency in the sample of 56 patients; C: Common; WD: Well Documented; I: Intermediate; NR: Not represented

For the adjusted accuracy analyses in strategies 3 and 4, there was an increase in correct imputations and a decrease in errors. In strategy 3, 26 donors (46.4% of the sample) who lacked allelic representation in the Caucasian population were removed, with the remaining sample of 30 achieving an accuracy of 99.67%. In strategy 4, 23 donors (41.1%) who lacked allelic representation in any of the populations available in the HaploStats software were excluded. This resulted in a final sample of 33 donors with 100% accuracy and no imputation errors.

4. DISCUSSION

The main finding of this study was the observation that imputation via HaploStats, which employs an expectation-maximization algorithm, did not demonstrate superiority over a method based on the direct decoding of NMDP codes and allele frequency analysis. The total accuracy of HaploStats, using all 56 included genotypes (imputation strategy 2), was 94.11%, whereas the NMDP decoding approach achieved 94.30% (imputation strategy 1). This suggests that the platform's statistical power is diminished when its reference database does not adequately reflect the population's haplotypes and allelic diversity, confirming that the underrepresentation of admixed populations is a problem for allele imputation software (13).

To evaluate the algorithm's performance under ideal conditions, donors with alleles not represented in the HaploStats reference panels were excluded from the analysis. The high exclusion rate, 26 of 56 donors (46.4%) in strategy 3 and 23 donors (41.1%) in strategy 4, reflects the lack of haplotype representativeness for a highly admixed population like Brazil's in the HaploStats database (14). Once the subset of donors whose genotypes were entirely recognized by the platform was isolated, the adjusted accuracy increased to 99.67% in strategy 3 and 100% in strategy 4. This increase, especially the perfect accuracy in strategy 4, proves that the HaploStats algorithm is indeed effective, but only when supplied with representative population databases. This highlights that the main barrier to precision in our population is not the imputation method itself, but the lack of a representative reference panel. These findings reinforce the need to expand and diversify segregated HLA haplotype databases.



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The three additional donors who were excluded from the Caucasian panel analysis but included in the global one represent individuals whose genotypes contained alleles absent from the Caucasian reference but present in other platform panels, such as those of African, Asian, or Hispanic ancestry. This subgroup perfectly exemplifies the admixed profile of the Brazilian population. The inability of the standard Caucasian panel to encompass these individuals highlights the risk of adopting a single-ethnic reference to analyze a diverse population, which underestimates its complexity and generates errors that could be avoided with a more comprehensive database.

The analysis of the 33 HaploStats errors from strategies 1 and 2 reveals that most of the erroneously imputed alleles are common in the Brazilian population. The case of the DQB1*02:02 allele, which is classified as common and yet was incorrectly imputed on seven occasions, is particularly controversial. This suggests that the error does not lie in the inability to recognize the allele in isolation, but in the absence of the specific haplotype in which this allele segregates in the Brazilian population. The platform assigns the common allele to a more frequent haplotype within its reference database (Caucasian), which results in the error.

5. CONCLUSION

This study demonstrated that the HaploStats platform's accuracy is limited for the Brazilian population, failing to surpass a direct decoding approach. This confirms the hypothesis that the failure lies not in the algorithm itself, but in the underrepresentation of Brazilian alleles and haplotypes in its reference panels. This was evidenced by the 46.4% sample exclusion required to achieve high precision. We conclude there is a critical need to develop imputation tools with Brazil's population specific reference panels to ensure their clinical applicability, a goal to be achieved through the continuation of this work.

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Keywords: brazilian population; computational tools; high-resolution.

Área temática: Etiofisiopatologia de doenças humanas e animais



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ISONIAZIDE-DERIVED MOLECULES WITH ANTI-*Mycobacterium tuberculosis* ACTIVITY

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1. INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by species of the *Mycobacterium tuberculosis* complex, with *M. tuberculosis* (*Mtb*) being the most relevant from a public health perspective (1). Recognized as one of the oldest and most persistent human diseases, TB affects individuals across all age groups and has become the leading cause of death from a single infectious agent worldwide, imposing a major global health burden. The transmission of *Mtb* occurs through airborne particles, produced when individuals with active pulmonary or laryngeal TB cough, speak, or sneeze, releasing aerosols containing viable bacilli (1,2).

The standard treatment for drug-susceptible TB consists of a four-drug regimen including isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB). This regimen requires prolonged treatment (6 months), which often compromises patient adherence and increases the risk of selecting resistant bacilli. INH, introduced into the TB treatment regimen (1952–1956), has persisted as a mainstay in TB therapy, with its efficacy firmly established over decades of clinical use (3).

INH is a prodrug that requires activation by the catalase–peroxidase enzyme (KatG), present in *Mtb*. Once activated, it forms an adduct with nicotinamide adenine dinucleotide (NAD), known as INH–NAD. This adduct inhibits the enoyl-acyl carrier protein reductase (InhA), an enzyme essential for the biosynthesis of mycolic acids, thereby compromising cell wall integrity, inducing DNA damage, and ultimately leading to bacillus death. Through these mechanisms, INH exerts strong bactericidal activity. In addition, the molecular structure of INH contains organic compounds, specifically N-acyl hydrazones, which display antimicrobial, antitumor, antiviral, and antiparasitic activities. Building on these properties, INH derivatives have been developed and have shown potential as novel and selective antitubercular candidates (4–9).

The minimum inhibitory concentration (MIC) of INH against *Mtb* is considered low (ranging from 0.02 to 0.20 µg/mL) and widely recognized as a safe drug with low toxicity when used at appropriate dosage and is generally associated with fewer adverse effects compared to other anti-TB agents. Its combination with other agents, such as RIF, further enhances treatment efficacy. Nevertheless, the global rise of INH-resistant *Mtb* strains poses a major challenge to current TB treatment strategies. The most frequent mechanism of resistance is related to mutations in the *katG* gene, which reduce KatG enzymatic activity by preventing the activation of the prodrug INH into its active metabolite (9), thereby posing a major challenge to global TB control efforts.

New therapeutic options against *Mtb* remain an important strategy in TB treatment and control, particularly approaches that could shorten the treatment time. Throughout the history of pharmacology, several molecules have undergone structural modifications to improve their therapeutic action. Such modifications may include changes in the chemical structure of the original compound to increase efficacy, minimize adverse effects, or improve bioavailability. Given its potency at low concentrations and favorable safety in patients without hepatopathy or renal impairment, INH serves as a valuable scaffold for the development of novel derivatives that may overcome mycobacterial resistance and reduce treatment time.



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(9). In this context, this study aims to investigate the activity of INH derivatives against the pan-susceptible *Mtb* reference strain H₃₇Rv and clinical isolates.

2. MATERIAL AND METHODS

2.1. INH derivatives

The 11 INH derivatives, designated as ACH-01, ACH-03, ACH-05, ACH-06, ACH-08, ACH-09, ACH-10, ACH-11, ACH-12, ACH-13, and ACH-16, were synthesized at the Federal Technological University of Parana, State of Parana, Brazil. The derivatives were dissolved in dimethyl sulfoxide (DMSO) and stock solutions prepared at 10.000 µg/mL. INH was purchased from Sigma-Aldrich, and stock solutions (10.000 µg/mL) were prepared according to the manufacturer's instructions.

2.2. Bacterial strains/clinical isolates and culture conditions

The pan-susceptible *M. tuberculosis* H₃₇Rv strain and five INH resistant *Mtb* clinical isolates from the Mycobacteria collection of the Medical Bacteriology Laboratory at the State University of Maringa (UEM) were studied. The bacilli were cultured in Middlebrook 7H9 medium (Difco Laboratories, Sparks, MD, USA) supplemented with 0.2% glycerol and 10% Middlebrook OADC enrichment (oleic acid, albumin, dextrose, and catalase) (BBL™ Becton Dickinson Microbiology Systems, MD, USA) for 15 days at 35°C.

2.3. *In silico* study - Absorption, distribution, metabolism and excretion

To evaluate the absorption, distribution, metabolism and excretion (ADME) of the derivatives, an *in silico* approach was performed using the free software SwissADME, OSIRIS Property Explorer, and Molinspiration. The physicochemical parameters, including molecular weight and the number of hydrogen bond donors and acceptors, were assessed according to Lipinski et al. (2001) (10). The OSIRIS Property Explorer provided toxicity data. The topological polar surface area (TPSA) value obtained using SwissADME was applied to calculate the percentage of absorption (%ABS = 109 – 0.345 × TPSA).

2.4. Minimum inhibitory concentration (MIC)

The MIC of each INH derivative was determined by the Resazurin Microtiter Assay Plate (REMA) method as described by Palomino et al. (2002) with modifications (11). All INH derivatives were two-fold serially diluted from 2.048 µg/mL to 0.12 µg/mL. The *Mtb* bacterial suspensions were adjusted to an optical density at 625 nm (OD₆₂₅ = 0.16–0.20) in a spectrophotometer and further diluted 1:20 in supplemented Middlebrook 7H9 medium. Then, 100 µL of each mycobacterial suspension was added to wells of the microplates containing the pre-diluted INH derivatives. Microplates were incubated at 35°C for seven days.

After the incubation period, 30 µL of 0.01% resazurin solution was added to each well, and the microplates were incubated again for 24 hours prior to visual reading. A color change from blue to pink indicated resazurin reduction, corresponding to bacterial growth. The MIC of each INH derivative was defined as the lowest derivative concentration in which no bacterial growth was observed (blue color of resazurin). The assays were performed in triplicate in different days. A negative, positive, INH, and RIF were included as controls in each assay.

2.5. Cytotoxicity assay

Cytotoxicity assays were performed in triplicate using the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) in Vero cells (African green monkey kidney cell line, *Chlorocebus* sp., ATCC CCL-81). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, MO, USA), supplemented with 10% fetal bovine serum (Gibco, Life Technologies, Paisley, UK). After cell detachment, the cell suspension was adjusted to 5.0 × 10⁵ cells/mL, seeded into 96-well microplates and incubated at 37°C for 24 hours at 5% CO₂. After this period, the supernatant was removed,



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and the compounds to be tested were diluted to 500 to 0.97 µg/mL and 100 µL added to the wells and microplates incubated at 37 °C for 24 hours at 5% CO₂. Subsequently, the supernatant was removed, and the cells were washed with Phosphate-Buffered Saline (PBS), after which 75 µL of MTT at 2 µg/mL was added and incubated for 3 hours under 5% CO₂ at 37°C protected from light, to allow formazan crystal formation. Then, 75 µL was removed from each well and 75 µL of DMSO was added. Plate reading was carried out at 550 nm in a Spectrophotometer Varioskan Lux (Thermo Scientific, USA), to determine cytotoxic activity (CC₅₀), defined as the concentration required to reduce cell viability by 50%. DMSO was used as the negative control in all assay.

2.6. Hemolysis assay

Firstly, defibrinated sheep red blood cells (SRBC) were washed twice with glucose saline solution (1% glucose and 0.9% NaCl, pH 7.25) and a 6% SRBC suspension was prepared. After, in a 96-well microplate, 100 µL of glucose saline (1% glucose and 0.9% NaCl pH 7.25) was added to each well, followed by the two-fold serially diluted INH-derivatives at concentrations ranging from 500 to 7.81 µg/mL. Subsequently, 100 µL of the 6% SRBC suspension was added to each well containing the diluted INH-derivatives, and the plates were incubated for 3 hours under 5% CO₂ at 37°C. After incubation time, the microplates were centrifuged at 1100 rpm for 3 minutes, and the supernatant was transferred to a new microplate for absorbance measurement. The microplate was read in a spectrophotometer at 550 nm using a microplate reader.

Triton X-100 solution (Merck, Darmstadt, Germany) was used as the positive control, while SRBC in saline served as negative control. The absorbance values obtained were used to estimate the concentration of each INH-derivative that lysed 50% of the SRBC (HC₅₀), calculated according to the formula:

$$\%Hemolysis = 100 - [(Ap - As)/(Ap - Ac) \times 100]$$

where Ap, As, and Ac correspond to the absorbance values of the positive control, the test compounds, and the negative control, respectively. The selectivity index (SI) was calculated as the ratio HC₅₀/MIC.

3. RESULTS

The *in silico* analysis conducted with INH and the 11 INH-derivatives, by SwissADME, showed results that none of them violate Lipinski's rules, according to the physicochemical parameters. The drug score ranged from 0.17 to 0.34 for INH-derivatives and 0.06 for INH, reinforcing the pharmacological potential of the INH-derivatives.

The anti-*Mtb* susceptibility evaluation (Table 1), revealed that the tested INH-derivatives displayed variable activity against *Mtb*, including the pan-susceptible H₃₇Rv and multidrug-resistant *Mtb*. In the susceptible *Mtb* H₃₇Rv, derivatives ACH-01, 03, 05, 09, 10, and 16 showed high activity (MICs ranging from 0.5–4 µg/mL). In general, for the resistant *Mtb*, from the 11 INH-derivatives studied, only the ACH-12 showed the highest MIC values (≥ 512 µg/mL). Notably, derivatives ACH-01 and ACH-16 showed the greatest activity against *Mtb*.

The analysis of the cytotoxicity of INH-derivatives in VERO cells revealed distinct profiles with CC₅₀ ranging from 120.7 to > 500 (Table 2). The SI determined considering the MIC values obtained with *Mtb* H₃₇Rv showed 10 INH-derivatives were more selective to *Mtb* H₃₇Rv. Only ACH-12 exhibited low selectivity (SI = 0.7) due to the high MIC value (512 µg/mL) against the *Mtb* H₃₇Rv.

In the hemolysis assays (Table 2), most INH-derivatives showed CC₅₀ values above 500 µg/mL. Derivatives ACH-01, 05, 09, 10, and 16 displayed high SI values (≥125), with ACH-01 standing out with an SI > 1000. Although the ACH-12 exhibited CC₅₀ 500 µg/mL, the SI was too low as 0.97 once its MIC against *Mtb* were high as 512 or 1024 µg/mL depending on the *Mtb* studied.



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Table 1. Minimum Inhibitory Concentration (MIC) of INH derivatives in susceptible and resistant *Mycobacterium tuberculosis* by Resazurin Microtiter Assay Plate.

Mtb	Profile	MIC Derivatives ACH												
		Drugs (µg/mL)												
		RIF	INH	01	03	05	06	08	09	10	11	12	13	16
H ₃₇ Rv	Susceptible	0.03	0.06	0.5	4	1	8	8	4	4	8	512	8	4
47S	INH ^R	0.03	2	0.5	8	16	8	8	8	4	16	512	8	4
4250	INH ^R (<i>katG</i>)	0.03	2	8	8	16	8	16	8	16	16	1024	32	2
3360	INH ^R /RIF ^R (<i>inhA</i> + <i>katG</i>)	0.03	1	2	8	16	8	8	2	2	2	1024	4	0.5
109	INH ^R /RIF ^R (MDR)	256	4	4	128	4	16	8	4	16	8	512	8	2
3614	INH ^R /RIF ^R /EMB ^R /ETH ^R /S ^R (MDR)	256	4	2	64	64	8	32	16	16	16	512	32	16

Note. *Mtb*: *Mycobacterium tuberculosis*; INH^R: isoniazid-resistant; INH^R (*KatG*): isoniazid-resistant strain due to *katG* mutation; INH^R/RIF^R (*inhA*+*KatG*): isoniazid- and rifampicin-resistant *Mtb* due to *inhA* and *katG* mutations; INH^R/RIF^R (MDR): multidrug-resistant *Mtb* (isoniazid- and rifampicin-resistant); INH^R/RIF^R/EMB^R/ETH^R/S^R (MDR): multidrug-resistant *Mtb* resistant to isoniazid, rifampicin, ethambutol, ethionamide, and streptomycin.

Table 2. Cytotoxicity of INH-derivatives determined in VERO and defibrinated sheep red blood cells.

Derivatives	VERO				Hemolysis		
	CC ₅₀ (µg/mL)	±SD (µg/mL)	MIC (µg/mL)	SI	MIC (µg/mL)	HC ₅₀ (µg/mL)	SI
ACH-01	238.6	±28.8	0.5	477.2	0.5	>500	>1000
ACH-03	375.6	±53.3	4	93.9	4	250	62.5
ACH-05	120.7	±37.2	1	120.7	1	>500	>125
ACH-06	139.9	±3.4	8	17.5	8	>500	>62.5
ACH-08	284.4	±9.6	8	35.6	8	>500	>62.5
ACH-09	135.0	±31.0	4	33.7	4	>500	>125
ACH-10	>500	ND	4	>1000	4.0	>500	>125
ACH-11	>500	ND	8	62.5	8	>500	>62.5
ACH-12	378.1	±61.6	512	0.7	512	500	0.97
ACH-13	322.4	±54.0	8	40.3	8	>500	>62.5
ACH-16	>500	±68.7	4	136.9	4	>500	>125
INH	>500	ND	0.06	>1000	0.06	500	>1000

Note. VERO: African green monkey kidney cell line; Hemolysis: hemolysis assay; CC₅₀: half-maximal cytotoxic concentration; SD: standard deviation; MIC: minimum inhibitory concentration; SI: selectivity index; HC₅₀: hemolytic concentration at 50%; ND: not determined.



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4. DISCUSSION

The INH-derivatives tested in this study demonstrated highly promising activity as anti-*Mtb* agents, inhibiting bacterial growth at low concentrations of INH-derivatives, corroborating studies conducted by Sampirou et al 2019 (7).

The *in silico* analysis conducted with INH and the 11 INH-derivatives, by SwissADME, showed results that none of them violate Lipinski's rules, according to the physicochemical parameters. Lipinski's rule of five points out physicochemical properties associated with 90% of oral medications that reach phase II clinical trials, making it possible to predict characteristics and assist in the selection of the next stages of study (12). In addition, most molecules showed equal or higher cellular absorption percentages. Studies show that altering the INH structure by inserting more lipophilic groups can promote penetration into the bacterial cell wall, contributing to increased efficacy against tuberculosis (13).

Cytotoxicity assessments in VERO cells and in SRBC (hemolysis assays) revealed selectivity to all *Mtb* in this study. Among the 11 INH-derivatives, ACH-10 showed the highest SI value, followed by ACH-01 corroborating to García et al. (2012), which indicates molecules with an SI greater than 10 and MICs below 10 µg/mL should be considered promising candidates for treatment (14, 15).

Furthermore, the hemolysis test results found in this study showed that most INH derivatives had an HC50 > 500 µg/mL, a value well above the MIC. Therefore, even if the derivatives needed to increase the MIC concentration, it would still not be toxic to red blood cells. Hemolysis assays further supported the low *in vitro* cytotoxicity of all INH-derivatives, which deserve greater attention to these INH-derivatives and continued studies, envisioning molecules that can be considered for future anti-TB medicine. The derivatives also exhibited MIC values comparable to those of reference drugs in the treatment of TB, INH, when tested against resistant *Mtb*, suggesting a possibly similar mechanism of action among them. This finding needs attention in the attempt to have an INH-derivative with a different mechanism of action to combat *Mtb* with INH resistance due to already known gene mutations (6–9).

5. CONCLUSION

The derivatives ACH-01 and ACH-10 emerge as promising anti-TB molecules, with significant antimicrobial activity against INH-susceptible, INH-resistant, and multidrug-resistant *Mtb*. Overall, these results support the continued investigation of INH-derivatives as valuable leads for the development of future anti-TB agents to complement and strengthen current therapeutic regimens.

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EVALUATION OF THE SYNERGISTIC ACTIVITY OF ANTIBACTERIALS AT THERAPEUTIC CONCENTRATIONS AGAINST *Klebsiella pneumoniae* PRODUCING CONCOMITANTLY KPC AND NDM: PROPOSAL OF A RAPID METHODOLOGY

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1. INTRODUCTION

The increase of multidrug-resistant (MDR) bacterial isolates is a concerning issue and one of the main challenges due to the lack of available antibacterial agents (1). Among MDR bacteria, carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) stands out, being listed as one of the most critical pathogens for the development and research of new drugs by the World Health Organization in 2024 (2). One of the main mechanisms of carbapenem resistance presented by CR-Kp is the production of carbapenemases, enzymes capable of hydrolyzing these drugs (1). The dissemination of carbapenemases in hospital environments can happen easily, as they are encoded by genes present in mobile genetic elements, such as conjugative plasmids, favoring the increase of isolates that produce these enzymes (1).

CR-Kp can produce almost all molecular classes of beta-lactamase proposed by Ambler (1980), from A to D (3). In Class A, the serine carbapenemase *Klebsiella pneumoniae* carbapenemase (KPC) stands out, which can hydrolyze most beta-lactams. In Class B, the metallo-beta-lactamases stand out, such as New-Delhi metallo-beta-lactamase (NDM). In Class C, there are the inducible plasmid enzymes of the AmpC type, and finally, in Class D, the oxacillinase-48 enzyme stands out for CR-Kp (3).

The treatment of CR-Kp is extremely complicated due to the lack of therapeutic options (1, 4). As an alternative, there is the "rescue" of old antibacterials, such as polymyxin B. However, this drug has high renal toxicity and promotes a rapid increase of resistant isolates, hence its use in monotherapy is not recommended. Its use in association with other antibacterials is therefore recommended (4). Among the new drug options, there is ceftazidime-avibactam (CAZ/AVI), launched in 2018. However, despite being effective against CR-Kp producing carbapenemases of Ambler's classes A and D, it is not effective when CR-Kp produces metallo-beta-lactamases. Therefore, when CR-KP produces both serine and metallo-beta-lactamases, the use of CAZ/AVI is limited, and other treatment options for infections caused by this pathogen would be ceftiderocol or combinations like aztreonam-avibactam, which are not available on the Brazilian market (5).

In this scenario, combined therapy stands out as the only viable option with the combination of existing drugs available on the Brazilian market. However, standardized methods such as checkerboard and time kill curve are methodologically complex, expensive, and demand prolonged incubation, reducing the clinical impact of their results.

Thus, the objective of this study was to standardize and propose a faster and easier methodology for the detection of antibacterial synergism, based on therapeutic concentrations that are applicable to the laboratory routine.



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2. MATERIAL AND METHODS

2.1. Isolates selection and characterization

From 17 *Klebsiella pneumoniae* isolates producing both KPC + NDM, five molecularly distinct isolates were selected and included in the study, differentiated by Enterobacterial Repetitive Intergenic Consensus-PCR. Carbapenemase detection was performed by multiplex-PCR, targeting the following genes: *bla_{KPC}* and *bla_{NDM}*.

These isolates came from samples of patients hospitalized at the Hospital Universitário Regional de Maringá (HUM) and were part of a continuous resistance gene surveillance project, approved by the Comitê Permanente de Ética em Pesquisa com Seres Humanos at State University of Maringá, under number: 7.676.954.

2.2. Bacterial Inoculum Preparation and Minimum Inhibitory Concentration (MIC) Determination

To determine the minimum inhibitory concentration (MIC), a bacterial suspension containing approximately 1.5×10^8 Colony Forming Units (CFU)/mL was prepared from fresh colonies of the selected isolates, standardized in a nephelometer. The inoculum concentration in the experiments was 5×10^4 – 5×10^5 CFU/mL, as described by the Clinical and Laboratory Standards Institute (CLSI) document M07-A12 (6).

The MIC for the four antimicrobials (meropenem, polymyxin B, fosfomycin, and tigecycline) was determined by the microdilution method in cation-adjusted Mueller-Hinton broth (MHB-CA), as described by CLSI document M07-A12 (6). Additionally, tests were performed in duplicate, and the interpretation of the results obtained was based on the breakpoints established by CLSI in document M100-S34 (7).

2.3. Synergism evaluation - *checkerboard*

The antimicrobial activity of the drug associations was evaluated in duplicate using the checkerboard method, as described by Eliopoulos *et al.* Combinations were made between the antimicrobials, using all associations among the four antimicrobials to evaluate synergistic action. The tests were performed in duplicate, and to analyze the synergistic effect, the Fractional Inhibitory Concentration Index (FICI) was used, with $FICI = (MIC \text{ of antimicrobial A in combination} / MIC \text{ antimicrobial A in monotherapy}) + (MIC \text{ of antimicrobial B in combination} / MIC \text{ of antimicrobial B in monotherapy})$. The FICI result was classified as follows: $FICI \leq 0.5$: synergism; $0.5 < FICI \leq 4$: indifferent; $FICI > 4$: antagonism.

2.4. Rapid methodology: Broth Disk Elution (BDE)

The concentrations used in the broth disk elution test (BDE) were therapeutic concentrations for a standard dose, as standardized by the recommendations for intravenous regimens, according to BrCAST (9). In standard doses, peak is considered the highest expected serum concentration for the drug, and trough is the lowest expected serum concentration for the drug. Based on the determination of the isolates' MICs, only the isolates that presented MICs higher than the trough of both antibacterials used in combination were selected for the disk elution test. For the recommended therapeutic doses, we obtained the peak and trough values for each antimicrobial as described below: meropenem - 2g/day in a single dose (peak: 40.9 µg/mL; trough: 4.3 µg/mL), polymyxin B - 25,000 IU/Kg/day (peak: 6.44 µg/mL; trough: 2.4 µg/mL), fosfomycin - 8g q8h (peak: 395 µg/mL; trough: 25 µg/mL), and tigecycline - initial dose 100 mg followed by 50 mg q12h (peak: 1.5 µg/mL; trough: 0.13 µg/mL).

Each antimicrobial disk has a distinct concentration based on its use in disk diffusion. Therefore, the preparation of each antimicrobial solution was performed 18 to 20 hours before the experiment in MHB-CA so that the drug's concentration was 3x higher than desired. The possible combinations were tested according to the following description: Combination 1: antimicrobial A peak + antimicrobial B peak; Combination 2: antimicrobial A peak + antimicrobial B trough; Combination 3: antimicrobial A trough + antimicrobial B peak; Combination 4: antimicrobial A trough + antimicrobial B trough.



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In a 96-well microplate, antimicrobial solutions were pipetted into each row as follows: 50 μ L of pure antimicrobial A, 50 μ L of pure antimicrobial B, and 50 μ L of inoculum (prepared according to item 2.2). After this, the microplate was incubated at 37°C for 4 hours. Then, 22 μ L of 0.05% resazurin solution was added to each well, and the microplate was returned to incubation, with results read within 1 hour. The color change of the resazurin, from blue to pink, indicates bacterial growth. These BDE tests were performed in duplicate.

3. RESULTS

The molecular characteristics of the five bacterial isolates used in the study, as well as the Minimum Inhibitory Concentrations (MIC) for the antibacterials used for synergism, are in Figure 01.

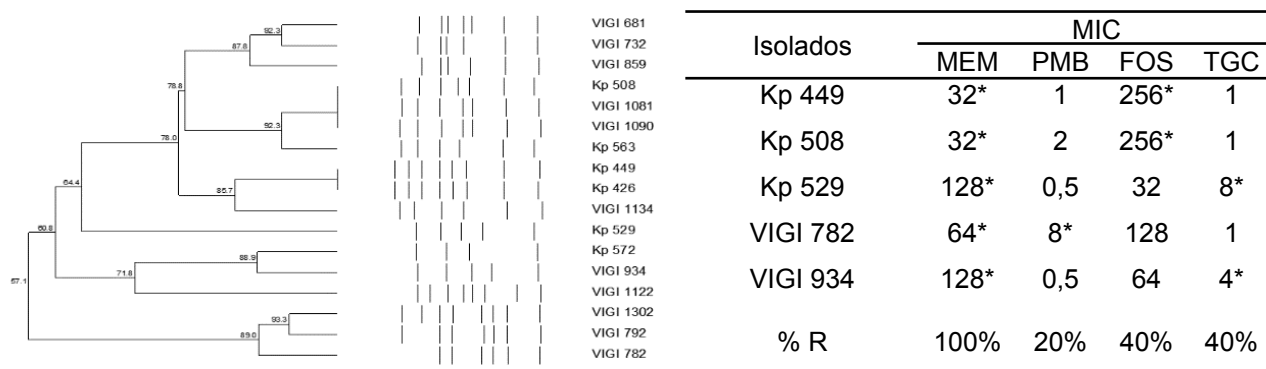


Figure 1. Molecular characteristics of the five selected isolates and MIC for the antimicrobials used in the synergism tests. MIC: Minimum Inhibitory Concentration. S (Susceptible, standard dosing regimen); I (-Susceptible, increased exposure); R (resistant); MEM-Meropenem (S $\leq$ 1, I=2, R $\geq$ 4); PMB-Polymyxin B (S $\leq$ 2, R $\geq$ 4); FOS-Fosfomycin (S $\leq$ 64, I=128, R $\geq$ 256); TGC-Tigecycline (S $\leq$ 1, R $\geq$ 2); * indicates resistance.

The highest MIC for polymyxin B was 8 μ g/mL, which was the only isolate with an MIC greater than the trough of polymyxin B, making it the only one to be tested in combinations involving this drug in the disk elution test. All isolates were resistant to meropenem. The results obtained by the checkerboard and the Fractional Inhibitory Concentration Index (FICI) are described in Table 1.

Table 1. Results obtained from the *in vitro* combination of antimicrobials in *Klebsiella pneumoniae* isolates using the checkerboard method.

Isolate	FICI (*)					
	MEM+PMB	MEM+FOS	MEM+TGC	PMB+FOS	PMB+TGC	FOS+TGC
Kp 449	0,75 (I)	0,1875 (S)	0,75 (I)	0,156 (S)	0,5 (S)	0,156 (S)
Kp 508	0,375 (S)	0,3125 (S)	0,75 (I)	0,09 (S)	0,375 (S)	0,75 (I)
Kp 529	1 (I)	0,1562 (S)	0,375 (S)	0,75 (I)	0,312 (S)	0,265 (S)
VIGI 782	0,1562 (S)	0,3125 (S)	0,75 (I)	0,625 (I)	0,265 (S)	0,312 (S)
VIGI 934	0,5312 (I)	0,1875 (S)	1 (I)	0,625 (I)	0,132 (S)	0,75 (I)
S%	40%	100%	20%	40%	100%	60%

MER: meropenem; PMB: polymyxin B; FOS: fosfomycin; TGC: tigecycline; FICI (*): Fractional Inhibitory Concentration Index and effect produced; S: synergism; I: indifference; S %: Percentage of isolates that showed synergism with the combination.

Based on the checkerboard method, the combinations of fosfomycin with meropenem, and tigecycline with polymyxin B showed the highest synergistic activity. Regardless of the presence of synergism, a reduction in the MIC was observed for all isolates and combinations. No cases of antagonism were found in our study between the associations.



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For the disk elution tests, only isolates with an MIC higher than at least the trough concentration of both antibacterials in the combination were used. Therefore, for all combinations involving polymyxin B, only the VIGI 782 isolate was used. For the other combinations, all isolates were tested. The results are shown in Table 2.

Table 2. Broth disk elution results according to the antimicrobial combination

Meropenem and Polymixin B										
Isolate	Monotherapy				Combination				Checkerboard	BDE
	MP	PT	PP	PT	MP+PT	MT+PP	MP+PP	MT+PT		
VIGI 782 Agreement	-	+	+	+	-	-	-	+	S	S 100%
Meropenem and Fosfomycin										
Isolate	Monotherapy				Combination				Checkerboard	BDE
	MP	MT	FP	FT	MP+FT	MT+FP	MP+FP	MT+FT		
Kp 449	+	+	-	+	-	-	-	-	S	S
Kp 508	-	+	-	+	-	-	-	-	S	S
Kp 529	+	+	-	-	-	-	-	-	S	I
VIGI 782	-	+	-	+	-	-	-	-	S	S
VIGI 934 Agreement	+	+	-	+	-	-	-	-	S	S 80%
Meropenem and Tigecycline										
Isolate	Monotherapy				Combination				Checkerboard	BDE
	MP	MT	TP	TT	MP+TT	MT+TP	MP+TP	MT+TT		
Kp 449	-	+	+	+	-	+	-	+	I	I
Kp 508	-	+	+	+	-	+	-	+	I	I
Kp 529	+	+	+	+	+	+	+	+	S	I
VIGI 782	-	+	+	+	-	+	-	+	I	I
VIGI 934 Agreement	+	+	+	+	+	+	+	+	I	I 80%
Polymixin B and Fosfomycin										
Isolate	Monotherapy				Combination				Checkerboard	BDE
	PP	PT	FP	FT	PP+FT	PT+FP	PP+FP	PT+FT		
VIGI 782 Agreement	+	+	-	+	-	-	-	-	S	S 100%
Polymixin B and Tigecycline										
Isolate	Monotherapy				Combination				Checkerboard	BDE
	PP	PT	TP	TT	PP+TT	PT+TP	PP+TP	PT+TT		
VIGI 782 Agreement	+	+	+	+	-	-	-	-	S	S 100%
Fosfomycin and Tigecycline										
Isolate	Monotherapy				Combination				Checkerboard	BDE
	FP	FT	TP	TT	FP+TT	FT+TP	FP+TP	FT+TT		
Kp 449	+	+	+	+	-	-	-	+	S	S
Kp 508	-	+	+	+	-	-	-	-	I	S
Kp 529	-	-	+	+	-	-	-	-	S	I
VIGI 782	-	+	+	+	-	-	-	+	S	S
VIGI 934 Agreement	-	+	+	+	-	-	-	-	I	S 40%

MP: Meropenem Peak; MT: Meropenem Trough; PP: Polymyxin B Peak; PT: Polymyxin B Trough; FP: Fosfomycin Peak; FT: Fosfomycin Trough; TP: Tigecycline Peak; TT: Tigecycline Trough; +: Indicates bacterial growth; -: Indicates no bacterial growth; Agreement: Percentage of agreement between checkerboard and the results; BDE: Broth disk elution method; S: Synergism; I: Indifferent.

All BDE tests involving polymyxin B associations showed 100% agreement with checkerboard method. The associations of meropenem and fosfomycin, meropenem and tigecycline showed 80%



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agreement with checkerboard, and fosfomycin and tigecycline presented agreement of 40% between their results and checkerboard.

4. DISCUSSION

The search for new therapeutic options has been a clinical challenge in the era of multidrug resistance, especially when the microorganism is *Klebsiella pneumoniae* carrying KPC and NDM (Kp/KPC+NDM). The present study used the broth disk elution (BDE) method with the use of resazurin, a simple, extremely rapid, and easy-to-perform method for detecting synergism of clinically used antibacterials at therapeutic concentrations.

The nature of our study is innovative since it aims to facilitate the verification of synergism in Kp/KPC+NDM. To our knowledge, no publications on synergism using traditional methods (time kill curve or checkerboard) or BDE have been conducted with therapeutic doses against Kp/KPC+NDM. Therefore, our discussion will be based primarily on the importance of the broth disk elution method for clinicians, and with limited possibilities for direct comparisons regarding the presence or absence of synergism for BDE.

The association of meropenem+fosfomycin was also tested by AL-Quraini and coworkers, with a synergism rate of 94%, however, their isolates produced only one carbapenemase (4). On our study, this association against Kp/KPC+NDM was great, with 100% synergism, and our BDE test was able to detect this synergism in just 5 hours, with 80% agreement with checkerboard. On the other hand, for our Kp/KPC+NDM isolates, the association of polymyxin B+tigecycline also had 100% synergism, while Kuai and coworkers had a 75% synergism rate for *K. pneumoniae* isolates producing KPC or NDM (10). For this combination, the proposed BDE was also able to detect synergism in 5 hours, with 100% agreement with checkerboard.

Based on the data from our study, we reinforce the synergistic activity of the associations between fosfomycin+meropenem, and between polymyxin+tigecycline. This is particularly notable as the isolates used in our tests were Kp/KPC+NDM, differently from the studies from AL-Quraini and coworkers, and Kuai and coworkers, which used *K. pneumoniae* producing only one carbapenemase (4, 10). In addition, we saw that BDE was able to detect synergism with 80% and 100% agreement with checkerboard results for these 100% synergistic associations in our study, while being less expensive and much faster, with results in just 5 hours. It is important to highlight that for all the antibacterials we used, despite maintaining antimicrobial activity in monotherapy, it is recommended that they are used in combination when the target is carbapenemase-producing bacteria, thus avoiding the possibility of resistant mutants (11).

To our knowledge, a rapid method for the detection of synergism using disk elution has been described only by Collar and coworkers. While they also used Kp/KPC+NDM, among other isolates, their study evaluated synergism between aztreonam/ceftazidime-avibactam, antibacterials that have a much higher cost (12). Additionally, they did not evaluate therapeutic doses. Collar and coworkers reported a 98% agreement between BDE and checkerboard, whereas in our study, we obtained agreements of 100% for combinations using polymyxin B, 80% for the meropenem+fosfomycin and meropenem+tigecycline combinations, and 40% for the fosfomycin+tigecycline combination. All these antimicrobials are simple, with previously detected activity, and are easily accessible, as they are already available to clinicians at our study hospital (HUM).

Furthermore, we observed that all associations involving polymyxin B had 100% agreement with the checkerboard method. For this association, only VIGI 782 was used, as it was the only isolate that had a higher MIC than the polymyxin B trough concentration. Rapid disk elution tests have already been reported for detecting the susceptibility or resistance of Gram-negative bacilli to polymyxin B, showing 90% agreement between their proposed test and the standardized method: broth microdilution (13).

The data obtained from our difficult-to-treat isolates, Kp/KPC+NDM, showed that the BDE method had a 100% agreement for combinations involving polymyxin B, and 80% for 2 associations and 40% for only 1 association. Thus, it is believed that, based on this research, BDE has proven to be an easily applicable method for the rapid, cost-effective, and simple detection of synergism.

For future studies and perspectives, we suggest using a time kill curve to analyze therapeutic concentrations and their associations to evaluate synergism, which would facilitate the comparison of results with BDE. Additionally, we also suggest a larger sample size with isolates from other bacterial species to demonstrate that the BDE method is comprehensive and not limited to Kp/KPC+NDM.



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5. CONCLUSION

Considering the results observed in our study, with *K. pneumoniae* isolates concomitantly producing KPC and NDM, and with the tested antimicrobial associations using therapeutic concentrations, we found that the BDE proposed in this study proved to be a method with high agreement, easy to perform for the detection of synergism, and rapid, with results in up to 5 hours, and can be easily implemented in routine and hospital laboratories.

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Keywords: *Klebsiella pneumoniae*; carbapenemase; drug synergism.

Área temática: Patógenos de interesse médico.

SYNERGISTIC EFFECT OF PHOTOCHEMOTHERAPY WITH CISPLATIN AND HYPERICIN ON CERVICAL CANCER CELLS

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1. INTRODUCTION

Cervical cancer is the most common gynecological cancer worldwide. Despite preventive measures based on HPV vaccination and population screening, in 2022, there were approximately 660,000 new cases and 350,000 deaths, representing the fourth most common cancer and the fourth leading cause of cancer death among women worldwide (1). In countries with low income, the situation is even more severe due to the lack of access to cervical cancer prevention, screening, and treatment. There are two main types of cervical cancer: invasive squamous cell carcinoma, mainly associated with persistent HPV16 infection and accounting for 70% of all cases; and adenocarcinoma, which accounts for about 25% of cervical cancer cases, often associated with HPV18, presenting a worse prognosis and a higher frequency of metastases (2).

The treatment of invasive cervical cancer depends on its stage of progression and currently involves surgery, radiotherapy, and chemotherapy. Chemotherapy plays an important role, especially in locally advanced, metastatic, or recurrent cervical cancer, with Cisplatin being the standard chemotherapeutic agent. However, Cisplatin-based treatment is associated with many side effects, ranging from malaise to neutropenia and nephrotoxicity (3). In addition, the effectiveness of current chemotherapy-based therapies is limited by drug resistance (4).

The use of drug combinations to achieve multiple targets, maximize therapeutic results, and overcome resistance to chemotherapy has been the basis of modern cancer treatment. In this context, the combination of photodynamic therapy (PDT) and chemotherapy could increase the effectiveness of antitumor action (5). PDT is a minimally invasive approach that utilizes a photosensitizer, light at an appropriate wavelength, and oxygen, which together initiate a photochemical reaction that culminates in the generation of a highly reactive product called singlet oxygen (1O_2) (6). Among the most studied photosensitizing agents, Hypericin stands out as a natural product isolated from St. John's wort (*Hypericum perforatum*), which has shown antitumor efficacy through PDT in different cancer cell lines and experimental animal models (7).

Although promising, Hypericin is highly hydrophobic, which increases the tendency for self-aggregation after administration, leading to a decrease in its photodynamic potential. This limitation has been overcome with the use of drug delivery systems based on poloxamers, such as Pluronic, which can solubilize and stabilize hydrophobic drugs through nanosystems (8). Thus, nanosystems based on Pluronic have great potential for the development of drug delivery platforms, and Pluronic 123 (P123) was chosen for use in this work, continuing the studies previously developed in our laboratory (9).

Despite the potential of photodynamic therapy to overcome the limitations of monotherapy in cancer treatment, to the best of our knowledge, no studies have reported the simultaneous or sequential administration of Hypericin and Cisplatin for the treatment of cervical cancer. In this study, we aimed to investigate the effect of combining Cisplatin with Hypericin-mediated PDT *in vitro*. For this purpose, we evaluated different treatment regimens and the use of P123 micelles for combination treatment with



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Hypericin and Cisplatin in the two main types of human cervical cancer cells, compared to a non-tumor cell line.

2. MATERIAL AND METHODS

2.1. Preparation of Hypericin and Cisplatin in Pluronic 123 micelles

Cisplatin (Cis-Diammineplatinum(II) dichloride, 99.9%, Merck-Sigma-Aldrich) 9.0 mg was solubilized in 10.0 mL of phosphate-buffered saline (PBS, pH 7.4) to obtain a stock solution of $3.0 \times 10^3 \mu\text{mol/L}$. Hypericin (1,3,4,6,8,13-hexahidroxy-10,11-dimethylphenanthro[1,10,9,8-opqra]perileno-7,14-diona, 98%, Chongqing Xingcan Pharmaceutical Technology Co.) was nanoencapsulated within colloidal copolymeric micelles of Pluronic 123 (Merck-Sigma-Aldrich) following the solid dispersion method (10) to obtain a final formulation of $100 \mu\text{mol/L}$ Hyp/0.025% P123. Finally, the micelles of Hypericin-Cisplatin and P123 were also obtained by solid dispersion in formulations comprising $20 \mu\text{mol/L}$ of Hypericin/ $100 \mu\text{mol/L}$ of Cisplatin/0.025% P123. To reach the final concentrations, the formulations were diluted in Dulbecco's modified Eagle's medium (DMEM) immediately before the *in vitro* experiments.

2.2. Light irradiation source

PDT was performed using a light source comprising 33 light-emitting diode units, emitting warm white light with maximum emission wavelengths at 450 and 580 nm, with an integrated power of 4.1 mW/cm^2 and providing a photon energy dose of 7.4 J/cm^2 , in 30 minutes of exposure. To prevent any interference, all irradiation tests were performed in a dark environment.

2.3. Cell lines and culture conditions

The cell lines SiHa (cervical carcinoma with HPV16 DNA integrated), HeLa (cervical adenocarcinoma with HPV18 DNA integrated), and HaCaT (spontaneously immortalized human keratinocytes) were cultured in DMEM enriched with 10% fetal bovine serum, 100 U/mL penicillin and streptomycin, and $2.5 \mu\text{g/mL}$ amphotericin B. The cells were maintained in a 5% CO_2 atmosphere, with 95% relative humidity, at 37°C .

2.4. Monotherapies and combined therapies

The cells were seeded at 2.5×10^5 cells/mL into 96-well plates and incubated overnight. Then, they were exposed only to Cisplatin or PDT with Hypericin/P123 to determine the appropriate individual concentrations to be evaluated in combination therapy. Next, combined therapies were performed under three conditions: Cisplatin followed by Hypericin/P123-PDT (Cis-Hyp+light), Hypericin/P123-PDT followed by Cisplatin (Hyp+light-Cis), and finally, the simultaneous administration of Hypericin and Cisplatin loaded in P123 (Nano Hyp/Cis+ light), as summarized in Table 1.

Table 1. Treatment regimen for Hypericin and Cisplatin in monotherapies and combination therapies.

Groups	Hyp (μM)	Cis (μM)	Incubation time	Light dose
Hyp alone	0.1-12.5	0	90 min	0 or 7.4 J/cm^2
Cis alone	0	2.5-62.5	24 h	0
Sequential therapy	0.5	2.5 or 12.5	90 min (Hyp), 24 h (Cis)	0 or 7.4 J/cm^2



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Simultaneous therapy	0.5	2.5 or 12.5	24 h	0 or 7.4 J/cm ²
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Hyp: Hypericin in Pluronic 123 micelles; Cis: Cisplatin

2.5. Cytotoxicity and phototoxicity assays

Cell viability was assessed by the Methylthiazol tetrazolium (MTT) assay. After the treatments, the cells were washed with PBS, 50 µL of MTT (2 mg/mL) was added to each well and incubated for 4 h in the absence of light. The resulting precipitate was dissolved in 150 µL of Dimethyl sulfoxide, and the absorbance was measured at 570 nm. The results were expressed as a percentage of viable cells, compared to cells exposed only to culture medium, which were considered 100% viable (11). The 50% inhibitory concentration (IC₅₀) was calculated based on a dose-response curve, applying a nonlinear regression.

2.6. Synergism assessment

The combination index (CI), based on the Chou-Talalay method (12), was calculated according to the equation:

$$CI = \frac{(D)_1}{(D_{50})_1} + \frac{(D)_2}{(D_{50})_2}$$

Where (D₅₀)₁ and (D₅₀)₂ are the individual concentrations of the drugs that are necessary to inhibit 50% of the cell viability. (D)₁ and (D)₂ indicate the concentrations of drugs used in combination to achieve the same result. Values of CI <, =, and > 1 represent synergism, additive, and antagonism effects, respectively.

2.7. Statistical analyses

The results were expressed as mean ± standard deviation (SD) of three independent experiments. Significant differences among means (p values < 0.05) were evaluated by multiple unpaired t tests with Holm–Šidák correction for multiple comparisons, using the software GraphPad Prism 8.0 (GraphPad, USA).

3. RESULTS

Cisplatin and PDT with Hypericin/P123 were evaluated as monotherapies to determine the optimal conditions to be employed in combination. The cell lines proved to be slightly sensitive to Cisplatin as a single agent (Figure 1, dotted lines). The concentrations of 2.5 and 12.5 µM of Cisplatin were selected for the combination treatments. The rationale for this choice was that 12.5 µM of Cisplatin as monotherapy maintained approximately 70–90% viability for the three cell lines, which provided an appropriate window to investigate whether additional agents could enhance its effect. Furthermore, the inclusion of the lower concentration (2.5 µM) was aimed at demonstrating the potential of combination therapy to increase efficacy at sub-effective doses.

Hypericin without light exposure did not significantly reduce cell viability (Figure 1, dotted lines). On the other hand, after PDT, cell viability decreased at the lower range of tested concentrations. At 0.5 µM, Hypericin/PDT reduced the viability of tumor cells to 17.5% and 31.5% for HeLa and SiHa cells, respectively (Figure 1A and B, dotted lines), maintaining the viability of the HaCaT control cells at 70.3% (Figure 1C, dotted line). Given these results, the concentration of 0.5 µM Hypericin was chosen for the combined treatments.

Combined treatments showed increased effects. Pretreatment with Hypericin/PDT followed by Cisplatin reduced the viability of HeLa cells to 5.5% (Figure 1A) and 8.9% for SiHa cells (Figure 1B), while HaCaT cells still had 10.5% viable cells (Figure 1C). In this case, the increase in Cisplatin dose did not result in a significant improvement. Pretreating with Cisplatin followed by Hypericin/PDT reduced cell viability up to 8.9%, 21.4%, and 62.9% for HeLa, SiHa, and HaCaT cells, respectively (Figure 1). The last approach



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evaluated was the simultaneous delivery of Hypericin and Cisplatin in P123 micelles, which resulted in viability of 11.3% for HeLa, 13.6% for SiHa, and 10.6% for HaCaT cells (Figure 1). These data showed that the Hyp+light-Cis regimen was significantly more effective in reducing cell viability than the exposure to Cisplatin before or at the same time as Hypericin using P123 micelles.

Because it proved to be the most promising, the Hyp+light-Cis treatment regimen was chosen for further investigation and used for synergy assessment. The IC₅₀ values of Hypericin/PDT and Cisplatin as monotherapies and combined in the Hyp+light-Cis regimen are described in Table 2. All the Combination Indices were lower than 1, which indicates a synergistic effect between Hypericin/PDT and Cisplatin in decreasing cell viability.

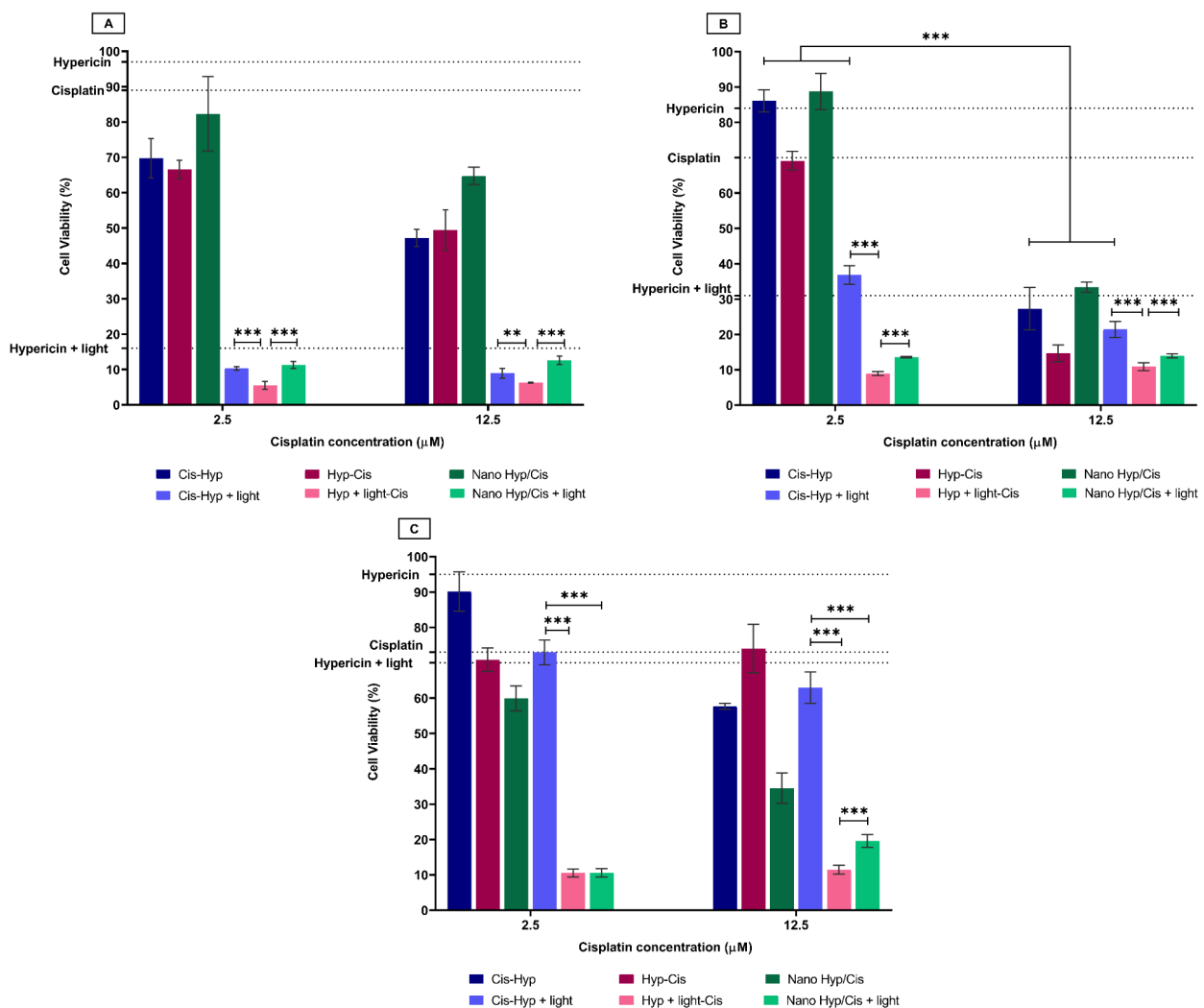


Figure 1. Cytotoxic effects of 12.5 μM Cisplatin (dotted lines), 0.5 μM Hyp/P123 with or without light (dotted lines), and combinations of Hyp/P123-PDT with Cisplatin (bars) on HeLa (A), SiHa (B), and HaCaT (C) cells. Each bar represents the mean ± SD of three independent experiments conducted in triplicate. Cis: Cisplatin; Hyp: Hypericin in Pluronic 123 micelles; Nano Hyp/Cis: Hypericin and Cisplatin in P123 micelles; **p < 0.01, ***p < 0.001.

Table 2. The 50% Inhibitory Concentration (IC₅₀) and the Combination Index (CI) calculated for the cervical cancer and control cell lines under monotherapies and Hyp+light-Cis combination treatment

Cell line	Hyp/PDT IC ₅₀ (μM)	Cis IC ₅₀ (μM)	Cis IC ₅₀ (μM) after Hyp 0.5 μM/PDT	CI
HeLa	2.0	38.5	1.7	0.3



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SiHa	3.4	40.7	1.8	0.2
HaCaT	4.7	36.6	1.8	0.1

Hyp: Hypericin in Pluronic 123 micelles; Cis: cisplatin.

4. DISCUSSION

The present study investigated the effect of combining Cisplatin with Hypericin-mediated PDT *in vitro* and assessed possible synergistic interactions. Different treatment regimens were evaluated, including the use of P123 micelles for simultaneous drug delivery, against cervical cancer cell lines and a non-tumor cell line. Overall, the results provide evidence that the combination treatment, especially when Hypericin/PDT is applied before Cisplatin, exerted synergistic antitumor effects.

Cisplatin is an alkylating antineoplastic agent used to treat several malignancies, including cervical cancer. Despite its anticancer activity, Cisplatin possesses serious dose-limiting side effects and is non-selective for tumor cells. De Freitas *et al.* (13) reported that HaCaT cells may be even more sensitive to Cisplatin than cervical cancer cell lines, which is consistent with the data found in our study. In this sense, the combination therapy was evaluated as an alternative to reduce the doses of Cisplatin, benefiting from its therapeutic effects while reducing adverse effects. Hypericin-mediated PDT was chosen to be used in this combination because of its effectiveness against cervical cancer cells in a selective and dose-dependent manner, as previously described by our research group (9) and confirmed in the results obtained in this study, which showed the absence of toxicity without light irradiation and the preservation of the viability of non-tumor control cells.

We evaluated combinations of Hypericin-mediated PDT with Cisplatin in an extension of modalities. We found that the optimal combination treatment was Hyp+light-Cis at 2.5 μ M of Cisplatin. This regimen resulted in a drastic reduction in the viability of both cancer-derived cell lines. It achieved similar results compared to Hyp+light-Cis at 12.5 μ M, indicating that the combination allows the use of lower doses of chemotherapeutic drugs to achieve satisfactory results. The Hyp+light-Cis regimen was significantly more effective in reducing cell viability than the application of Cisplatin before or at the same time as Hypericin, which is consistent with other studies on cervical cancer cells that also demonstrated that the sequence of treatment administration influenced the response and efficacy of combination therapies (13).

In an innovative strategy, we applied a nanosystem for the simultaneous delivery of Hypericin and Cisplatin for antitumor treatment. Pluronic 123 has been one of the most promising polymers for targeting and controlling drug delivery. It has been used as a pharmaceutical ingredient, demonstrating safety and non-toxicity in cells and animal models (14). Although it has the potential to deliver chemotherapeutic and photoactive drugs, modulating their physicochemical properties, in our study, the simultaneous delivery did not demonstrate advantages in terms of cytotoxic effect. In addition, the concurrent administration does not allow for the exploitation of different routes of administration or exposure times for the two components, which have been shown to play an important role in the treatment outcome.

Synergism was evaluated when Hypericin/P123-PDT was followed by Cisplatin (Hyp+light-Cis). Under these conditions, CI showed a synergistic effect between Hypericin/PDT and Cisplatin, demonstrating that the combined effects are greater than expected from the sum of their individual effects. This may enhance therapeutic efficacy, allow dose reduction, minimize toxicity, and overcome mechanisms of drug resistance. Theodossiou *et al.* (15) have reported a strong synergistic effect between Hypericin/PDT and Tamoxifen against breast cancer cells, showing that the synergistic modality with traditional cancer treatments can lay the foundation for new anticancer therapies in various types of tumors.

Regarding the different types of cervical cancer cell lines, HeLa cells showed the highest sensitivity to the combined therapy, highlighting the promising potential of this combination to treat cervical adenocarcinoma, which is more aggressive and presents a poorer prognosis than squamous cell carcinoma (16).

5. CONCLUSION

Hypericin-mediated PDT in combination with Cisplatin effectively reduced the viability of cervical cancer cells compared to monotherapies. Our findings suggest a synergistic and sequence-dependent



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strategy for targeting cervical cancer cells, with Hypericin/P123-PDT followed by Cisplatin being more effective. These results reinforce the therapeutic potential of combining PDT with lower doses of Cisplatin, potentially reducing side effects, and support the need for further investigation to understand its mechanism of action and evaluate its effects *in vivo*.

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Keywords: cervical cancer; photochemotherapy; combined modality therapy.

Thematic Area: Etiopathophysiology of human and animal diseases

AUTOIMMUNE DISEASES AND THEIR RELATIONSHIP WITH THE INTESTINAL MICROBIOTA

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1. INTRODUCTION

Autoimmune diseases affect approximately 5% to 8% of the world's population and encompass a spectrum of 80 to 100 different clinical conditions (1). The etiology of these disorders results from a complex interaction among genetic predisposition, environmental factors, and alterations in the gut microbiota (2). Among autoimmune diseases are rheumatoid arthritis (RA), spondyloarthritis (SpA), systemic lupus erythematosus (SLE), and Sjögren's syndrome (SS).

Patients with autoimmune diseases often present intestinal dysfunction, with increased epithelial permeability facilitating bacterial translocation. The presence of bacteria or their products in the circulation confirms this translocation, which may activate pro-inflammatory immune responses and exacerbate disease progression (3). In this context, the intestinal microbiota plays a fundamental role in essential biological processes such as metabolic interactions and host immune responses (4–5).

Intestinal dysbiosis, characterized by alterations in microbial composition, has been associated with autoimmune diseases through mechanisms such as exacerbated activation of innate and adaptive immune responses, molecular mimicry, dysregulated immune activation, and autoantibody production (6).

Mousa et al. (7) provide several examples of microorganisms associated with autoimmune diseases. The genus *Prevotella*, for instance, has been linked to increased inflammation in rheumatoid arthritis due to its ability to stimulate pro-inflammatory Th17 responses, whereas *Akkermansia muciniphila* has been studied for its regulatory potential, influencing multiple sclerosis by modulating immune responses and reducing disease severity. The reduction of *Faecalibacterium prausnitzii*, an anti-inflammatory bacterium, has been related to a higher incidence of inflammatory bowel diseases, including Crohn's disease. Finally, *Bacteroides thetaiotaomicron* contributes to the regulation of host immune responses, and its dysbiosis has been implicated in the development of autoimmunity in conditions such as type 1 diabetes.

The identification of specific gut microorganisms consistently depleted or enriched in disease states across different populations suggests a pathway for future interventions or therapies, as well as for addressing causality (8). Evidence from the literature highlights how alterations in microbial composition can trigger or modulate autoimmune responses in the body. However, this research gap remains unresolved, and individual studies are not always sufficient to address it. Therefore, this systematic review evaluated the gut microorganisms commonly associated with autoimmune diseases and their relationship with immunological markers, contributing to the development of therapeutic strategies targeting the gut–immunity axis.



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2. MATERIALS AND METHODS

This systematic review was conducted in accordance with the PRISMA guidelines (9) (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and its protocol was registered in the PROSPERO platform under the number CRD42025645006 (<https://www.crd.york.ac.uk/PROSPERO/view/CRD42025645006>).

A systematic search was performed in the PubMed, Web of Science, Scopus, Embase, Virtual Health Library (VHL), Cochrane Library, and Research for Life (R4F) databases. The search strategy was constructed based on a combination of descriptors and MeSH terms involving autoimmune diseases and gut microbiota. For example, the PubMed strategy combined two blocks of terms: block 1: Arthritis, Rheumatoid OR Spondylarthritis OR Sjögren's Syndrome OR Lupus Erythematosus, Systemic and block 2: Metagenome OR RNA, Ribosomal, 16S OR Dysbiosis OR Gastrointestinal Microbiome. Searches were conducted in April 2024, restricted to human studies published in English.

We included observational studies (cross-sectional, case-control, cohort) and clinical trials that analyzed the baseline gut microbiota profile of adults using exclusively 16S rRNA sequencing, metagenomics, or alpha and beta diversity measures, along with immunological assessment of blood samples (T and B lymphocytes, cytokines, autoantibodies), and taxonomic identification up to the species level. Excluded were studies involving children or animals, interventions without baseline profiling, non-original publications, and studies without clear methodology or species characterization.

The initial screening was carried out by a group of researchers (Gessilda, Jorge, Magali, and Karla), with disagreements resolved by consensus. Study screening was conducted in two phases: initial reviewers (Karla, Magali, Amanda, Ueslei, Angela, and Daniele) performed title, abstract, and keyword screening, followed by full-text reading (PDF). Final eligibility of the articles was validated by a judging committee composed of the other reviewers (Danieli, Gessilda, Felipe, Bianca, and Áquila).

Data from the selected studies were extracted and systematically organized according to population and sampling characteristics; methodologies employed for microbiota analysis (16S rRNA sequencing, metagenomics, and alpha/beta diversity metrics); identified microbial species; and assessed immunological biomarkers.

The tools used were STROBE and CONSORT, the latter applied only to clinical trial studies.

3. RESULTS

A total of 1,460 articles were initially retrieved from the database search. After applying the inclusion and exclusion criteria, 15 studies were included, focusing on immunity and gut microbiota in rheumatoid arthritis (RA) (10–15), spondyloarthritis (SpA) (16–17), systemic lupus erythematosus (SLE) (18–21), and Sjögren's syndrome (SS) (22–24), as illustrated in Figure 1 of the PRISMA flow diagram.

Regarding study location, slightly more than 50% of the studies were conducted in China, 20% in the USA, 6.6% in Spain, and 6.6% in Brazil. The studies were published between 2018 and 2024. Most study designs were cross-sectional, with equal representation of case-control studies and clinical trials, and one cohort study was included.

Based on data extraction from the articles, participants were divided into patients with autoimmune diseases (n = 977) and healthy controls (n = 695). All patients met the diagnostic criteria for each respective disease: ACR/EULAR (combined criteria of the American College of Rheumatology and the European League Against Rheumatism), SLEDAI (Systemic Lupus Erythematosus Disease Activity Index), ASAS (Assessment of Spondyloarthritis Symptoms), ACR (American College of Rheumatology), and SLICC (Systemic Lupus International Collaborating Clinics classification criteria for lupus).

Participants were exclusively female, with variation in age and additional variables, including lifestyle, comorbidities from other ongoing conditions, preexisting autoimmune diseases, and the use of immunosuppressive medications.

Fecal sample processing and analysis of gut microbiota composition were primarily performed using 16S ribosomal RNA sequencing, with operational taxonomic units (OTUs) as the standard approach in the majority of studies. Amplicon sequence variants (ASVs) were reported in some studies on SpA, mostly targeting the V3–V4 regions. Microorganisms were presented in a hierarchical taxonomic order (family, phylum, genus, and species).

Results from patients with RA showed increased Actinobacteria and Proteobacteria and reduced Firmicutes and Fusobacteriota. Elevated genera included *Faecalibacterium*, *Blautia*, *Escherichia–Shigella*,



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and *Ruminococcus*, while reduced genera included *Bacteroides*, *Coprococcus*, *Lachnospiraceae*, *Prevotella*, *Clostridium*, *Roseburia*, and *Dialister* (10–11). Positive correlations were observed between *Megamonas* and *Monoglobus* with CD4⁺ T cell counts and pro- and anti-inflammatory cytokine levels (IL-2, IL-4, IL-6, IL-10, IL-17, TNF- α , IFN- γ), while negative correlations were noted for *Blautia* and *Ruminococcus* with T and B cell subpopulations (10–11). In the study by Qiao et al. (14), patients with a *Prevotella* enterotype exhibited a better response to methotrexate compared to those with a *Bacteroides* enterotype, indicating potential for personalized therapy.

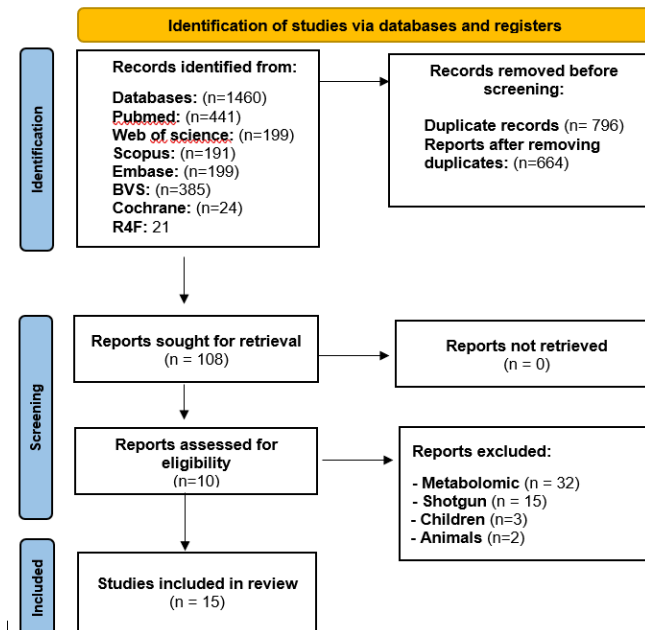


Figure 1. Prisma Flowchart

In the study by Liu et al. (16), patients with ankylosing spondylitis exhibited intestinal dysbiosis, characterized by increased phyla such as Cyanobacteria and Actinobacteriota, and reduced Proteobacteria and Verrucomicrobiota, was associated with increased serum levels of pro-inflammatory cytokines (IL-23, IL-17, IFN- γ). Intraepithelial lymphocytes (IELs) are T cells residing in the intestinal epithelium, where they recognize and eliminate pathogens and regulate tolerance to commensal antigens. Dysbiosis, or alterations in gut microbiota composition, can lead to IEL dysfunction. Normally, IELs help maintain epithelial homeostasis and respond in a regulated manner to pathogens and resident antigens. Aberrant activation of IELs results in increased TNF- α production, contributing to local inflammation and epithelial barrier dysfunction. A reduction in total IELs may also reflect cellular exhaustion due to continuous activation or systemic migration of immune cells between the mucosa and peripheral circulation, as suggested by the negative correlation between circulating lymphocytes and IELs in the mucosa in SpA (17).

In studies on SLE, intestinal dysbiosis was characterized by reduced microbial diversity and rapid microbial blooms, particularly of *Ruminococcus gnavus*, during nephritic flares, accompanied by a reduction in protective species such as *Bacteroides uniformis*. Additionally, increased intestinal permeability was observed through markers such as zonulin, fecal calprotectin, soluble CD14 (sCD14), and α 1-acid glycoprotein. Local immune responses included secretory IgA (sIgA), while systemic responses included IgG anti-*R. gnavus* and anti-DNA antibodies, which activated toll-like receptor 2 (TLR2), playing a critical role in innate immunity (19,21).

Xin et al. (22) and Cano-Ortiz et al. (24) showed that patients with primary Sjögren's syndrome (pSS) exhibited intestinal dysbiosis characterized by reduced microbial diversity and richness, decreased butyrate-producing bacteria (*Roseburia*, *Coprococcus*, *Faecalibacterium*), and increased opportunistic pathogens (*Escherichia*, *Prevotella*, *Enterobacter*). These changes were associated with higher production of pro-inflammatory cytokines (IL-6, IL-17, TNF- α), increased intestinal permeability (zonulin), and reduced FOXP3 expression, which is essential for the development and function of regulatory T cells (Tregs) that



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suppress immune responses. The loss of anti-inflammatory metabolites, such as butyrate, contributed to Th17/Treg imbalance and systemic inflammation in pSS.

4. DISCUSSION

Intestinal dysbiosis may precede the clinical onset of rheumatoid arthritis (RA), acting as a relevant environmental modulator in its pathogenesis. Recent studies have shown that individuals in the pre-clinical phase exhibit an increased abundance of *Prevotella spp.*, supporting the hypothesis that microbiota alterations precede the emergence of autoimmunity (25). In contrast, other studies of patients with established RA have reported a reduction in butyrate-producing bacteria and an expansion of microbes that consume this metabolite. This microbial imbalance compromises the anti-inflammatory effects of butyrate, whose mechanisms include balancing T-cell subpopulations and reducing autoantibody production through both epigenetic pathways and gene expression modulation (26–27).

In patients with spondyloarthritis (SpA), other reports highlight the expansion of barrier-protective lymphocyte populations, including intraepithelial lymphocytes (IELs) in the intestinal mucosa. These findings underscore the role of IELs in maintaining homeostasis and illustrate how their pathogenic activation in the context of dysbiosis contributes to inflammatory activity (28).

Similarly, in systemic lupus erythematosus (SLE), Hevia et al. (29) demonstrated reduced microbial diversity and increased abundance of *Ruminococcus*, associated with the presence of autoantibodies and disease activity. Complementing these findings, Manfredo et al. (30) showed that isolated *R. gnavus* strains were capable of inducing anti-DNA autoantibody production in murine models. The association between increased intestinal permeability and autoimmunity has also been emphasized: Fasano et al. (31) highlighted the role of zonulin in autoimmune diseases, including SLE, and Ma et al. (32) further reinforced this link by identifying elevated fecal calprotectin and soluble CD14 (sCD14) levels in patients with active disease.

In primary Sjögren's syndrome (pSS), Wang et al. (33) reported that reduced levels of short-chain fatty acids (SCFAs)—particularly butyrate—diminished both local and systemic anti-inflammatory regulation, favoring prolonged macrophage activation and sustained production of pro-inflammatory cytokines (IL-6, IL-17, TNF- α). This created a permissive environment for the immunological imbalance characteristic of pSS. The deficit of these metabolites ultimately led to a breakdown in immune tolerance and disease progression. Moreover, elevated serum zonulin, which has been linked to intestinal barrier dysfunction in RA and ankylosing spondylitis, suggests a common mechanistic pathway that may also be operative in pSS (34).

5. CONCLUSION

Intestinal dysbiosis was found to be closely associated with the immune system in autoimmune diseases, promoting an imbalance in inflammatory responses. Additionally, the loss of intestinal barrier integrity, accompanied by increased permeability, allows greater exposure of the immune system to microbial antigens. Our review reinforces the central role of the gut–immunity axis in the pathophysiology of autoimmune diseases and highlights the importance of therapeutic strategies aimed at restoring eubiosis and strengthening the intestinal barrier.

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Keywords: microbiome, autoimmune diseases, dysbiosis.

Thematic Area: Eco-epidemiology and disease control.



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LACTOFERRIN IN HUMAN MILK: BIOLOGICAL PROPERTIES AND QUANTIFICATION BY HPLC-DAD

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1. INTRODUCTION

Worldwide, 40% of newborns (under six months old) are exclusively breastfed (1). In Brazil, the prevalence of exclusive breastfeeding increased from 3% in 1986 to 45.8% in 2021, according to the National Infant Feeding and Nutrition Study (2). The World Health Organization (WHO) recommends that by 2025, at least 50% of children be exclusively breastfed, and that this percentage reach 70% by 2030 (3).

Exclusive breastfeeding can reduce infant mortality from preventable causes by up to 13% (3), an effect mainly attributed to the properties of human milk (HM), a highly complex fluid recognized as the ideal food for newborns and infants (1). Its dynamic composition adapts to the baby's needs according to the stage of lactation. In the first days after birth (up to about seven days), colostrum is secreted — a thick, yellowish fluid rich in proteins, immunoglobulins, cytokines, antimicrobials, and growth factors. This is followed by transitional human milk (between the seventh and the 14th day), which gradually gives way to mature milk (after 15 days), mainly responsible for meeting the infant's energy demands (4,5).

Among the bioactive components of HM, lactoferrin (LF) stands out for its multifunctional properties. It is a glycoprotein of the transferrin family, approximately 80 kDa in size, that binds iron and is present in all stages of lactation, with higher concentrations in colostrum and lower levels in mature milk (6). Depending on its saturation with iron, it can assume different structural forms, and these variations influence its thermal and enzymatic stability as well as its biological functions, including iron homeostasis, modulation of the immune system, and antimicrobial activity (7,8).

In addition to its role in iron metabolism, LF exhibits antimicrobial, antifungal, anti-inflammatory, immunomodulatory, anticancer, and antioxidant activities, being a multifunctional protein. It also contributes to intestinal barrier integrity by stimulating the proliferation of intestinal epithelial cells, cytokine production, and modulating immune responses (10). In recent years, LF has been used as an iron supplement, since it shares structural similarities with transferrin, exhibits high iron-binding affinity across a wide pH range, and retains these properties after heating. Due to its iron-binding capacity, LF is proposed to enhance intestinal iron absorption and improve hemoglobin production (11).

Several analytical methods are currently being improved and developed for the qualitative and quantitative analysis of human milk whey proteins, particularly LF, including immunological techniques such as enzyme-linked immunosorbent assay (ELISA), nephelometric immunoassays, and surface plasmon resonance (SPR)-based immunosensors. However, immunological methods lack reliable reproducibility and involve the high cost of specific antibodies (12). Therefore, chromatographic techniques, especially high-performance liquid chromatography (HPLC), are widely used due to their high reproducibility, good resolution, and ability to separate target proteins from complex mixtures of human milk whey.



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In this context, the present study aims to present the biological properties of LF and analyze this protein quantitatively, determining its concentration in the three stages of human milk lactation using HPLC coupled to a diode array detector (DAD).

2. MATERIALS AND METHODS

2.1. Ethics Committee Approval

The study was approved under the Certificate of Presentation for Ethical Consideration (CAAE) No. 93048818.0.0000.0104, integrated into the APLE-A-CNPq research group.

2.2. Milk Samples

Human milk samples from the three lactation stages—colostrum (0 to 6 days), transitional (7 to 14 days), and mature (above 15 days)—were collected at the Regional University Hospital of Maringá. The classification of lactation stages followed the guidelines of the Brazilian Health Regulatory Agency (ANVISA) (13). For this study, only raw human milk samples classified as presenting impurities and therefore not meeting quality control standards for distribution to infants were included. Samples that met standards for distribution were excluded. All included samples underwent no processing. The selected samples were transported under refrigeration and stored at -18°C until analysis.

2.3. Chromatographic Conditions

The lactoferrin content in human milk was determined by HPLC (Alliance e2695 model, Waters, USA), coupled to a DAD detector (2998 model, Waters, USA), at a wavelength of 280 nm, following the methodology described by FRUEH et al. (2024), with modifications.

The method included a calibration curve using human lactoferrin (hLF) (85% purity, Sigma-Aldrich, USA), prepared at concentrations of 5 ppm, 10 ppm, 50 ppm, 100 ppm, 300 ppm, 500 ppm, and 700 ppm in 1 mL dilutions with sodium phosphate dibasic buffer (Na_2HPO_4) at 28.4 g/L, adjusted to pH 8 with phosphoric acid.

Samples were prepared using 5 mL of human milk from each lactation stage, homogenized with 12 mL of sodium phosphate dibasic buffer for 60 min under vortex agitation at 200 rpm (Fisatom 761-5), and then centrifuged at 6000 rpm, 4°C for 20 min (Harrier 18/80 model, Sanyo MSE, Kent, UK). The supernatant from the intermediate fraction was collected and transferred to a 25 mL volumetric flask, completed with the same buffer. The prepared sample was filtered through a $0.22\ \mu\text{m}$ membrane and analyzed using the equipment.

The analysis was performed using a Nucleodur C8 column ($150 \times 4.6\ \text{mm}$, $5\ \mu\text{m}$ particle size) purchased from Macherey-Nagel (Düren, Germany), with an injection volume of $30\ \mu\text{L}$. The mobile phase consisted of 0.1% trifluoroacetic acid in water (A) and acetonitrile (B), with a linear gradient elution mode: 70% A : 30% B (0–5 min), 55% A : 45% B (5–10 min), 40% A : 60% B (10–12 min), 30% A : 70% B (12–14 min), and 70% A : 30% B (14–16 min). The flow rate was 0.5 mL/min, with a run time of 16 minutes, and chromatograms were monitored at 280 nm.

The results were calculated based on the coefficient of determination of the linear regression, showing adequate linearity with $R^2 > 0.9919$. The determination equation obtained was $y = 3601.2x - 64677$, which was used to calculate the LF content, expressed in g/L.

3. RESULTS

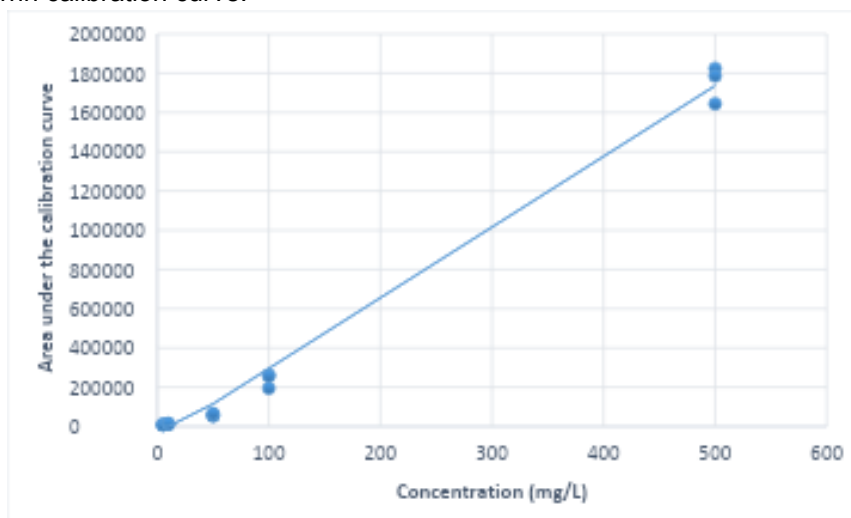
The lactoferrin content was determined based on a standard curve (5 to 500 mg/L) with $R^2 > 0.9919$ and the determination equation $y = 3601,2\ x - 64677$, as shown in Figure 1 of the calibration curve of lactoferrin analysis.



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Figure 1. Lactoferrin calibration curve.



The quantification results are presented in Table 1. Indicating differences in protein levels according to the lactation stage, ranging from 2,33 g/L (mature) to 2,90 g/L (transitional). These findings confirm the dynamic nature of human milk, whose composition is constantly modulated to meet the nutritional and immunological needs of the infant at each stage of development.

Table 1. Lactoferrin concentration (g/L) in human milk lactation stages using HPLC-DAD.

Samples	Lactoferrin content (g/L) ^a
Colostrum	2,85 ± 0,43
Transitional	2,9 ± 0,87
Mature	2,33 ± 0,16

^aMean ± Standard Deviation

4. DISCUSSION

The results obtained in this study are consistent with the values reported in the literature for LF content in human milk, generally ranging between 2,2 e 3,3 g/L (14). Previous studies also report similar behavior, indicating LF levels in preterm human milk of 3,16 g/L in colostrum, 3,29 g/L in transitional milk, and 1,03 g/L in mature milk (15). In terms of mothers, reported values were 6,7 mg/mL, 3,7 mg/mL, and 2,6 mg/mL for colostrum, transitional, and mature milk, respectively (16). Although variations exist among studies, the trend of decreasing LF throughout lactation is consistent and reflects the physiological adaptation of milk to the infant's nutritional and immunological demands. According to the literature, the highest LF concentration is usually observed in colostrum, when the demand for immunological and antimicrobial proteins is greatest, as the newborn undergoes adaptation to the extrauterine environment and presents immune immaturity (17).

The high concentration of LF at the beginning of lactation is directly associated with its biological functions, including antimicrobial, anti-inflammatory, and immunomodulatory activities, in addition to the regulation of iron metabolism (9). Even though its levels progressively decrease, LF remains functionally relevant in mature human milk, contributing to intestinal mucosa integrity, protection against infections, and support of immune system development. Studies indicate that this reduction can reach approximately 1 g/L in mature milk (18).

In addition to the lactation stage, maternal factors may also influence LF levels. For example, the intake of eggs, proteins, energy, cholesterol, and vitamin A has been associated with higher concentrations in mature milk. Moreover, regional differences also describe variations in LF content (19).). On the other



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hand, there is no evidence that the collection method (manual or pump) significantly alters its quantification, reinforcing the robustness of analyses regardless of the sampling procedure (20).

From a methodological perspective, the application of HPLC-DAD proved efficient and reproducible for LF quantification, being a robust alternative to immunological methods, which often present high costs and lower reproducibility. In the literature, different detectors have been used in association with HPLC, such as UV, fluorescence, diode array (DAD), and mass spectrometry (MS/MS), usually with C4 or C18 columns and mobile phases composed of water and acetonitrile, acidified with formic or trifluoroacetic acid (21–23). It is worth noting that the quantification of milk proteins, such as LF, often requires pre-treatment steps for the removal of fat and caseins (centrifugation, precipitation, or filtration), which may result in yield loss or reduced protein activity (24).

In summary, the results confirm the dynamic behavior of LF throughout lactation stages, highlighting its initial abundance as an immunological protection mechanism and its persistent importance during breastfeeding. Furthermore, this study demonstrates the feasibility of using HPLC-DAD as an accurate analytical tool, capable of generating reproducible data and comparable results across different application contexts.

5. CONCLUSION

The results evidenced the modulation of LF content throughout lactation stages, with higher concentrations at the beginning, especially in the transitional phase, reflecting the adaptation of human milk to the specific needs of the infant. Despite the reduction observed in mature human milk, LF remains an essential bioactive component, and its antimicrobial, anti-inflammatory, and immunoregulatory properties reinforce its relevance throughout breastfeeding. In addition, the methodology employed for LF quantification by HPLC-DAD proved sensitive, reproducible, and feasible, representing a robust alternative to immunological methods. Thus, the findings of this study contribute to advancing knowledge about the dynamic composition of human milk, provide support for the standardization of analytical methodologies, and strengthen the understanding of lactoferrin's role as one of the main bioactive factors involved in promoting infant health.

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EFFECTS OF EXTRACELLULAR VESICLES ON ESOPHAGUS, COLON AND HEART OF BALB/C MICE INFECTED WITH *Trypanosoma cruzi*

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1. INTRODUCTION

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is endemic in approximately 21 countries in the Americas, affecting approximately 6 million people and causing more than 12,000 deaths annually. Previously restricted to rural areas, its distribution has changed due to human mobility, reaching urban regions and other continents. The disease has an acute phase, and a chronic phase with cardiac and/or digestive complications. Pathophysiology involves an interaction between the immune response and parasitic factors (1) (3).

The heart is one of the main organs affected, with structural remodeling and dysfunction resulting from oxidative, immunological, and molecular mechanisms. In its digestive form, enteric neuropathy leads to dilation and dysfunction of the gastrointestinal tract, associated with neuronal loss and parasite persistence. The *T. cruzi*-host cell interaction stimulates the release of extracellular vesicles (EVs), which modulate infection and the immune response, with proteomic composition varying depending on the strain (2) (10).

Given the importance of the gastrointestinal tract as a reservoir for the parasite in the chronic phase (3) (11), this study evaluated the effect of EV administration on tissue damage caused by infection with two genetically distinct strains of *T. cruzi* (CL Brener and Dm28c). Murine myoblast (C2C12) and human intestinal epithelial (Caco-2) cell lines were used to obtain EVs, which were co-administered with the parasite in mice. Changes in the colon and heart 28 days after infection were analyzed to understand how different strains and cellular origins of EVs influence pathogenesis.

2. MATERIALS AND METHODS

2.1 Ethical Aspects

All procedures were previously approved by the Animal Use Ethics Committee of the State University of Maringá (CEUA 5864311022).

2.2 Induction and Purification of Extracellular Vesicles (EVs)

EVs were obtained from co-cultures of *Trypanosoma cruzi* tissue culture-derived trypomastigotes (TCTs; CL Brener and Dm28c strains) with C2C12 myoblasts or Caco-2 intestinal epithelial cells at a 5:1 ratio (parasites:cells), following (12). After 2 h of interaction in serum-free RPMI at 37 °C and 5% CO₂, the supernatants were sequentially centrifuged (500 × g for 5 min, 4,000 × g for 30 min, and 11,000 × g for 120 min at 4 °C) to isolate large EVs. Pellets were resuspended in PBS, and protein concentrations were determined using the Pierce-BCA assay.



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2.3 Experimental Design

Sixty-day-old male BALB/c mice were randomly allocated into six groups (n=5): an uninfected control group, one infected with *T. cruzi* only, and four groups co-inoculated with parasites and EVs derived from C2C12 or Caco-2 interactions. Inoculation was performed intravenously (penile vein) under isoflurane anesthesia with 1×10^5 trypomastigotes and/or 5 µg of EVs. Groups were designated as G1 (*T. cruzi* only), CLC2 (CL Brener + C2C12 EVs), CLCa (CL Brener + Caco-2 EVs), DmC2 (Dm28c + C2C12 EVs), and DmCa (Dm28c + Caco-2 EVs). Animals were housed under controlled temperature and light/dark cycles with food and water ad libitum.

2.4 Parasitemia and Clinical Signs

Parasitemia was quantified daily from day 4 to 27 post-infection by microscopic examination of tail vein blood (5 µL). Clinical signs such as skin lesions, alopecia, piloerection, inflammation, and other atypical manifestations were recorded daily. Body weight was measured every two days, and food consumption was calculated from the difference in food weight before and after feeding.

2.5 Euthanasia

On day 28, mice were euthanized under deep isoflurane anesthesia. Laparotomy was performed to collect heart, colon, and esophagus samples. The heart was sectioned above the atrioventricular junction, and the colon length was measured. Tissues were fixed in 4% paraformaldehyde (PFA) for histology.

2.6 Histological Processing

Fixed tissues were dehydrated, paraffin-embedded, and sectioned at 5 µm. Hematoxylin and eosin (HE) staining was used for histopathological and morphometric analyses. Picrosirius red staining identified collagen fibers in the colon, while Weigert's Resorcinol Fuchsin was used for esophageal elastic fibers.

2.7 Histopathological Analysis of the Heart and Colon

Histopathological analyses. Cardiac parasitism was assessed in five HE-stained sections per animal by quantifying amastigote nests across 50 microscopic fields (40×). Lesion severity (edema, hypertrophy, tissue disorganization, inflammation) was scored semi-quantitatively as absent (0), mild (+), moderate (++), or severe (+++). Colon histopathology was evaluated in five sections per animal, considering edema, hyperemia, and inflammatory infiltrate. Scores were assigned as absent (0), mild (1 focus/section), moderate (2 foci/section), or severe (>3 foci/section). Results were expressed as mean scores and percentage increase compared with controls. All analyses were performed blindly.

2.8 Morphometric Analysis

Sixteen images per section were captured using an Olympus BX50 microscope. Sixty-four measurements were obtained for intestinal wall thickness, muscle and submucosal layers, and crypt dimensions. Collagen type I and III areas were quantified under polarized light using Image-Pro® Plus software.

2.9 qPCR for Quantification of *T. cruzi* in the Colon



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T. cruzi DNA in colon tissue was quantified by real-time PCR using primers specific for kinetoplast DNA. Reactions were performed with QuantiNova SYBR Green Master Mix on a LightCycler™ 480 (Roche). Standard curves were generated from purified parasite DNA (100–0.001 ng), with parasite load expressed as DNA equivalent (200 fg per parasite) (13).

2.10 Statistical Analyses

Statistical tests were performed using Graphpad Prism™ 7 software. Data normality was verified by the D'Agostino–Pearson test. Since all variables were normally distributed, comparisons between groups were made using one-way ANOVA followed by Tukey's post hoc test, with significance set at $p < 0.05$.

3.RESULTS

Infected mice showed lethargy when walking and developed ruffled fur from the second week of infection. Furthermore, some groups presented cachexia from day 15 post-infection (p.i.), with a significant weight reduction of approximately 20% in EV-inoculated mice compared to the control group (CG). This weight loss coincided with a decrease in food intake, particularly around the second week of infection (Figure 1).

Parasitemia peaks occurred simultaneously in all groups from day 12 p.i., decreasing by day 25 p.i. However, significant differences were observed in the number of trypomastigotes present in the blood between the groups. For example, on day 13 p.i., the parasitemia level in the CLCa group was 191% higher compared to the IG group. This increase remained evident on days 15 (180%), 16 (105%), and 19 (207%) p.i. Notably, on day 21 p.i., only the CLC2 group experienced a peak in parasitemia, with a 295% increase compared to the previous day. Two days later, this led to the death of two animals in this group (Figure 1).

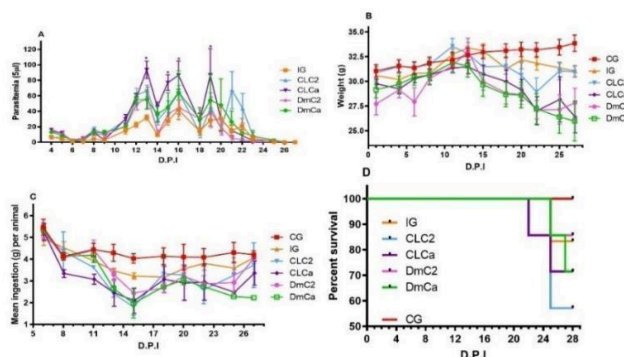


Figure 1. Parasitaemia (A), body weight (B), food consumption (C), and survival rate (D) of mice infected with the CL Brener strain of *T. cruzi* and administered extracellular vesicles (EVs) of different origins. CLC2: EVs derived from CL Brener cultured in C2C12 cells; CLCa: EVs derived from CL Brener cultured in Caco-2 cells; DmC2: EVs derived from Dm28c cultured in C2C12 cells; DmCa: EVs derived from Dm28c cultured in Caco-2 cells. CG: Control group; IG: Infected group. Data presented as mean $\pm$ SD ($n = 5$) (* $p < 0.05$, ANOVA followed by the Tukey test).

Throughout the experiment, some animals in infected groups did not survive to the scheduled euthanasia date (Figure 1A). One mouse each in the IG and DmC2 groups died, two mice each in the CLCa and DmCa groups, and three mice each in the CLC2 group died. Therefore, statistical analyses were conducted using a sample size of five mice per group.

Although the primary focus of this study is the colon *T. cruzi*, infection can induce inflammation and tissue damage in multiple organs, with the heart being the primary target due to the parasite's cardiotropism. Consequently, histopathological analysis of the heart was performed as a complementary assessment, as cardiac alterations can indicate the progression of systemic disease, thus contributing to a more comprehensive understanding of the effects of infection on the body.

The results demonstrated a significant increase in tissue edema, which was approximately threefold greater in the CLCa group and twice as great in the DmC2 and DmCa groups compared to the CG. The



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CLCa group showed an approximately twofold increase in edema compared to the IG. Regarding the analysis of inflammatory infiltrate, a significant increase was observed in the CLCa and DmCa groups, with the CLCa group showing a twofold increase compared to the IG. Furthermore, the DmCa group had more amastigote nests compared to the CLC2 and CLCa groups. No significant changes were observed in the hypertrophy parameter. Analysis of hyperemia revealed a threefold increase in the CLCa group and an approximately 2.5-fold increase in the DmC2 and DmCa groups compared with the CG, along with a twofold reduction in the CLCa group compared with the IG (Figure 2). These changes led to disorganization of cardiac histoarchitecture, with the most pronounced changes occurring in the CLCa group compared with the CG.

No macroscopic changes such as ulceration, redness, or colonic dilation were observed. However, a reduction in colon length was observed, with an 18% decrease in the CLC2 group and a 6% decrease in the DmCa group compared to the IG group.

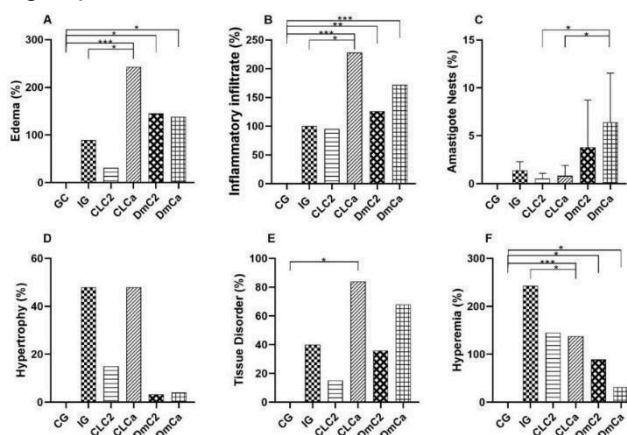


Figure 2. Histopathological scoring (%) of cardiac tissue from mice infected with the *T. cruzi* CL Brener strain and administered with EVs. A) Oedema; B) Inflammatory infiltrate; C) Amastigotes; D) Hypertrophy; E) Tissue disorganization; F) Hyperaemia.

To assess the microscopic changes induced by the infection, histopathological analysis was performed. The IG group presented pronounced edema, inflammatory infiltrate, and histoarchitectonic alterations. Edema was significantly greater in the IG and CLCa groups compared to the CG. Furthermore, hyperemia was observed in the CLC2 and CLCa groups compared to the CG. Regarding the analysis of the inflammatory infiltrate, the IG, CLC2, DmC2, and DmCa groups presented greater infiltration compared to the CG. No amastigote nests were detected in the colon sections analyzed (Figure 3).

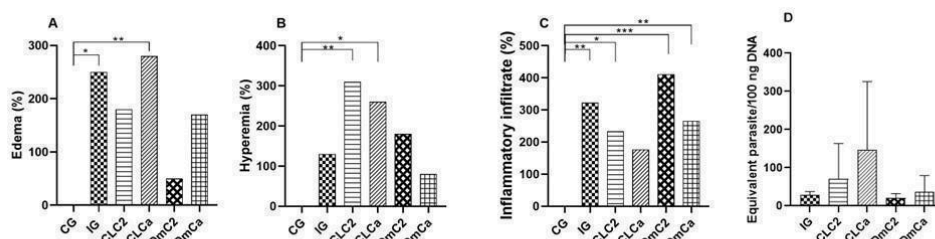


Figure 3.

Histopathological scoring (%) and detection of *T. cruzi* DNA in the colon by qPCR in mice infected with the *T. cruzi* CL Brener strain and administered with EVs. (A) Oedema. (B) Hyperemia. (C) Inflammatory infiltrate. (D) Parasite load in the colon of BALB/c mice (parasite/mL).

To elucidate the effects of infection on the presence and absence of EVs on colonic structure, several morphometric measurements of the colonic wall were performed. The IG group showed a greater than 60% increase in muscular layer thickness compared to the CG. In contrast, the EV-treated groups showed statistically lower values than the IG (Figure 4). Furthermore, the submucosal layer thickness was



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reduced by approximately 40% in the CLC2 group compared to the IG (Figure 4). No significant changes in crypt depth or width were observed.

To elucidate the effects of infection on the presence and absence of EVs in the esophageal structure, several morphometric measurements of the esophageal wall were performed. The CLC2 group showed an increase of more than 50% in the thickness of the muscular layer compared to the CG (Figure 4). No significant changes were observed in the total wall, total muscular layer, submucosa, crypt depth, or width.

Considering the changes in the esophagus caused by the infection, we evaluated tissue remodeling by analyzing the deposition of type III collagen fibers. An approximately 60% increase in the area occupied by type III collagen fibers was observed in the IG and CLC2 groups. The CLCa and DmC2 groups demonstrated a decrease in the occupied area compared to CLC2. No alteration was found in the area occupied by elastic fibers.

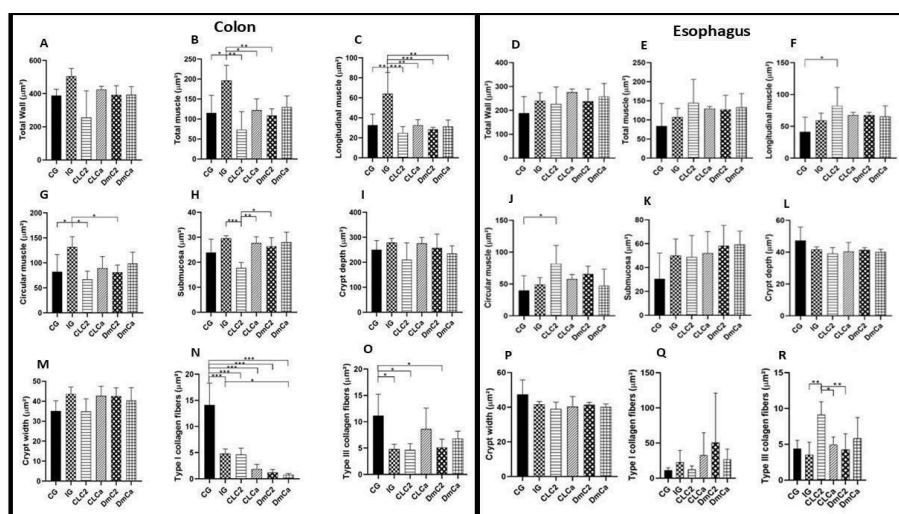


Figure 4. Morphometric analysis of the colon and esophagus wall in mice infected with the CL Brener strain of *T. cruzi* and administered with EVs. (A) Total Wall, (B) Total muscle, (C) Longitudinal muscle (D) Total Wall (E) Total muscle (F) Longitudinal muscle (G) Circular muscle (H) Submucosa (I) Crypt depth (J) Circular muscle (K) Submucosa (L) Crypt depth (M) Crypt width (N) Area occupied (μm^2) by type I and (O) III collagen fibers. (P) Crypt width (Q) Area occupied (μm^2) by type I and (R) III collagen fibers.

4.DISCUSSION

Invasion of host cells by *Trypanosoma cruzi* results in the release of extracellular vesicles (EVs), which influence infection by mediating cell-to-cell communication, modulating the inflammatory response, and promoting immune evasion. In *in vitro* studies, EVs enhance cellular infection by promoting increased permeability, Ca^{2+} mobilization, and cytoskeletal remodeling, which increases parasite proliferation (4). *In vivo*, EV inoculation is associated with increased parasitemia and mortality (5). The composition of EVs varies according to the evolutionary form of the parasite, strain, and host cell type, as demonstrated in interactions between DM28c and CL Brenner strains with C2C12 and Caco-2 cells (6). Co-administration of EVs during infection increases the number of circulating parasites, likely by inhibiting complement-mediated parasite lysis and increasing parasite infection, resulting in increased parasite release from cells. This can cause pathological damage to target organs, such as the liver and heart, evidenced by inflammatory infiltrates, hyperemia, edema, and tissue disorganization, especially in groups receiving EVs and parasites (7).

In the colon, although no amastigote nests were found, *T. cruzi* DNA was detected in all infected animals, which presented inflammation with edema and infiltrates in the mucosal, submucosal, and muscular layers. Submucosal inflammation resulted in reduced collagen deposition, possibly due to the action of collagenases released by inflammatory cells, which also modulate immune responses and recruit leukocytes (8). High levels of proinflammatory cytokines, such as IFN- γ and TNF- α , contribute to this collagen dynamic.



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Intestinal inflammation in Chagas disease is associated with the accumulation of lymphocytes in the muscle layers and damage to the enteric nervous system, including the loss of neurons in the myenteric and submucosal plexuses, a phenomenon observed in the chronic phase of the disease and corroborated in this study (9).

Esophageal morphometric changes suggest a structural adaptation of muscle tissue in response to infection-induced inflammatory stress, possibly aimed at maintaining organ motility and functionality in models of chronic inflammatory diseases. The absence of significant changes in the other layers of the esophageal wall indicates a muscle-specific tissue response, which may reflect an early or localized stage of the pathological response which highlight the selective involvement of muscle structures in esophageal inflammatory processes. The active process of tissue remodeling and fibrosis in response to infection. The increased presence of type III collagen, associated with the early phases of healing and extracellular matrix remodeling, suggests that esophageal tissue is undergoing a chronic state of repair, which may compromise its elasticity and functionality in the long term (9).

5. CONCLUSION

Our results suggest that during the subacute phase, EVs promote *T. cruzi* survival, worsen clinical symptoms, and increase mortality, raising the need to further investigate their composition and mechanisms of action.

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Keywords: extracellular vesicles; *Trypanosoma cruzi*; digestive Chagas disease.

Thematic area: Etiopathophysiology of human and animal diseases



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EVALUATION BY THE CHECKERBOARD METHOD OF PROMASTIGOTES OF *Leishmania (Leishmania) amazonensis* AND A SYSTEMATIC REVIEW OF COINFECTIONS

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1. INTRODUCTION

Leishmaniasis are neglected tropical diseases caused by the bite of a female sand fly infected with the protozoan of the genus *Leishmania*. The disease is endemic in more than 89 countries. In 2023, Brazil recorded 12,910 cases of the cutaneous form and 1,461 cases of the visceral form (1,2). Clinical manifestations depend on the species of parasite and the parasite-host relationship. Cutaneous leishmaniasis (CL) causes skin lesions and is the most common form, while visceral leishmaniasis (VL) affects the spleen, liver, and lymph nodes and is the most severe form (1,3). *Leishmania* is considered an obligate intracellular parasite, infecting mainly macrophages, cells of the innate immune response responsible for phagocytosis. During the disease, in addition to the innate response, the adaptive response is also present, primarily mediated by Th1-type cells (CD4+) and characterized by the production of interleukins to combat *Leishmania* (4,5).

Currently, treatment is carried out with pentavalent antimonials. However, these do not completely cure the disease and can cause toxicity and serious side effects to the patient, in addition to relapses (6). To improve treatment, photodynamic therapy (PDT) emerged as a technique that uses photosensitizers (PS), which, when activated by adequate intensity and length of light, lead to changes in cellular functions, ultimately resulting in the death of the parasite (7,8).

Curcumin (CUR) is a bioactive found in *Curcuma longa*, has anti-inflammatory and healing effects, and can modulate cellular pathways. It is used as a PS and has a synergistic action against pathogens (9,10). The essential oil (EO) of *Pectis brevipedunculata* contains citral as one of its main components, exhibiting a calming effect and antiparasitic potential in leishmaniasis (11). The PDT is a practical therapeutic approach for several dermatological conditions, including CL, and may be effective against different species of the parasite. This method offers advantages such as reduced side effects, being safer for the patient, non-invasive, lower cost, and highly effective (12).

To further understand the immune system of patients with leishmaniasis, a systematic review is being developed that addresses studies on leishmaniasis and coinfection. The immune system is responsible for recognizing and eliminating foreign substances and pathogens present in the body, including *Leishmania*. Coinfection can lead to alterations in the immune system, thereby affecting the disease's outcome. Therefore, it is necessary to gather studies that describe which other infections can co-occur with leishmaniasis and how they influence the immune system, potentially impacting diagnosis and treatment.

Since leishmaniasis is an infectious disease that occupies an important place at the public health, political, and socioeconomic levels, with limitations in treatment and changes in coinfection, this work aims to



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investigate the anti-leishmanial activity in vitro, using CUR as FS in PDT together with the EO of *Pectis brevipedunculata* to evaluate the synergism against promastigote forms of *Leishmania* (*Leishmania*) *amazonensis* (LLa) and to understand the immune system during coinfections with leishmaniasis through a systematic review.

2. MATERIAL AND METHODS

2.1. Compounds and cells

The compounds used were curcumin ($\geq 99\%$) (Sigma-Aldrich®) and *Pectis brevipedunculata* essential oil, obtained from the Chemistry Laboratory of the Federal University of Maranhão (UFMA) campus in São Luís, Maranhão, and extracted by hydrodistillation. Promastigote forms of LLa (PH8 Fiocruz) were used to evaluate the compounds.

2.2. Evaluation of the effect of *Pectis brevipedunculata* and curcumin on LLa promastigotes using the checkerboard technique

LLa promastigotes (2×10^7 *Leishmania*/mL) were treated in 96-well plates with different combinations of *Pectis brevipedunculata* EO (250-3.9 $\mu\text{g/mL}$) and the photosensitizer curcumin (125-1.95 $\mu\text{g/mL}$). One plate was kept in the dark, and the other was irradiated with a blue LED for 30 min. Subsequently, both were incubated at 25°C for 24 h. After incubation, the plates were analyzed spectrophotometrically using 450/620 nm filters, and parasite viability was determined using the XTT colorimetric method. The percentage inhibition was calculated by comparing it with untreated cells. Amphotericin B was used as a positive control. Dose-effect relationships were evaluated using the Chou-Talalay combination index method, as implemented in the Synergy Finder software (13), to determine the concentrations that exhibited synergism. Synergistic combinations were considered when the combination index was less than 1. The experiments were performed in triplicate under the same conditions, with and without light.

2.3. In Silico Analysis

To begin the *in silico* analysis, we searched for the 1D structure of curcumin (CID: 969516) in PubChem (14). Reverse docking analysis was performed using the online software, SEA (Similarity Ensemble Approach) (15). The enzymes indicated as potential targets of curcumin in redocking were analyzed for structural similarity with those of *Leishmania* on the NCBI structures website (16). Among these, the CPG DNA methylase enzyme from *Haemophilus parahaemolyticus* (Uniprot: P15840) showed similarity to *Leishmania major* pteridine reductase 1 (LmPTR1) (PDB id: 6CRX), which was used to simulate conventional docking using the AutoDock Vina program implemented in the PyRx-0.9.8 graphical interface (17). The dimensions used for x, y, and z were -54,941, 19,524, and 2,128, respectively. The results were analyzed based on RMSD (Root Mean Square Distance) and binding affinity values.

2.4. Systematic Review

A systematic review was conducted to answer the following question: "What happens to the immune response to a coinfection between *Leishmania* and other pathogens?" Independent research groups conducted each stage of the review. The data extracted from the articles were tabulated and synthesized for analysis. The review was registered on the PROSPERO platform (18) (CRD42023425941) and is being conducted based on the criteria defined by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (19). The criteria of inclusion was original articles that addressed coinfection between leishmaniasis, of any type and species, and bacteria or viruses or fungi or parasites, with abstract available and English language, that addressed the immune response in humans, without time limitation. The criteria of exclusion was article that did not address coinfection or did not study/



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discuss the immune response during it, dealt only with *in vitro* or animal methodologies, reviews, letters, guidelines, comments, book chapters, article without abstracts available, case reports, clinical trials, news and conference abstracts.

For the review, the screening of articles was performed in six databases: PubMed, Embase, Scopus, Web of Science, Cochrane Library, and the Latin American and Caribbean Health Sciences Literature (LILACS). Four groups of terms joined by the Boolean term "AND" were used: Group 1: Leishmaniasis OR Leishmania; Group 2: Immune system OR Immune system phenomena; Group 3: Coinfection OR Cross infection; and Group 4: Viral diseases OR Bacterial infections and Mycoses OR Parasitic diseases.

3. RESULTS

3.1: Evaluation of the antileishmanial activity of the compounds using the checkerboard method

Tests conducted with *Leishmania* promastigotes allowed us to evaluate the effects of CUR and *Pectis brevipedunculata* EO, both in combination and separately, using the checkerboard method. The tests were conducted in the presence and absence of light, using a 24 h incubation time. The percentage of kill was calculated for each combination and tabulated using SynergyFinder software. In addition to the combination index, scores were also evaluated using the Bliss plot, where scores above 10 indicated strong synergism. The parameters were analyzed together for greater robustness. Several concentrations, in the presence of light, showed synergism, with concentrations of 7.81 µg/mL (CUR) and 3.9 µg/mL (EO) standing out, indicating a high synergistic score (26.36) with cellular inhibition of 73.75%. In the absence of light, the same concentration showed a low synergistic score (9.85) and 59.75% cellular inhibition. The compounds isolated in the presence of light showed a death percentage of 37% and 16.5% for curcumin and *P. brevipedunculata* essential oil, respectively. In the absence of light, the death percentage was 40% and 16% for curcumin and *P. brevipedunculata* essential oil, respectively.

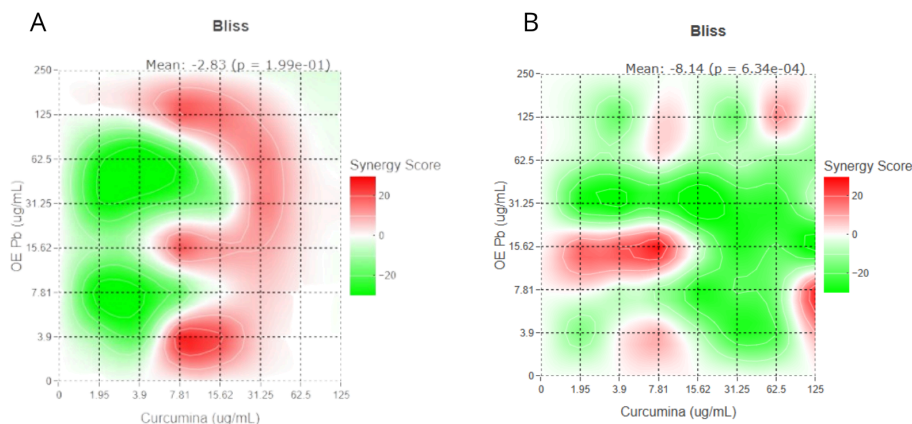


Figure 01: 2D Heatmap of the Bliss synergistic score for the combinations of Curcumin and EO from *P. brevipedunculata*, in the presence (A) and absence (B) of light. SynergyFinder.

3.2: *In silico* analysis

The CPG DNA methylase enzyme showed 11% structural similarity to LmPTR1, which was used for computational analyses. Conventional docking indicated that curcumin binds to LmPTR1, presenting an RMSD of zero, confirming a mechanical fit between the enzyme's binding site and curcumin. The compound also exhibited a binding affinity of -8.7 kcal/mol, comparable to that of the known inhibitor KMK (PDB ID: 6CRX), which had a binding affinity of -8.8 kcal/mol.

3.3: Systematic Review

Initially, 762 articles were found. After manually removing duplicates using EndNote software, 716 articles were selected for title and abstract reading, and 86 articles were read in full. Of these, 25 met the inclusion criteria and were accepted for review.



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Among the accepted articles, 10 were published in Brazil, with a year range from 1993 to 2024. Visceral leishmaniasis was presented in 21 articles, and the most frequently reported coinfection was with HIV with 21 articles, 2 were about helminths and 2 about protozoa. The components of the immune response analyzed varied between cells, soluble molecules, and receptors and ligands. In patients coinfecting with HIV, CD4⁺ T cells decreased, particularly in cases of VL coinfection. Depending on the patient's history and the clinical manifestation of leishmaniasis, interleukins could be higher or lower. Coinfection with *Trypanosoma cruzi* resulted in a reduction in differentiated CD4⁺ T cells and an increase in senescent CD4⁺, CD8⁺, and CD57⁺ lymphocytes, indicating an exhaustion profile. Coinfections with different intestinal parasites did not alter the Th1 or Th2 cell profiles. Finally, in *Plasmodium falciparum* and VL coinfection, increased levels of IFN- γ , TNF- α , IL-2, IL-13, and IL-17A, with the latter being associated explicitly with malaria.

4. DISCUSSION

One of the main objectives of this study was to evaluate, using the checkerboard, whether curcumin combined with *P. brevipedunculata* EO at different concentrations showed synergism against promastigote forms of *LLa*. The work presented here showed that these two substances present good activity together, suggesting synergy. These results corroborate another study that evaluated the gel formulation (20). Studies performed in combination with curcumin and other compounds have shown synergism against fungi and bacteria (21–23), corroborating our results, which demonstrated a synergistic effect in PDT at various combined concentrations. The concentrations of 7.81 $\mu\text{g/mL}$ (CUR) and 3.9 $\mu\text{g/mL}$ (EO) showed the greatest synergism, as assessed by the Bliss model, with combination index values below 1. Another point demonstrated was the effective action of PDT. On the plate without light, the synergism and percentage of inhibition were lower when compared to the plate irradiated with blue LED light. This finding again corroborates other studies demonstrating the effective action of curcumin in combination with PDT (10,24). Our study showed no significant difference in the percentage of inhibition between the compounds isolated with and without light. Other studies on the action of *P. brevipedunculata* EO also demonstrate its effect on *Leishmania* promastigotes (11,25).

The enzyme LmPTR1 is involved in the *Leishmania* metabolic pathway, and its inhibition impairs the cells' ability to acquire nucleotides for DNA synthesis, leading to cell death (26). Considering the inhibitory effect of curcumin observed on promastigotes in *LLa*, the essential role of PTR1 in the protozoan's metabolism, and the docking observed in LmPTR1, indicate a possible inhibitory mechanism of action.

Still focusing on parasitic infection, this systematic review aims to clarify changes in the immune response during coinfection between leishmaniasis and other pathogens. Among the accepted studies, most were conducted in Brazil, which is explained by the country's endemic status for the disease (2). Regarding the clinical manifestation of leishmaniasis, we observed a predominance of the visceral form, which is more severe and lethal, leading to greater alterations in the immune system. The predominant coinfection was with HIV, a virus considered immunosuppressive, increasing the patient's susceptibility to developing other infections, leading to worsening of the clinical condition (27). The immune system responds differently during co-infection with *Leishmania* and other pathogens, depending on the patient's clinical condition, the likelihood of relapse, and the treatment regimen. The changes are most often seen in co-infections with HIV, in decreasing the CD4⁺ T cells count. Co-infections with protozoa result in a non pronounced immunological alteration. Despite this, in both cases, there is a modification of the immune system in comparison to a single infection.

5. CONCLUSION

Our studies concluded that curcumin, combined with *P. brevipedunculata* EO, is more effective in PDT than either alone or without the use of light, demonstrating that they are a valuable compound for new therapies against cutaneous leishmaniasis. *In silico* analyses indicate that the LmPTR1 enzyme can be inhibited by curcumin, and its mechanism of action is related to alterations in DNA synthesis, leading to parasite death. Further studies are needed for confirmation. The findings of the systematic review indicate that coinfections involving leishmaniasis modulate the immune response. This variation depends on the type of pathogen, the clinical manifestation of leishmaniasis, and the patient's clinical history, highlighting the complexity of immunological interactions.

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Keywords: *Leishmaniasis*; checkerboard; coinfection.

Thematic area: Pathogens of clinical relevance.



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ACETAMINOPHEN POISONINGS RECORDED AT A TOXICOLOGICAL ASSISTANCE CENTER

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1. INTRODUCTION

In the healthcare field, medications play an indispensable role, as they are used in the treatment and cure of diseases, in prophylaxis, palliative care, and diagnosis, making them fundamental tools in the health-disease process (1,2). However, inappropriate or abusive use, especially due to the ease of access to over-the-counter medications (OTCs), contributes significantly to the increase in poisoning cases (3). According to data from the Department of Informatics of the Unified Health System (DATASUS), between 2023 and 2025, approximately 56% of reported exogenous poisoning cases in Brazil were attributed to medication use, highlighting this group as the main agent involved in poisoning episodes (4).

Among OTCs, paracetamol, also known as Acetaminophen, stands out as a drug with analgesic and antipyretic properties. It is traditionally classified as a non-steroidal anti-inflammatory drug (NSAID); however, its anti-inflammatory activity is minimal compared to other representatives such as ibuprofen or naproxen. Furthermore, paracetamol's adverse effect profile differs, with less interference in platelet aggregation and a lower ulcerogenic potential, indicating a distinct mechanism of action. These characteristics make paracetamol a widely used drug, both for its analgesic and antipyretic efficacy and its relatively favorable safety profile (5,6).

Globally, paracetamol is one of the leading causes of acute poisoning, particularly in developed countries such as the United States and the United Kingdom (7). In the United States, paracetamol toxicity is the leading cause of liver failure, with 50% of overdose cases being unintentional (8).

Paracetamol is well absorbed orally, reaching peak plasma concentrations approximately two hours after ingestion. About 90% of paracetamol is metabolized in the liver through three main pathways: glucuronidation, sulfation, and oxidation. Sulfation is the primary conjugation pathway in children, while glucuronidation predominates in adults, a process that becomes fully developed around the age of 12. When doses exceed 4g per day, the main metabolic pathways become saturated, leading to activation of the oxidative pathway and the subsequent formation of the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI) (5,6).

Under therapeutic conditions, NAPQI is rapidly neutralized through conjugation with endogenous glutathione, forming cysteine and mercapturic acid conjugates that are subsequently eliminated from the body. This conjugation process is crucial to prevent liver toxicity associated with paracetamol. In overdose cases, depletion of endogenous glutathione allows NAPQI to accumulate, resulting in hepatocellular necrosis and impaired liver function (5,9,10). In cases of poisoning, the antidote used is N-acetylcysteine (NAC), which increases glutathione production, restoring the liver's conjugation capacity and preventing or minimizing hepatic damage (11,12).

Symptoms resulting from paracetamol poisoning vary depending on severity. In the early stages, typically within the first few hours after excessive ingestion, gastrointestinal manifestations such as nausea, vomiting, and abdominal discomfort predominate. In more severe cases, significant systemic complications may arise, including liver injury and hemorrhages. Paracetamol poisoning can be divided into four clinical phases. The first phase, occurring within the first 24 hours post-ingestion, may be asymptomatic or present nonspecific signs such as malaise, nausea, vomiting, and sweating. The second phase, between 24 and 72



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hours, may remain asymptomatic or present symptoms similar to the first phase, accompanied by evidence of hepatic injury. The third phase, occurring between 72 and 96 hours, is characterized by hepatotoxicity, which may progress to liver failure, acute respiratory distress syndrome (ARDS), cerebral edema, and hemorrhagic disorders, with a potential risk of death. Finally, the fourth phase, between 96 hours and two weeks, involves the regeneration of hepatic tissue, generally associated with complete clinical recovery in patients who survive hepatotoxicity, without sequelae (8,11).

The purpose of this study was to characterize the epidemiological profile of cases of paracetamol poisoning, using records from a Toxicological Assistance Center (CIATOX), in order to identify the factors associated with these poisonings, assess the severity of the cases and outline patterns of medication use, supporting prevention strategies, optimizing clinical management and improving the quality of patient care.

2. MATERIALS AND METHODS

2.1. Study Design

This is a quantitative, descriptive, cross-sectional study involving a retrospective documentary analysis of paracetamol-related poisoning cases reported to a Toxicology Information and Assistance Center in the state of Paraná, Brazil (13,14). The information used in this study was obtained from patient records of paracetamol poisoning cases that occurred between September 2023 and August 2025. Data collection was performed using the DATATOX digital platform, a tool developed for systematizing and analyzing toxicological data, allowing for greater accuracy in risk assessment and supporting decision-making processes in the field of toxicology. This system was implemented in September 2023, replacing the previously used manual recording method, which allowed for greater standardization, faster service delivery, and improved data collection.

2.2. Inclusion Criteria

The study included all reported cases of paracetamol poisoning, regardless of age group, that presented clinical, laboratory, and/or epidemiological data related to acute or chronic intoxication by this substance.

2.3 Variables analyzed

Sociodemographic: biological sex, age group, occupation, educational level, and race. *Clinical characteristics*: symptoms presented, severity of the condition (mild, moderate, severe), time between ingestion and medical care. *Toxicological information*: reason for poisoning, type of exposure, route of administration, co-ingestion with other substances. *Management and treatment*: use of antidote, type of treatment (activated charcoal, gastric lavage, etc.), clinical outcome (prognosis).

2.4 Ethical aspects

This study received approval from the Research Ethics Committee under approval number 6.940.629, CAAE: 78158724.9.0000.0104.

3. RESULTS

Between September 2023 and August 2025, a total of 245 cases of poisoning related to paracetamol use were reported, with 50 cases in 2023, 123 in 2024, and 72 notifications in 2025. Regarding biological sex, there was a predominance of female patients, accounting for approximately 66% of the occurrences. In terms of race/color, most cases were recorded among white individuals (53%), followed by mixed-race individuals (33%). As for educational level, individuals with completed high school represented 37% of the



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cases, followed by those with incomplete high school and incomplete elementary education, each comprising approximately 20% of the cases. Patients with higher education accounted for only 2% of the reported cases.

Analysis of occupational status revealed that 31.4% of patients were employed, while 29.8% were students - covering elementary, secondary, and higher education levels. Unemployed individuals represented 15% of the records, a percentage similar to that observed in the group classified as “ignored” or “not applicable,” which also accounted for 15%.

Patient age and circumstances related to age are detailed in Table 1. Overall, the main motivation for poisoning was suicide attempt (SA), representing approximately 88.5% of reported cases, followed by accidental poisonings, which accounted for 4.9%. Temporal exposure analysis showed that 33% of patients received medical care more than one hour after ingestion; 25.7% were treated within one hour, and 17.5% in less than one hour. Cases with exposure ranging from more than five hours to one day represented 21.2%. Regarding the type of exposure, single acute exposure was the most prevalent, totaling 204 notifications, while repeated acute exposure accounted for 11.43% of cases.

Table 1 – Age profile related to the circumstances of paracetamol poisoning cases recorded between 2023 and 2025

Age	Nº. of cases	%	Circumstance
<1	4	1.63	Accidental or medication error
1-4	8	3.27	Accidental or medication error
5-9	3	1.22	Accidental or self-medication
10-14	24	9.80	98.8% SA
15-19	59	24.08	
20-29	88	35.92	95.8% SA;
30-39	30	12.24	4.2% Abuse
40-49	18	7.35	7.14% Abuse, 3.57% Accidental, 3.57% Adverse Reaction, 85.71% SA
50-59	6	2.45	
60-69	4	1.63	

*SA: Suicide Attempt

The association between paracetamol poisoning and other medications is illustrated in Figure 1. Among the initial clinical symptoms observed, the most frequent were drowsiness (31% of cases), emesis (20%), nausea (12.2%), and abdominal pain/epigastralgia (7.8%).

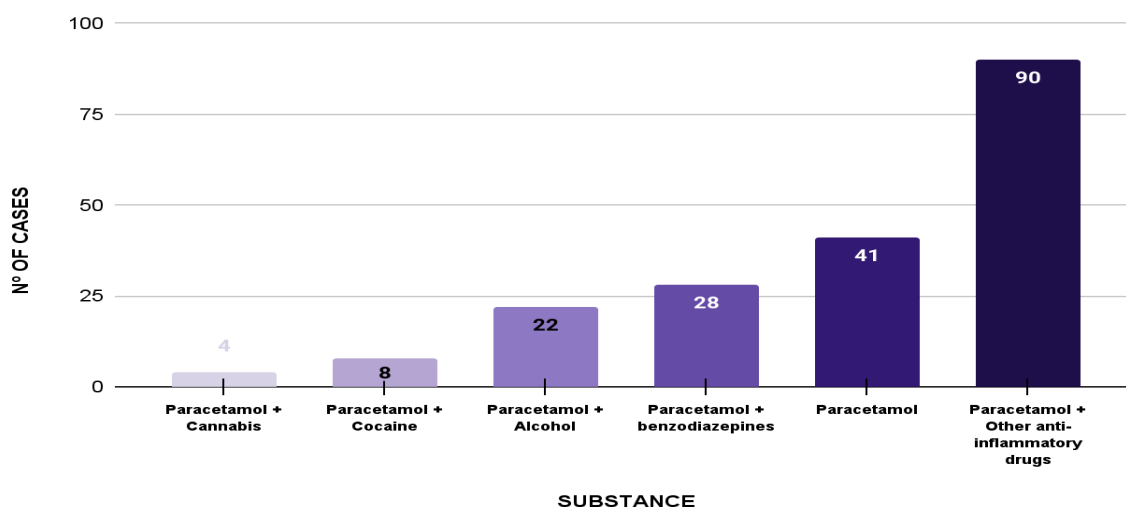


Figure 1 – Drug associations involving cases of paracetamol poisoning recorded between 2023 and 2025



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Treatment of poisoning cases showed that 29.4% of patients received activated charcoal more than one hour after exposure, and gastric lavage was performed within the same time frame in 29.8% of cases. Among patients treated within one hour of exposure, 19.2% received activated charcoal and 24.1% underwent gastric lavage. Repeated doses of activated charcoal were administered in approximately 15% of cases. It is important to note that all individuals received symptomatic treatment and clinical support. The antidote N-acetylcysteine (NAC) was used in 21.2% of cases. Regarding severity, 67.8% of poisonings were classified as mild, 24.1% as moderate, and 4.1% as severe. One death was recorded during the evaluated period. In terms of clinical outcome, 79.6% of patients achieved probable recovery, while in approximately 13.5% of records, the outcome was either unreported or marked as unknown.

4. DISCUSSION

The year 2024 accounted for the highest number of poisoning cases; however, it was the only year fully evaluated due to the recent implementation of the DATATOX system. Analysis of the sociodemographic profile of patients revealed that individuals most vulnerable to paracetamol poisoning were female, a finding consistent with other epidemiological studies on medication-related poisonings, which suggest greater vulnerability among women, particularly in cases involving SA (15,16,17). Furthermore, data from DATASUS indicate that approximately 73% of reported medication-related cases involve female patients, of which 56% are attributed to SA (4). This association was also confirmed in the present study, as 88.5% of cases were related to intentional ingestion, reinforcing the need for prevention strategies and mental health support, with emphasis on female populations.

Analysis of educational level and occupation indicated that individuals of economically active age or in school represented the majority of cases, implying a significant social impact. According to data from the Brazilian Institute of Geography and Statistics (IBGE), most of the Brazilian population has at least completed basic education, with only 20.5% having higher education (18). The results of this study suggest that lower socioeconomic conditions may constitute risk factors for SA. Moreover, poisoning events did not significantly affect individuals with higher education, indicating that such episodes are more frequent among groups with lower educational attainment and possibly greater social vulnerability (15).

Regarding clinical manifestations, gastrointestinal symptoms (emesis, nausea, and abdominal pain) were most frequently observed, which aligns with classical descriptions of paracetamol poisoning, typically beginning with nonspecific signs within the first hours after ingestion (8). Drowsiness is not a direct effect of paracetamol but rather results from its association with other central nervous system depressants, such as alcohol and benzodiazepines. Among the drug associations with paracetamol, the most significant was with other anti-inflammatory drugs. As these are available over the counter, their ease of access contributes to increased misuse and a heightened risk of hepatotoxicity (3). Additionally, studies have indicated a serious interaction between paracetamol and other NSAIDs, corroborating the notion that easy access and limited information on toxicity may lead to excessive use and subsequent poisoning (17).

Other important associations include substances of abuse such as cocaine, cannabis, and alcohol, which directly affect hepatic metabolism. Both chronic and acute alcohol consumption are associated with an increased risk of paracetamol toxicity, even at therapeutic doses. Alcohol competes with the hepatic oxidative system; when ingested with paracetamol, the formation of its toxic metabolite NAPQI is reduced, initially providing protection against liver injury. However, as plasma alcohol levels decline, this protective effect diminishes, rendering the body more susceptible to paracetamol toxicity and potential hepatocellular damage (19).

Treatment in cases of drug poisoning follows specific protocols and includes supportive and symptomatic care, as well as measures to eliminate the toxic agent or administer antidotes (15). In this study, gastric lavage and activated charcoal—when indicated based on the duration of exposure - were shown to be effective in preventing drug absorption. The use of NAC is essential to reduce mortality associated with acetaminophen poisoning when toxic doses are reached, as determined by the Rumack-Matthew nomogram. NAC allows the neutralization of NAPQI and prevents liver injury (15). NAC was administered in 21.2% of cases, indicating its use primarily in poisonings that met established toxicity criteria and confirming the appropriate administration of antidotes in patients at real risk of liver injury.

Most cases were classified as mild, and clinical outcomes revealed that the majority of patients progressed to probable recovery, although approximately 13.5% of records had unreported or missing



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outcomes. This gap highlights a limitation in the use of secondary data sources, as information loss and discontinuity in follow-up compromise the quality of results.

5. CONCLUSION

The findings of this study highlight that drug poisonings, particularly those involving paracetamol, constitute a serious public health issue. When paracetamol poisoning occurs in association with other medications or chemical substances, the risk of hepatotoxicity increases significantly. Moreover, prompt and effective clinical management plays a crucial role in achieving favorable outcomes. It is expected that these results will support the development of more effective public policies and promote the rational use of over-the-counter medications, thereby minimizing the risks of poisoning and its adverse outcomes, especially among vulnerable population groups.

FUNDING

CAPES

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HLA CLASS I AND II IN HOSPITALIZED AND NON-HOSPITALIZED PATIENTS WITH COVID-19 IN MARINGÁ, PARANÁ, BRAZIL

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1. INTRODUCTION

The Pandemic of Coronavirus Disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), rapidly escalated into a public health emergency of unprecedented scale (1). The disease exhibits a highly heterogeneous range of clinical outcomes, spanning from asymptomatic infection to life-threatening acute respiratory distress syndrome and mortality. While advanced age and pre-existing chronic conditions, such as cardiovascular disease and type 2 diabetes, are well-established risk factors for severe disease, a significant degree of inter-individual and inter-population variability in disease presentation exists (2,3). This clinical heterogeneity underscores the critical importance of host-specific factors, particularly those of immunological and genetic origin, in modulating the host's response to SARS-CoV-2 and determining disease severity (4).

The host immune system is a central determinant in shaping the outcome of viral infections. A robust and well-coordinated adaptive immune response is essential for effective viral clearance and the establishment of long-term immunological memory (5). A pivotal component of this adaptive response is the Human Leukocyte Antigen (HLA) system, also known as the Major Histocompatibility Complex (MHC) in other species. HLA molecules are highly polymorphic cell-surface glycoproteins that function to present antigenic peptides to T lymphocytes, thereby initiating antigen-specific immunity (6). The extensive polymorphism of HLA genes, which are the most polymorphic loci in the human genome, results in a diverse array of alleles that dictate the repertoire of peptides that can be presented to the immune system (7).

The HLA system is divided into Class I and Class II molecules, each with distinct roles in immune defense. Class I molecules (HLA-A, -B, -C) are found on nearly all nucleated cells and present internal antigens, such as viral proteins, to CD8⁺ cytotoxic T cells. This interaction activates the T cells, enabling them to destroy infected cells and control viral replication. Class II molecules (HLA-DR, -DP, -DQ) are expressed on specialized antigen-presenting cells like macrophages and dendritic cells. They present external antigens to CD4⁺ helper T cells, which then initiate and coordinate the adaptive immune response by promoting antibody production and supporting CD8⁺ T cell function (6,7).

Numerous studies in diverse populations have established associations between specific HLA alleles and a range of disease severities, underscoring the population-specific impact of HLA variants on clinical outcomes. This study was thus designed to address this question within a specific demographic context. The primary objective was to investigate the relationship between polymorphisms in the *HLA Class I (A and B)* and *HLA Class II (DRB1)* genes and the clinical severity of COVID-19 in a population from Maringá, Paraná.



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2. METHODS

2.1. Study Population

This cross-sectional study was performed before the implementation of COVID-19 vaccination and involved patients with SARS-CoV-2 infection confirmed by Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-qPCR). Recruitment took place between May 2020 and April 2021 in Maringá, Southern Brazil. The study protocol was approved by the Ethics Committee of Unicesumar—The University Center of Maringá (CAAE: 37322420.4.0000.5539, approval date: September 10, 2020). Written informed consent was obtained from all participants or their legal representatives for critically ill individuals. A total of 240 unvaccinated patients were included. Based on clinical records and disease progression, participants were divided into two groups: non-hospitalized ($n = 146$) and hospitalized ($n = 94$). An analysis of demographic and clinical characteristics revealed significant differences between these groups.

2.2. Sample Collection and DNA Extraction

Peripheral blood samples were collected in EDTA tubes and processed at the Laboratory of Immunogenetics of the State University of Maringá (UEM). Genomic DNA was isolated using the BIOPUR® extraction kit (Biometrix, Curitiba, Brazil) according to the manufacturer's protocol, and its quality and concentration were assessed with a Nanodrop 2000® spectrophotometer (Wilmington, DE, USA).

2.3. HLA Genotyping

Genotyping of *HLA-A*, *HLA-B*, and *HLA-DRB1* loci was performed using Polymerase Chain Reaction with Sequence-Specific Oligonucleotide Probe (PCR-SSO) combined with Luminex™ technology. The LABType™ CWD kit (One Lambda Inc., Canoga Park, CA, USA) was used at low-to-medium resolution following the manufacturer's guidelines. PCR amplification was carried out using biotinylated primers, followed by hybridization with fluorescent microsphere-bound probes. Detection relied on R-phycoerythrin-conjugated streptavidin (SAPE), and fluorescence signals were read with the FLEXMAP 3D™ system. Genotype assignment was performed using HLA Fusion software, version 4.6 (One Lambda Inc., Canoga Park, CA, USA).

2.4. Statistical Analysis

All statistical procedures were conducted in the R environment (version 4.2.1; R Foundation for Statistical Computing, Vienna, Austria). Demographic and clinical variables, including age, sex, smoking status, and comorbidities, were analyzed using the “stats” package. Continuous variables were compared with Student's t-tests, and categorical variables were assessed by chi-square tests. Associations between HLA alleles and hospitalization status were examined using logistic regression models implemented with the “midashLA” package, adjusting for potential confounders such as age, sex, and comorbidities.

3. RESULTS

An analysis of patient characteristics revealed significant differences between those hospitalized ($N=94$) and those not hospitalized ($N=146$). Hospitalized patients were, on average, significantly older (mean 55.91 years) and were more likely to be male (66%) and over 50 years of age (64%), all with $p<0.01$. Furthermore, hospitalization was significantly associated with several comorbidities. A greater proportion of hospitalized patients had cardiovascular disease (47%), obesity (63%), and were smokers (18%) compared to non-hospitalized patients (19%, 45%, and 3.4%, respectively), with all these differences being statistically significant ($p<0.01$). The prevalence of diabetes was higher in the hospitalized group (14% vs 7.5%), but this difference was not statistically significant ($p=0.11$) (Table 1).



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Table 1. Characteristics patients with Covid-19

Characteristics	non-hospitalized - N: 146	hospitalized - N: 94	P Value
Age			
Mean ± Sd	46.84 (14.37)	55.91 (14.41)	<0.01^a
≤50	81 (55%)	34 (36%)	<0.01^b
>50	65 (45%)	60 (64%)	
Sex			
Female	91 (62%)	32 (34%)	<0.01^b
Male	55 (38%)	62 (66%)	
CVD			
No	118 (81%)	50 (53%)	<0.01^b
Yes	28 (19%)	44 (47%)	
Diabetes			
No	135 (92%)	81 (86%)	0.11 ^b
Yes	11 (7.5%)	13 (14%)	
Obesity			
No	81 (55%)	35 (37%)	<0.01
Yes	65 (45%)	59 (63%)	
Smoking			
No	141 (97%)	77 (82%)	<0.01
Yes	5 (3.4%)	17 (18%)	

Abbreviations: Sd: Standard deviation; CVD: Cardiovascular disease; a: Statistical analysis was performed by T test; b: Statistical analysis was performed by χ^2 -test.

Among the analyzed HLA alleles, only *HLA-B*44* (Table 2) demonstrated a significant association with COVID-19 severity, being more frequent in hospitalized patients compared to non-hospitalized ones (OR = 2.15, 95% CI: 1.02–4.58, $p = 0.04$). This association remained significant even after adjusting for potential confounding factors such as age, sex, and comorbidities. *HLA-A* or *HLA-DRB1* alleles showed no statistically significant differences between the groups.

Table 2. *HLA-B* Allele Frequencies in Non-hospitalized and Hospitalized COVID-19 Patients

Allele	Non-hospitalized n (%)	Hospitalized n (%)	OR (95% CI)	p-value
<i>B*07</i>	18 (6.2%)	14 (7.4%)	1.30 (0.55–2.97)	0.54
<i>B*08</i>	14 (4.8%)	5 (2.7%)	0.65 (0.19–1.92)	0.45
<i>B*13</i>	4 (1.4%)	3 (1.6%)	1.55 (0.27–8.06)	0.60
<i>B*14</i>	18 (6.2%)	11 (5.9%)	0.84 (0.35–1.95)	0.69
<i>B*15</i>	23 (7.9%)	10 (5.3%)	0.54 (0.21–1.26)	0.16
<i>B*18</i>	19 (6.5%)	13 (6.9%)	1.16 (0.49–2.69)	0.72
<i>B*27</i>	8 (2.7%)	2 (1.1%)	0.54 (0.07–2.67)	0.50
<i>B*35</i>	40 (13.7%)	27 (14.4%)	1.13 (0.60–2.12)	0.70
<i>B*37</i>	5 (1.7%)	1 (0.5%)	0.19 (0.01–1.44)	0.15
<i>B*38</i>	7 (2.4%)	7 (3.7%)	1.01 (0.29–3.46)	0.98
<i>B*39</i>	9 (3.1%)	6 (3.2%)	1.04 (0.32–3.20)	0.94



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<i>B*40</i>	13 (4.5%)	10 (5.3%)	0.89 (0.32–2.40)	0.82
<i>B*44</i>	21 (7.2%)	21 (11.2%)	2.15 (1.02–4.58)	0.04
<i>B*45</i>	4 (1.4%)	2 (1.1%)	0.84 (0.09–5.46)	0.86
<i>B*49</i>	5 (1.7%)	8 (4.3%)	2.50 (0.74–9.19)	0.14
<i>B*50</i>	8 (2.7%)	6 (3.2%)	0.97 (0.27–3.35)	0.96
<i>B*51</i>	28 (9.6%)	13 (6.9%)	0.86 (0.39–1.86)	0.71
<i>B*52</i>	5 (1.7%)	7 (3.7%)	1.95 (0.51–7.83)	0.32
<i>B*53</i>	3 (1.0%)	2 (1.1%)	1.14 (0.12–8.31)	0.89
<i>B*55</i>	3 (1.0%)	2 (1.1%)	0.49 (0.06–3.47)	0.47
<i>B*57</i>	8 (2.7%)	4 (2.1%)	0.58 (0.13–2.19)	0.43
<i>B*58</i>	5 (1.7%)	5 (2.7%)	1.89 (0.43–8.07)	0.38

Abbreviations: N, total number of alleles; (%), proportion of alleles; OR, odds ratio; CI, confidence interval; p-value, statistical analysis was performed by logistic regression.

4. DISCUSSION

The COVID-19 pandemic has disproportionately affected individuals with underlying conditions. Factors like older age, male sex, and comorbidities such as cardiovascular disease and diabetes are linked to worse outcomes (8). In our cohort, these traits were more common in hospitalized group, aligning with known risk profiles. Hospitalization was significantly associated with several comorbidities, such as cardiovascular disease and diabetes, that it was higher in this group (14% vs 7.5%), but this difference was not statistically significant ($p=0.11$) (Table 1). Beyond clinical factors, emerging evidence suggests genetic and immune components also influence disease severity (9,10).

SARS-CoV-2's interaction with the immune system-especially via HLA molecules-shapes the body's response and disease progression (8). While many HLA alleles have been linked to COVID-19 outcomes, findings remain inconsistent, likely due to ethnic variation in allele frequencies (9–11). This highlights the need for research in genetically diverse populations like Brazil's.

In this study, while *HLA-A* alleles have no statistically significant differences between the groups, among *HLA-B* alleles, only *HLA-B*44* showed association with severity in the hospitalized group, remaining after adjusting for factors (Table 2). Some papers have evaluated the role of this allele, showing the *HLA-B*44* alleles are highly susceptible to the disease. *HLA-B*44* and *HLA-C*01* may help predict COVID-19 risk and guide clinical management, including vaccination and prevention strategies (12). Tymoniuk, in Poland, demonstrated association with symptomatic disease and presence of *HLA-B*44* (13), similarly with findings of Correale et al in Italy (14). In contrast, *HLA-B*44:03* implicated with relative protection from disease in USA (15).

HLA class II molecules, especially those from the *DRB1* locus, play a central role in the adaptive immune response to COVID-19. Reduced *HLA-DR* expression on monocytes (mHLA-DR) is consistently linked to immunosuppression, impaired antigen presentation, and worse outcomes in critically ill patients (16).

Our findings showed *HLA-DRB1* alleles had no statistically significant differences between the groups, although two important alleles in the literature are *HLA-DRB1*15* and *DRB1*11*. Studies on *HLA-DRB1*11* have yielded mixed findings. Research in Sardinia, Italy, found the allele more common in non-severe COVID-19 cases, though the association lost significance after Bonferroni correction (17). Similarly, a Spanish study reported higher *DRB1*11:01* frequency in healthy donors than in hospitalized patients, suggesting a possible protective effect that also did not remain statistically significant (18). A study from Burkina Faso showed that carriers of *HLA-DRB1*11* had a sixfold increased risk of asymptomatic infection compared to COVID-negative individuals (19). In contrast to earlier findings, Iranian studies reported *HLA-DRB1*11* and its subtype *DRB1*11:01* as more frequent in severe COVID-19 cases, suggesting a link to disease severity, while *DRB1*11:05* was more common in healthy individuals (20, 21).

Multiple studies have explored the association between *HLA-DRB1*15* and COVID-19 severity. In Italy and the UAE, the *HLA-DRB1*15:01* allele was significantly linked to severe and critical cases (22, 23).



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In the UK, Poulton et al. found a similar association, though it lost significance after Bonferroni correction (24). Despite regional variation, these findings suggest *HLA-DRB1*15* may be a genetic indicator of susceptibility to severe COVID-19.

5. CONCLUSION

The hospitalized patient group exhibited a higher mean age, greater prevalence of male sex, and more comorbidities linked to disease severity. The presence of the *HLA-B*44* allele was statistically significant in this group, suggesting it may be associated with poorer clinical outcomes.

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EMPLOYING NATURAL BIO CERAMICS ENRICHED WITH Nb_2O_5 AND PRP IN REGENERATIVE MEDICINE

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1. INTRODUCTION

The reconstruction of lost bone remains one of the major challenges in medicine and dentistry. Although small bone defects can spontaneously regenerate, critical-size defects (CSDs) exceed the body's repair capacity and require surgical interventions with the use of grafts (1). The CSD model, frequently employed in experimental research, reproduces clinical conditions of extensive bone loss resulting from trauma, neoplasms, or infections. In such scenarios, bone regeneration is slow due to poor blood supply and the limited availability of essential minerals, favoring fibroblast invasion over osteoblastic activity (2). CSDs provide a reliable means to evaluate the effectiveness of biomaterials prior to their therapeutic application (3).

In this context, the use of biocompatible and bioactive biomaterials emerges as a promising alternative to optimize bone regeneration, providing both structural support and biological stimulation for tissue repair (4). Calcium phosphate (CaP)-based bioceramics have gained prominence due to their chemical and structural similarity to the mineral phase of bones and teeth, combining biocompatibility, osteoconductivity, and osteoinductive potential (5,6). The main examples include hydroxyapatite (HAp), tricalcium phosphates (α -TCP, β -TCP), octacalcium phosphate (OCP), amorphous calcium phosphate (ACP), and biphasic calcium phosphate (BCP). The latter, resulting from the combination of HAp and β -TCP in variable ratios, allows modulation of its degradation rate and bioactivity (7-9).

Despite their advantages, the mechanical strength of bioceramics still represents a limitation, especially in situations of higher functional load. In this regard, the incorporation of metallic elements such as niobium (Nb), in the form of niobium pentoxide (Nb_2O_5), has been investigated as a strategy to improve the physicochemical and mechanical properties of ceramics, including compressive strength, hardness, and Young's modulus (10). Beyond structural reinforcement, there is evidence that niobium pentoxide in the form of porous discs positively influenced the bioactivity of ceramic composites (11), contributing to a more favorable microenvironment for bone regeneration (12).

Another approach consists of combining bioceramics with platelet-rich plasma (PRP), an autologous concentrate rich in growth factors capable of recruiting mesenchymal stem cells, stimulating pre-osteoblast proliferation, and enhancing local angiogenesis (13). A recent study from the research group further demonstrated that the addition of PRP can potentiate the osteoregenerative effects of biphasic calcium phosphate (BCP) grafts of natural origin, obtained from fish bones, promoting an acceleration of the repair process (14).

Therefore, this study aims to evaluate biocompatibility and bone regeneration in critical-size defects (CSDs) in rat calvaria, in vivo, through histological analysis and micro-computed tomography (μ CT). The proposal seeks to contribute to the understanding of the role of Nb_2O_5 and PRP as potential adjuvants in



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bone regeneration mediated by BCP derived from fish bones, providing insights for future clinical applications.

2. MATERIALS AND METHODS

2.1. Production of BCP and BCP/Nb Bioceramics

Biphasic calcium phosphate (BCP), composed of 34% β -tricalcium phosphate (β -TCP) and 66% hydroxyapatite (HAp), was produced from Nile tilapia (*Oreochromis niloticus*) bones at the Laboratory of Metallic Materials and Biomaterials/DFI-UEM, through a calcination and milling method (16). Niobium pentoxide (Nb_2O_5) was supplied by the Brazilian Metallurgy and Mining Company (CBMM). The Nb_2O_5 composite was produced according to powder metallurgy techniques by mixing the respective powders, BCP and Nb_2O_5 , in a 1:1 ratio (vol.%), and was designated as BCP/Nb. The mixture was then milled in a Retsch® PM 100 high-energy ball mill (Haan, Germany) for 3 h at 300 rpm, using a zirconia grinding jar and zirconia balls with a ball-to-powder mass ratio of 6:1 (17,18).

From the BCP and BCP/Nb powders, porous granules (scaffolds) of approximately 1–2 mm in diameter were produced and used as grafting materials. The method for obtaining the granules is under patent application and, for this reason, will not be described.

2.2. Preparation of Platelet-Rich Plasma (PRP)

PRP was obtained according to Wilson (2006) (19). Allogeneic PRP was collected and used on the same day as the animal procedures, without prior activation. The buffy coat method (19) generates non-activated platelets, which become activated upon contact with tissues, forming a fibrin network and clot (20). Ten male Wistar rats (550–600 g) were anesthetized with 2% xylazine (20 mg/kg) and 10% ketamine (100 mg/kg) in a 1:1 ratio, intramuscularly. Blood was collected via cardiac puncture into tubes containing 3.2% sodium citrate (3 ml), centrifuged at 1,800 rpm for 15 min, and 600 μL of PRP was then obtained directly from the buffy coat. Platelet counts were performed at a commercial laboratory, and the material was used within 24 h, stored at 4 °C. Platelet quantification in whole blood was also performed as a control.

2.3. In Vivo Experimental Procedure

Thirty male Wistar rats were used (CEUA n° 6263280422). Following preventive analgesia (0.06 mg/kg fentanyl IM) 20 minutes before the procedure, the animals were anesthetized intramuscularly with a combination of 80–100 mg/kg ketamine, 5–10 mg/kg xylazine, and 1 mg/kg acepromazine. After hair removal from the cranial region and antisepsis with 10% povidone-iodine, a transverse skin incision was made from the base of one ear to the other to expose the periosteum and reach the calvaria.

In each animal, a critical-size defect (CSD) of 8 mm in diameter and 0.8 mm in depth was created in the parietal bones, using the sagittal suture as a reference. The defect was produced with a trephine drill (Neodent®) mounted on a straight handpiece (Kavo®) connected to a surgical electric motor (Branemark System®) at 1,500 rpm under abundant irrigation with sterile saline.

Porous granules of bioceramics were grafted into each defect. Based on the material used, the following groups were established: (1) BCP, (2) BCP/PRP, (3) BCP/Nb, and (4) BCP/Nb/PRP. In the PRP groups, 50 μL of allogeneic PRP was added after grafting the bioceramics. The periosteum was then repositioned, the skin sutured with 4-0 silk (Ethicon®), and 1% aqueous povidone-iodine solution was applied topically.

After recovery from anesthesia, the animals were housed individually under controlled environmental conditions (12-hour light/dark cycle, 22 ± 2 °C, 50% humidity) with free access to food and water. At 45 days post-grafting, the animals were euthanized with an overdose (double dose) of intramuscular ketamine and xylazine.

Histopathology: Following euthanasia, calvarial samples were collected, washed, and fixed in 4% paraformaldehyde, then decalcified in Morse solution for 20 days. After decalcification, the samples were



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processed for paraffin embedding. Semi-serial sections of 7 μ m thickness were obtained and stained with Hematoxylin-Eosin and Azan.

Microcomputed Tomography (μ CT): Samples were analyzed by microcomputed tomography (μ CT) (SkyScan 1272; School of Dentistry, São Paulo State University 'Júlio de Mesquita Filho,' Araçatuba Campus) to quantify bone formation in critical-size defects. For this purpose, the samples were fixed in 4% paraformaldehyde and stored in 70% ethanol. Excess liquid was air-dried for 15 minutes before image acquisition, and the samples were positioned in the sample holder. Nrecon, DataViewer, and CTAn software were used for reconstruction, visualization, and quantitative analyses. Quantitative analyses are currently in progress.

3. RESULTS

Microcomputed Tomography: Micro-CT images revealed new bone formation within and around the granules, indicating that the materials function as a structural support (osteoconductivity), and showed bone formation in the connective tissue between the granules, demonstrating osteoinduction (induction of undifferentiated cells into bone cells). The identification of new bone tissue in the groups enriched with niobium pentoxide (Nb_2O_5) was challenging due to the high radiopacity of the metal. In contrast, in the groups that received only BCP (biphasic calcium phosphate), with or without platelet-rich plasma, bone formation was clearly evident. Quantitative analyses of these images are still in progress (Figure 1).

Histological Study: Histological analysis confirmed the presence of new bone formation in all groups. Cell and vessel migration into the pores was evident in the images. Different degrees of osteogenic matrix maturation were observed within the pores across all animal groups. In the BCP/Nb and BCP/Nb-PRP groups, a considerable number of multinucleated giant cells were observed. No fibrosis or inflammatory response related to the material was detected. In animals that received PRP, numerous erythrocytes were still visible in the connective tissue between the bioceramic granules (Figure 2).

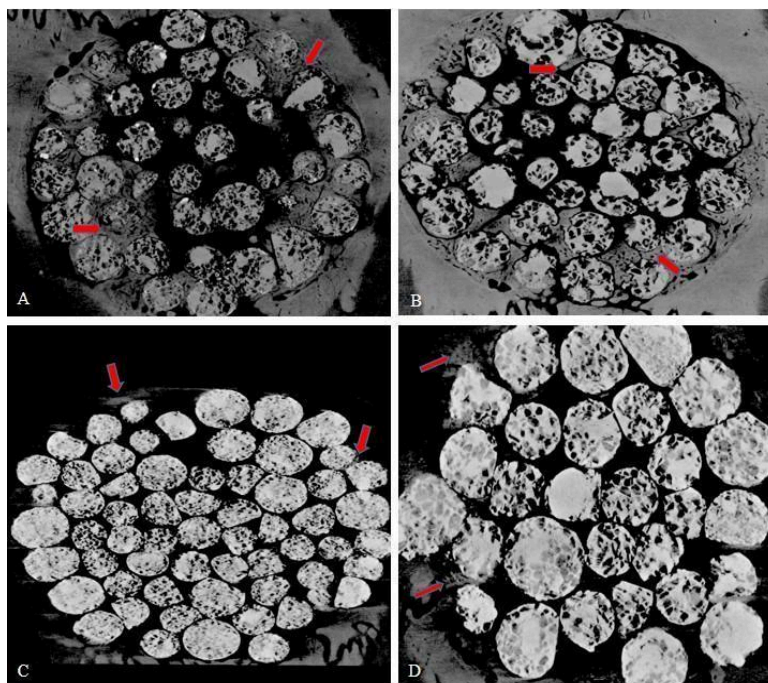


Figure 1. Representative photomicrographs of Critical-size calvarial defects in rats, 45 days post-grafting. (A) Defects grafted with BCP; (B) BCP-PRP; (C) BCP/Nb; (D) BCP/Nb-PRP. Red arrows indicate areas where newly formed bone tissue can be observed.



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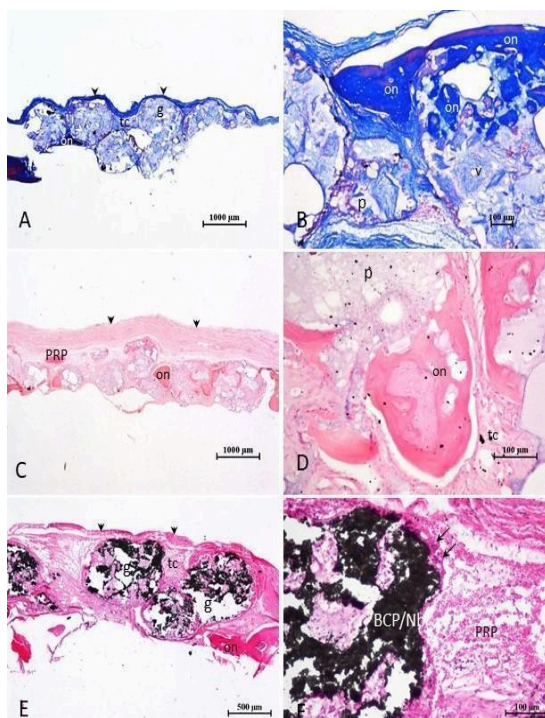


Figure 2. Representative photomicrographs of rat calvaria after craniotomy, grafted with BCP (A–B), BCP-PRP (C–D), and BCP/Nb-PRP (E–F). The images show the distribution of granules (g) with connective tissue (ct) present between them, and newly formed bone (nb). In B, higher magnification reveals more abundant newly formed bone and the presence of material pores (p). In C, newly formed bone (nb) can be seen within the pores and PRP (PRP) between the granules, with the periosteum indicated by an arrowhead; in D, the higher magnification highlights newly formed bone (nb), surrounding connective tissue (ct), and material pores (p). In E, newly formed bone at the periphery (nb), granules (g), and connective tissue (ct) between them are observed, while in F, PRP (PRP) is seen surrounding the BCP/Nb material. Staining: AZAN (A–B) and H&E (C–F).

4. DISCUSSION

The results of this study demonstrated that BCP derived from fish bones, enriched or not with Nb_2O_5 or PRP, exhibited both osteoconductive and osteoinductive properties. This finding corroborates previous studies describing calcium phosphate bioceramics as biocompatible and bioactive materials with the potential to integrate with host tissue (4,6,9). Moreover, their natural origin reinforces the sustainability of the production process, aligning with current perspectives in biomaterials research (16). These descriptive results are preliminary. Quantitative analyses by histomorphometry and microcomputed tomography are ongoing to assess whether enrichment with PRP or Nb_2O_5 has an additive effect on bone formation.

We have studied the biological and physicochemical properties of calcium phosphate ceramics extracted from fish bones, enriched or not with niobium pentoxide (Nb_2O_5). Non-porous (bulk) implants of BCP and its composite with Nb_2O_5 (BCP/ Nb_2O_5), when implanted in critical-size defects in rat calvaria, were biocompatible, bioactive, and osteoconductive, and were capable of osteointegration with the living tissue (21,22). In the form of scaffolds, disc-shaped implants functioned as a framework for cells, matrix, and newly formed bone (23). To evaluate the osteoinductive potential of the implants, two ectopic implantation models were studied: subcutaneous and subfascial, in which a bone-like matrix was observed within the material pores (24). In the present study, we are evaluating the osteogenic potential of these materials in the form of porous granules to be used as bone substitutes in grafting procedures.

The efficacy of combining calcium phosphate-based materials with PRP in bone regeneration has not yet been fully established, with conflicting results in the literature, sometimes reporting increased bone formation rates (25) and sometimes showing no significant differences (26). In a recent study (unpublished data), we demonstrated that the addition of PRP to BCP promoted a remarkable transient boost in



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osteogenesis at 45 days, highlighting the potential of PRP to accelerate the early stages of bone regeneration (14).

5. CONCLUSIONS

The study demonstrated that biphasic calcium phosphate (BCP) bioceramics, sustainably derived from fish bones, are promising biomaterials, as they exhibit both osteoconductive properties (serving as a scaffold for bone growth) and osteoinductive properties (stimulating new bone formation). Histological and microcomputed tomography analyses suggest that their association with platelet-rich plasma (PRP) and/or niobium pentoxide (Nb_2O_5) does not hinder new bone tissue formation, with bone trabeculae and mineralized matrix observed in close association with the graft particles. The results of this study also highlight the importance of employing multiple analytical approaches to interpret bone regeneration in experimental studies.

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Thematic área: Pathogens of clinical relevance.



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SYSTEMIC MASTOCYTOSIS ASSOCIATED WITH PRIMARY MYELOFIBROSIS: A RARE CASE REPORT WITH MOLECULAR EVIDENCE

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1. INTRODUCTION

Systemic mastocytosis (SM) is a rare clonal disorder defined by abnormal proliferation and accumulation of mast cells in one or more organs [1]. The World Health Organization recognizes six entities within the SM spectrum [2]. Its clinical presentation ranges from indolent variants to aggressive forms with organ damage. The advanced forms comprise systemic mastocytosis with an associated hematologic neoplasm (SM-AHN), aggressive systemic mastocytosis, and mast cell leukemia, with SM-AHN being the most frequent. SM-AHN is defined as clonal systemic mastocytosis coexisting with an independent hematologic neoplasm — most often myeloid — in which the AHN component must be diagnosed and graded by its own WHO criteria, in addition to fulfilling diagnostic criteria for SM [1–2]. The hematologic neoplasms most frequently associated with SM are predominantly myeloid and include chronic myelomonocytic leukemia (CMML), other MDS/MPN overlap neoplasms (e.g., atypical CML, MDS/MPN-U), myelodysplastic syndromes and classical myeloproliferative neoplasms (essential thrombocythemia and polycythemia vera); acute myeloid leukemia is less common [1,3] (Fig1). Primary myelofibrosis (PMF) is a chronic myeloproliferative neoplasm driven by mutually exclusive mutations in JAK2, CALR, or MPL [1–2], and is characterized histologically by marrow hypercellularity with atypical megakaryocytic proliferation and graded reticulin/collagen fibrosis (MF-0 to MF-3) [2]. SM is defined histologically by multifocal dense aggregates of mast cells (≥ 15 cells per cluster), mast-cell atypia, and aberrant expression of CD25/CD2 (and sometimes CD30) with elevated baseline serum tryptase in the appropriate context [1–2]. The coexistence of SM and PMF is exceptionally rare, and to our knowledge—consistent with Xu and Xu (2018). This association appears exceptionally rare in the literature and, to our knowledge, this is the first reported case to include next-generation sequencing–based mutational profiling [4].

In this context, we describe a unique case of SM associated with PMF, supported by histopathological and molecular findings, and discuss its clinical relevance [1,3]. The incidence of advanced systemic mastocytosis is approximately 0.9 cases per million inhabitants [5].

2. MATERIALS AND METHODS

We conducted a clinical case investigation with systematic collection of medical history, physical examination, and laboratory data, followed by imaging and bone marrow studies. Bone marrow trephine biopsy was processed with hematoxylin–eosin and reticulin staining, and immunohistochemistry included CD117 and CD30 to highlight mast-cell infiltrates and aberrant antigen expression. Peripheral-blood next-generation sequencing employed a validated myeloid panel (DNA and RNA) with high depth of coverage and predefined thresholds for variant calling, followed by expert review of raw data to adjudicate subthreshold but clinically relevant variants. Therapeutic interventions—including cytoreductive cladribine for prompt control of hepatic dysfunction, initiation of midostaurin on 29 October 2023, and allogeneic hematopoietic stem-cell transplantation in August 2024—were abstracted from the clinical record for longitudinal assessment.

3. RESULTS



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Across published series, the hematologic neoplasms associated with systemic mastocytosis (SM-AHN) cluster predominantly within the myeloid compartment: chronic myelomonocytic leukemia (CMML) and other MDS/MPN overlap entities constitute the largest share, followed by myelodysplastic syndromes and classical myeloproliferative neoplasms (essential thrombocythemia and polycythemia vera); acute myeloid leukemia is comparatively infrequent, and lymphoid AHN are rare. Primary myelofibrosis (PMF) is deliberately omitted from Figure 1 to underscore its exceptional rarity among SM-AHN presentations. In our case, the bone marrow biopsy was diffusely hypercellular (~100%) with granulocytic hyperplasia and striking dysmegakaryopoiesis—large, pleomorphic, hyperchromatic megakaryocytes arranged in cohesive clusters—consistent with a PMF-like morphology; reticulin staining showed MF-1 fibrosis with focal MF-2, and osteosclerosis was graded as 2. Numerous mast cells formed aggregates exceeding fifteen cells, displaying spindle and epithelioid morphology; immunohistochemistry showed diffuse CD117 positivity with partial aberrant CD30 expression. Together, these findings supported PMF in fibrotic evolution coexisting with SM in the same marrow sample. Supporting systemic mastocytosis in the same specimen. (Figure 2A and 2B). Next-generation sequencing detected MPL c.1544G>T (p.Trp515Leu; VAF 23.2%) and KIT c.2447A>T (p.Asp816Val, D816V; VAF 4.8%), consistent with the PMF and SM components, respectively; additional variants included TET2 p.Gln1051ArgfsTer4 (43.7%) and p.Gln1348Pro (37.6%), SRSF2 p.Pro95Arg (39.5%), and SH2B3 p.Gly220Trp (22.1%). A technical addendum noted that KIT D816V—initially below the routine reporting threshold (~5%)—was confirmed by manual review of raw reads (307/6,414).(Table 1)

3.1 Prevalence

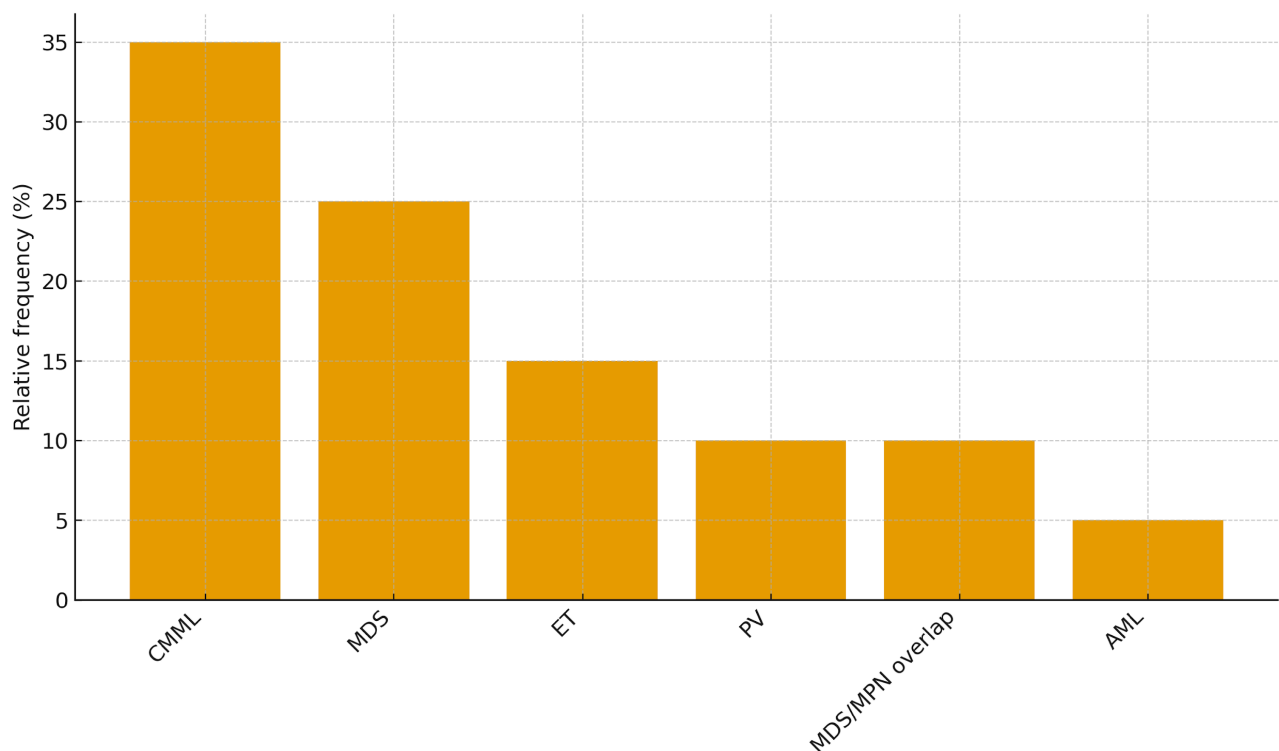


Figure 1. Most common hematologic neoplasms associated with systemic mastocytosis (SM-AHN). Illustrative proportions adapted from published cohorts; distribution varies. Primary myelofibrosis is rare and intentionally excluded.

3.2 Bone marrow findings



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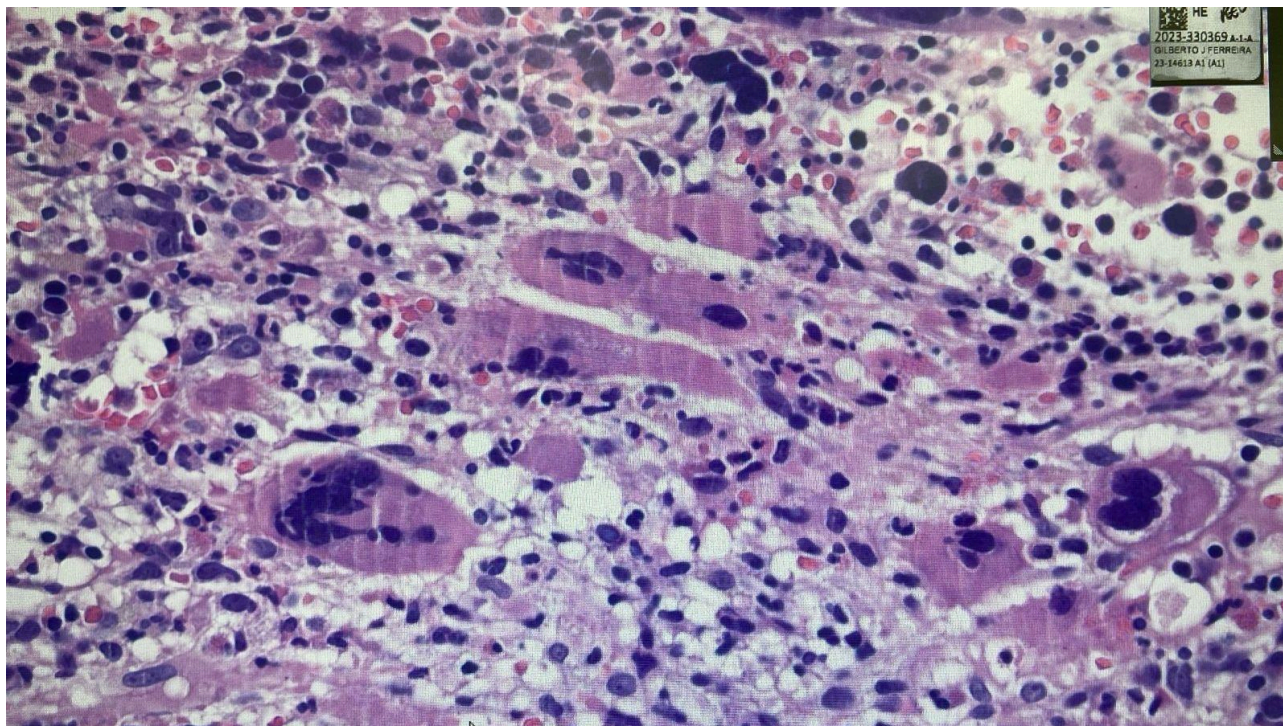


Figure 2A. Bone marrow biopsy (H&E). Representative high-power field showing clusters of atypical megakaryocytes within a fibrotic marrow, alongside mast-cell aggregates (>15 cells).

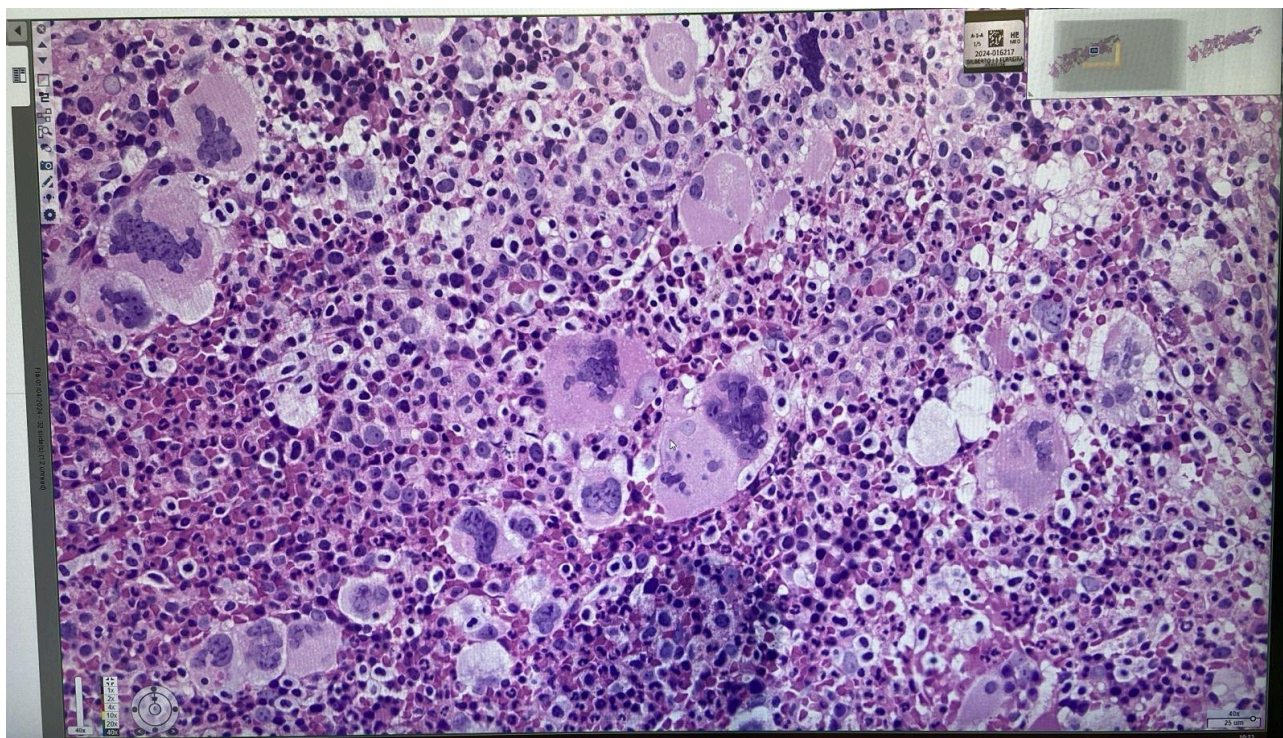


Figure 2B. Bone marrow biopsy (H&E). Hypercellular marrow with clustered pleomorphic megakaryocytes and background mast-cell infiltrates; features consistent with PMF coexisting with SM.



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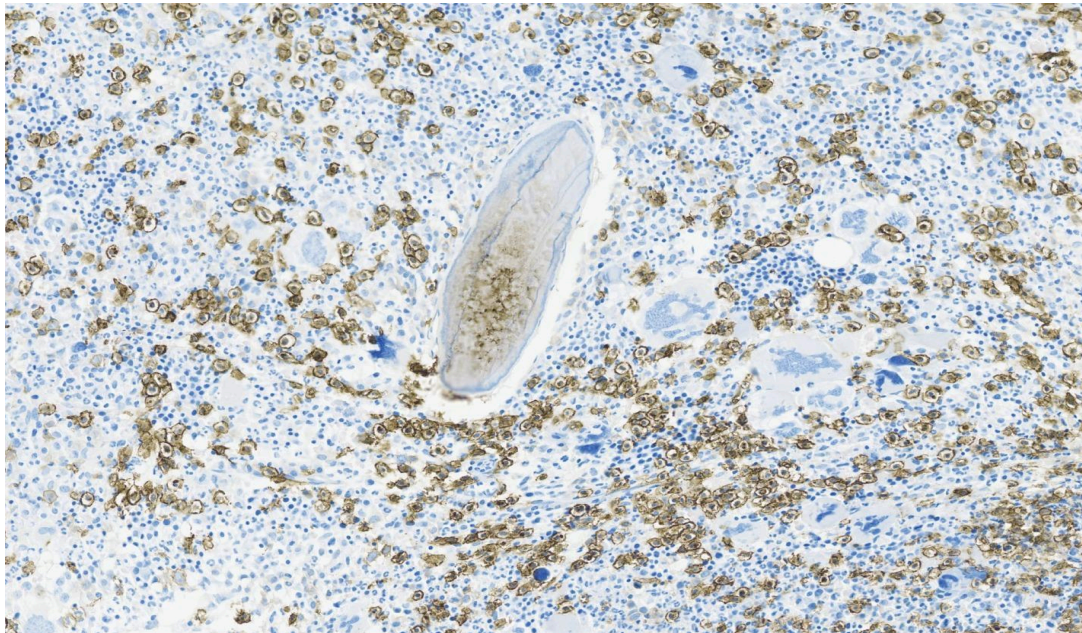


Figure 2C. Bone marrow biopsy. Immunohistochemistry showed diffuse CD117 positivity.

3.3 Next-generation sequencing

Table 1. Summary of somatic variants detected by next-generation sequencing

Gene	cDNA	Protein	VAF (%)	Tier	Interpretation
MPL	c.1544G>T	p.Trp515Leu	23.2	1	PMF driver
KIT	c.2447A>T	p.Asp816Val (D816V)	4.8	1	SM hallmark
TET2	c.3150del	p.Gln1051ArgfsTer4	43.7	1	Tumor suppressor
TET2	c.4043A>C	p.Gln1348Pro	37.6	3	Variant of uncertain significance
SRSF2	c.284C>G	p.Pro95Arg	39.5	1	Splicing factor
SH2B3	c.658G>T	p.Gly220Trp	22.1	3	Variant of uncertain significance

VAF: variant allele frequency; Tier per ASCO/CAP/AMP; KIT D816V initially filtered for VAF slightly below 5%, but confirmed on manual review.

3.4 Clinical course

Initial management comprised cytoreductive cladribine to achieve rapid control of hepatic dysfunction, followed by initiation of midostaurin on 29 October 2023 with clinical stabilization. Given disease risk in SM-AHN, the patient underwent allogeneic hematopoietic stem-cell transplantation in August 2024, but primary graft failure ensued. After graft failure, there was recurrence of systemic mastocytosis; midostaurin was reintroduced—this medication is typically withheld in the peri-transplant period in expectation of cure—with renewed disease control [1, 6–7]. To increase the probability of engraftment before a second attempt, splenectomy was elected, and a second unrelated-donor allogeneic transplantation is scheduled for early 2026. Primary myelofibrosis, given its comparatively indolent clinical course, does not currently require disease-specific therapy; however, its only curative option is likewise allogeneic transplantation [1]. Accordingly, the patient's only curative avenue for both diseases remains allogeneic hematopoietic stem-cell transplantation. He currently remains in excellent general condition while receiving midostaurin. Responses to midostaurin in advanced systemic mastocytosis can be durable in responders:



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median durations of response around two years have been reported, and control lasting approximately 2–5 years or longer is observed in real-world series [6–7].

4. DISCUSSION

This case underscores the central role of bone marrow examination in the recognition of concomitant SM and PMF. Recognition of dense mast-cell aggregates with atypical morphology, along with aberrant CD30 expression and KIT D816V, was pivotal to establishing SM, while megakaryocytic morphology and graded reticulin fibrosis substantiated PMF. The molecular profile supports a model of shared clonal hematopoiesis with lineage divergence: MPL W515L/K, a defining PMF driver, and KIT D816V, a canonical SM lesion, co-occurred with TET2 and SRSF2, which are frequently mutated across myeloid neoplasms. From a nosologic standpoint, the overlap between SM and PMF remains exceptionally rare. Consistent with Xu and Xu (2018), the present case appears to be only the second reported instance to date, the first being identified solely in a bone marrow specimen without longitudinal clinical characterization. Therapeutically, goals must be reconciled across both components: cytoreductive agents (e.g., cladribine) may provide rapid control of mast-cell-related organ dysfunction, KIT-directed therapy (e.g., midostaurin) can reduce mast-cell burden, and transplant candidacy hinges on PMF risk stratification, comutation profile and patient fitness. The graft failure observed here illustrates the guarded prognosis and reinforces the importance of meticulous pre-transplant optimization and vigilant post-transplant surveillance. Methodologically, this case highlights the value of manual review for subthreshold variants—exemplified by the KIT D816V call—and of integrating histology, immunohistochemistry and genomics to overcome the interpretive challenges posed by fibrosis and multilineage involvement.

5. CONCLUSION

We describe an exceptionally rare coexistence of systemic mastocytosis and primary myelofibrosis substantiated by histopathology and targeted genomics. Bone marrow biopsy was decisive, and the combined detection of KIT D816V and MPL W515L supports a shared clonal origin with divergent differentiation. To our knowledge, this appears to be the second reported case to date, the first having been identified solely on a bone marrow specimen. These observations reinforce the value of integrated diagnostics and multidisciplinary management in complex marrow presentations.

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Keywords: associated hematologic neoplasm (sm-ahn); bone marrow biopsy; next-generation sequencing.

Thematic area: Etiology and physiopathology of human and animal diseases.



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GENETIC POLYMORPHISMS IN *GSTP1* AND *XRCC1* GENES AND THEIR ASSOCIATION WITH BUCCAL CELL ANOMALIES

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1. INTRODUCTION

The extensive and often unregulated use of pesticides, in agricultural powerhouses like Brazil, represents a significant public health concern. As one of the world's largest consumers of these chemicals, Brazil's economy relies heavily on both large-scale agribusiness and family farming. This economic growth has consequently led to a substantial increase in occupational pesticide exposure for agricultural Workers (1). This vulnerable population group experiences continuous and often simultaneous contact with multiple compounds over prolonged periods, leading to exposure through various routes, including skin contact, inhalation, or ingestion (2).

While the toxic effects of pesticides are well-documented, the individual response to this exposure can vary significantly. This variability is largely influenced by a combination of physiological and genetic factors, including diet, age, and genetic polymorphisms. Crucially, variations in genes that encode enzymes for xenobiotic metabolism and DNA repair play a pivotal role (1). The glutathione transferases (GSTs), which are central to the detoxification of various pesticides, are highly polymorphic. When these detoxification pathways are rendered less effective due to genetic polymorphisms (in the *GSTP1* gene), toxic metabolites can accumulate and cause damage to intracellular molecules. Similarly, genes involved in DNA repair, such as X-ray repair cross-complementing group 1 (*XRCC1*), are critical for mitigating genotoxic damage. Polymorphisms in *XRCC1*, such as those at exon 6 (Arg194Trp) and exon 10 (Arg399Gln), have been consistently linked to an elevated risk of DNA damage in pesticide-exposed populations. These genetic variations, therefore, represent key biomarkers of susceptibility that can help identify individuals at greater risk (3).

To assess the genotoxic impact of this occupational exposure, biomonitoring tools are essential. The Buccal Micronucleus Cytome (BM cyt) assay is a particularly valuable method. As a minimally invasive technique, it not only detects micronuclei (MN), which are well-established indicators of chromosomal damage, but also provides a comprehensive view of cellular health by identifying other nuclear abnormalities such as binucleated cells, nuclear buds, and pyknosis. This assay also offers insights into cell proliferation, making it a robust and comprehensive tool for evaluating genotoxic risk in exposed populations (4).

Despite extensive research, a significant gap remains in understanding the specific interplay between these genetic susceptibility factors and the measurable genotoxic effects in agricultural workers. A deeper understanding of how specific genotypes correlate with biomarkers of genotoxic damage, such as those measured by the BM cyt assay, is crucial for developing targeted public health strategies and improving occupational safety standards. The aim of this study was to evaluate association of DNA damage with polymorphisms in the *GSTP1* and *XRCC1* genes in workers occupationally exposed to pesticides in Northwest of Paraná.

2. MATERIALS AND METHODS



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2.1. Study population

We studied 64 pesticide applicators and 19 non-applicators. Both groups were recruited from the northwest of Paraná State, Brazil. The study was approved by the Brazilian National Research Ethics Committee (CAAE 31596620.9.0000.0104) and informed consent was obtained from each subject. Questionnaires (personal, occupational, environmental, and medical data) were completed during a personal interview.

2.2. Genotyping

A blood sample (2 mL) was collected from each participant by venipuncture for DNA extraction.

Genomic DNA was extracted from whole blood samples using a spin-column-based method with the Kit Mini Spin Plus (Mobius Life). All procedures were carried out following the protocol provided by the manufacturer. The Ile to Val substitution in *GSTP1* exon 5 (codon 105) was amplified using the following conditions: denaturation at 94°C for 30s, annealing at 61°C for 30s, and extension at 72°C for 30s. The PCR products were digested with *BsmA*I for 24h at 37°C and then electrophoresed on 3,5% agarose. Arg to Trp substitution in *XRCC1* exon 6 (códon 194) amplification was conducted at 95°C for 30s for denaturation, annealing at 59°C for 35s, and extension at 72°C for 45s followed by digestion with *Pvu*II. The product was identified by gel electrophoresis using 2,5% agarose. Arg to Gln substitution in exon 10 (codon 399) amplification was done at 95°C for 30s for denaturation, annealing at 61 °C for 35s, and extension at 72°C for 45s. The PCR products were digested with *Msp*I and analyzed on 2% agarose. All digested PCR products were analyzed in agarose gel and detected with 10 mg/ml ethidium bromide. Electrophoresis was conducted at 70 V.

2.4. Cytogenetic Analysis: Buccal Micronucleus Cytome (BMCyt) Assay

Genotoxic damage and cellular alterations in the buccal mucosa were evaluated using the Buccal Micronucleus Cytome (BMCyt) assay, a minimally invasive and well-validated method. The protocol was performed according to Benedetti et al. (2013) with some modifications. Buccal epithelial cells were collected from the participants and used to prepare smears. The slides were subsequently fixed and stained using the Schiff method, which is highly specific for DNA, allowing for a precise identification of nuclear structures and micronuclei.

2.5. Statistical analysis

Statistical analysis was performed with Stata version 17.0. The relationship between genetic polymorphisms and cellular anomalies was evaluated using the Negative Binomial regression model for count data. This model was chosen to account for overdispersion in the data. Additionally, a Chi-square test was performed to examine the association between the exposure status and the distribution of each genotype. All statistical analyses were conducted with a significance level of $p < 0.05$.

3. RESULTS

The results indicate that there was no statistically significant association between pesticide exposure and the frequency of nuclear anomalies (IRR= 0.651; 95% CI: 0.356 - 1.192; $p = 0.164$). This finding suggests that, based on our preliminary data, the rate of nuclear anomalies in the non-applicator group was approximately 35% lower than in the applicator group, though this difference did not reach statistical significance.

Table 1. Incidence Rate Ratio (IRR) of Nuclear Anomalies in Pesticide Applicators and Non-Applicators



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Variable	Group	n	IRR (95% CI)	p-value
Nuclear Anomalies	Non-Applicators	19	0.651 (0.356 - 1.192)	0.164
	Applicators	64	1.00 (Reference)	

To investigate the role of genetic susceptibility factors, a Negative Binomial regression model was fitted to analyze the association between genetic polymorphisms and the frequency of nuclear anomalies. The analysis revealed a statistically significant association between the *XRCC1* Arg194Trp polymorphism and nuclear anomalies. Individuals carrying the Arg/Trp genotype showed an Incidence Rate Ratio (IRR) of 2.155 (95% CI: 1.074 - 4.324, $p = 0.031$), indicating that the rate of anomalies in this group was more than double the rate observed in the wild-type (Arg/Arg) group. No statistically significant associations were found for the *GSTP1* polymorphism ($p = 0.310$ for the Ile/Val and $p = 0.544$ for the Val/Val genotypes) or the *XRCC1* exon 10 (Arg399Gln) polymorphism ($p = 0.112$ for the Arg/Arg and $p = 0.485$ for the Gln/Gln genotypes). These findings suggest that the *XRCC1* Arg194Trp gene variant may be a key determinant of an individual's susceptibility to genetic damage in this exposed population.

Table 2. Incidence Rate Ratios (IRRs) of Nuclear Anomalies in Relation to Genetic Polymorphisms in *GSTP1* and *XRCC1* Genes.

Variable	Genotype	IRR (95% CI)	p-value
<i>GSTP1</i>			
(Reference = Ile/Ile)	Ile/Val	0.725 (0.389 - 1.350)	0.310
	Val/Val	1.316 (0.542 - 3.191)	0.544
<i>XRCC1</i> (Arg194Trp)			
(Reference = Arg/Arg)	Arg/Trp	2.155 (1.074 - 4.324)	0.031*
<i>XRCC1</i> exon 10 (Arg399Gln)			
(Reference = Arg/Gln)	Arg/Arg	0.606 (0.326 - 1.124)	0.112
	Gln/Gln	0.688 (0.241 - 1.965)	0.485

4. DISCUSSION

Previous studies have been conducted to investigate the genotoxicity of pesticides in human biomonitoring assessment(5, 6). However, there is little information in the scientific literature regarding the assessment of mutagenic occupational exposure using the buccal micronucleus assay associated with *GSTP1* and *XRCC1* genes.

Although the association between pesticide exposure and the frequency of nuclear anomalies did not reach statistical significance ($p = 0.164$), it is notable that the non-applicator group exhibited a lower rate of anomalies compared to the applicators (IRR = 0.651). This finding suggests a potential trend that warrants further investigation, especially in studies with larger sample sizes that can provide greater statistical power. These results are consistent with the hypothesis that chronic occupational exposure may contribute to the accumulation of cytogenetic damage, a process that can be further influenced by an individual's genetic background.

The *XRCC1* gene encodes a protein that is fundamental to the repair of single-strand DNA breaks, a crucial process for maintaining genomic stability. The variation in the *XRCC1* gene can influence DNA repair capacity. This occurs because the presence of the variant allele may decrease the protein's binding affinity to



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DNA damage, impacting the effectiveness of the repair process(7). The present study revealed a statistically significant association between the *XRCC1* Arg194Trp polymorphism and the frequency of nuclear anomalies in rural workers exposed to pesticides. It was demonstrated that individuals carrying the Arg/Trp genotype have an incidence rate of anomalies more than twice that of the wild-type genotype.

Although the association between exposure and anomalies did not reach statistical significance in our simple model, nor did the variations in the *GSTP1* and *XRCC1* exon 10 genes, this could be attributed to several factors. The relatively small sample size may have limited the statistical power of the study to detect effects of smaller magnitude.

However, these polymorphisms have been associated with increased DNA damage in studies conducted on populations exposed to pesticides, such as that by Saad-Hussein et al. (2017), who found an association with the *GSTP1* Ile-Ile and *XRCC1* 399 Arg-Arg genotypes, and by Aldana-Salazar et al. (2024), who associated heterozygous *GSTP1* and *XRCC1* genotypes with greater chromosomal instability.

These discrepancies can be attributed to multiple factors. Methodological differences, such as the use of the buccal micronucleus cytome assay in this study as opposed to the comet assay in lymphocytes, can influence the results. Genetic variation between populations, as well as the diversity of pesticide types and exposure levels, are also determining factors that can explain the differences between studies.

Despite these divergences, our results reinforce the importance of occupational genetic risk assessment. *XRCC1* is crucial in base excision repair, while *GSTP1* plays a fundamental role in the detoxification of xenobiotics. The relevance of this research is underscored by the pesticide exposure in the public health context in Brazil, which is one of the world's largest consumers of these substances. The identification of the *XRCC1* Arg194Trp polymorphism as a susceptibility biomarker, contributes significantly to the field of rural worker health, highlighting the need to consider the genetic profile for the prevention and monitoring of adverse health effects related to chronic pesticide exposure.

5. CONCLUSION

This study demonstrated that the *XRCC1* Arg194Trp polymorphism is significantly associated with increased genotoxic damage in farmers chronically exposed to pesticides. The detection of a higher incidence of nuclear anomalies in individuals with the Arg/Trp genotype highlights the importance of considering individual genetic susceptibility in occupational risk assessment. The findings contribute to knowledge on the interaction between genetic factors and environmental exposure. The identification of this susceptibility biomarker in a Brazilian population reinforces the need for the implementation of genetic monitoring and health prevention measures aimed at rural workers.

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Keywords: genetic polymorphism; genotoxicity; pesticide exposure.

Thematic area: Eco-epidemiology and disease control.



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RESUMO

SIMPLES





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AValiação da Ação de Metabólitos Especializados de *Aspergillus fumigatus* EM Modelos Alternativos aos Animais

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Introdução: Biofilmes são comunidades microbianas aderidas a superfícies, protegidas por uma matriz de biofilme (MB) que oferece resistência a condições adversas. O fungo filamentoso *Aspergillus fumigatus* forma biofilmes bem estruturados, nos quais a matriz desempenha papel essencial na adesão e na virulência. Já a levedura polimórfica *Candida albicans* destaca-se como patógeno oportunista de grande relevância clínica, especialmente por causar infecções em indivíduos imunocomprometidos. Métodos alternativos aos animais, como modelos *in vitro* e invertebrados, são essenciais para pesquisas científicas, em conformidade aos padrões éticos e regulatórios. Entre essas estratégias destaca-se o uso de larvas de *Tenebrio molitor*. **Objetivo:** Avaliar os efeitos dos metabólitos especializados de *A. fumigatus* em modelo alternativo ao uso de animais.

Material e métodos: Foram utilizados os fungos *A. fumigatus* (CMRP 5666) e *C. albicans* (ATCC 90028). Biofilmes de *A. fumigatus* foram formados em caldo sabouraud dextrose, seguidos de extração da MB por sonicação e filtração. Testes de suscetibilidade determinaram a concentração inibitória mínima (CIM) e fungicida mínima (CFM) contra *C. albicans*, além da avaliação da biomassa do biofilme por cristal violeta. Foram usadas larvas de *T. molitor* como modelo alternativo para testar a letalidade da MB e do fungo, com acompanhamento da sobrevivência por 10 dias. **Resultados:** Os metabólitos especializados de *A. fumigatus* não apresentaram atividade inibitória direta contra *C. albicans*, porém modularam a formação do biofilme da levedura, exibindo efeito antagonista em baixas concentrações e estimulante em concentrações elevadas. Na avaliação em larvas de *T. molitor*, a MB causou 52% de mortalidade, superior aos 35% observados com inoculação fúngica, demonstrando toxicidade significativa desses metabólitos no modelo invertebrado. **Conclusão:** Os metabólitos especializados de *A. fumigatus* não inibem diretamente *C. albicans*, mas modulam sua formação de biofilme, interferindo em um importante mecanismo de virulência. Além disso, apresentam toxicidade em larvas de *T. molitor*, evidenciando potencial patogênico. Esses resultados destacam a relevância da busca por novas terapias antifúngicas mais eficazes.

Palavras-chave: biofilme; *Tenebrio molitor*; antifúngico.

Área temática: Patógenos de interesse médico.



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PERFIL DOS CASOS DE INTOXICAÇÕES POR AGROTÓXICOS NO PARANÁ NA ÚLTIMA DÉCADA

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Introdução: A utilização de agrotóxicos tanto na agricultura como nas residências e demais atividades, é comum e amplamente praticada no País. Embora utilizados para obter maior produtividade, seu uso inadequado, a exposição acidental e a ingestão desses produtos representam sérios riscos à saúde podendo levar a graves sequelas e até mesmo à morte. Desse modo, é importante compreender o perfil das vítimas e identificar as principais circunstâncias envolvidas. **Objetivo:** Analisar o perfil dos casos de intoxicações por agrotóxicos notificadas no estado do Paraná nos últimos dez anos.

Material e métodos: Estudo descritivo com base em dados secundários do Sistema de Informação de Agravos de Notificação, referentes às notificações de intoxicação exógena por agrotóxicos e raticidas no período de 2015 a 2024 no Estado do Paraná, com o uso dos filtros: sexo, circunstância, relação com o trabalho e faixa etária. **Resultados:** Entre 2015 e 2024, foram registrados 14.143 casos de intoxicação por agrotóxicos, dos quais 60,5% (n=8.562) envolveram homens e 39,5% (n=5.581) mulheres, com a faixa etária prevalente de 20 a 39 anos 40,7% (n= 5.766). As principais circunstâncias notificadas foram: tentativa de suicídio (n= 6.467; 45,7%); causas acidentais (n= 5.123; 36,2%) e uso habitual (n= 1.283; 9,07%). Do total de casos, 21,3% (n=3.007) tiveram relação com a exposição no trabalho. **Conclusão:** Com base nos dados obtidos, observou-se predominância de casos entre homens jovens adultos, com destaque para tentativa de suicídio e acidentes como principais causas, sendo a maioria não relacionada ao trabalho. Esses resultados ressaltam a importância de estratégias de prevenção para esse público em questão.

Palavras-chave: agrotóxico; intoxicação; epidemiologia.

Financiador (es): Fundação Araucária - Programa Universidade Sem Fronteiras e CAPES.

Área temática: Eco-epidemiologia e controle de doenças.



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VESÍCULAS EXTRACELULARES DE *Trypanosoma cruzi* E SUA INFLUÊNCIA NA PATOGENIA DA DOENÇA

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Introdução: A doença de Chagas, causada por *Trypanosoma cruzi*, apresenta fase aguda frequentemente assintomática e fase crônica com lesões cardíacas e digestórias. Vesículas extracelulares de *T. cruzi* podem modular mastócitos intestinais, células-chave da rede neuroimune, influenciando degranulação e liberação de mediadores inflamatórios. Contudo, os efeitos específicos dessas vesículas extracelulares (VEs) sobre mastócitos permanecem restritos, representando um ponto crítico para compreender a patogênese gastrointestinal da doença de Chagas. **Objetivo:** Avaliar os efeitos de VEs derivadas da interação entre *T. cruzi* (cepas CL Brener e Dm28c) e cultura celular (C2C12) no processo patogênico de doença no jejuno de camundongos. A hipótese foi de que as VEs modulam a resposta imune intestinal e contribuem para alterações no sistema nervoso entérico. **Material e métodos:** Trata-se de um estudo experimental aprovado por Comitê de Ética (nº58644322022), realizado com 9 camundongos Balb/c, distribuídos em 3 grupos (n=3): Grupo controle, grupo infectado CL Brener e infectado tratado com VEs (Dm28c-C2C12). Após 28 dias de infecção, os animais foram eutanasiados, coletado jejuno e processado histologicamente. Cortes semi-seriados foram corados em Azul de Toluidina para quantificação de mastócitos em 100 campos microscópicos em objetiva de 100x. Considerando-se o valor de $p < 0,05$. **Resultados:** A análise da contagem de mastócitos no jejuno de camundongos infectados com *T. cruzi* e tratados com diferentes VEs não revelou diferenças estatisticamente significativas entre os grupos avaliados. **Conclusão:** Os resultados sugerem que, nas condições experimentais empregadas, as VEs derivadas de *T. cruzi* e de culturas celulares associadas à inoculação do parasito não exerceram impacto significativo na densidade de mastócitos do jejuno. Dessa forma, a hipótese de que as VEs modulam diretamente a resposta imune intestinal. Estudos adicionais, com maior número amostral e diferentes condições experimentais, são necessários para elucidar o papel das VEs na patogênese da doença de Chagas.

Palavras-chave: doença de Chagas; vesículas extracelulares; mastócitos.

Área temática: Patógenos de interesse médico.



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INTOXICAÇÃO AGUDA POR METANFETAMINAS: RELATO DE CASO E DESAFIOS NA ASSISTÊNCIA

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Introdução: O uso de substâncias psicoativas cada vez mais se torna um problema para a saúde pública no Brasil, com números crescentes de usuários, acarretando problemas para a sociedade em geral. A metanfetamina, considerada uma droga ilícita, tem efeitos estimulantes no sistema nervoso central com um alto potencial tóxico, seu consumo recreativo vem aumentando, resultando muitas vezes em casos graves de intoxicação aguda. **Objetivo:** Relatar um caso de intoxicação aguda por metanfetamina em paciente usuário de multidrogas, notificado a um Centro de Assistência e Informação Toxicológica (CIATox), com ênfase em características clínicas, procedimentos realizados e evolução do paciente. **Material e métodos:** Estudo de caso realizado por meio de análise da ficha de exposição humana notificada a um CIATox. Projeto aprovado pelo Comitê de Ética em Pesquisa, sob parecer n. 6.940.629. **Resultados:** Paciente de 46 anos, usuário de crack, foi atendido em serviço de saúde após ser encontrado desacordado em casa e socorrido via SAMU, apresentando paradas cardiorrespiratórias, alteração no nível de consciência e crises convulsivas. Na admissão foi entubado e submetido a ventilação mecânica, lavagem gástrica e uma dose de carvão ativado, exames laboratoriais complementares e triagem toxicológica, que resultou positiva para metanfetamina. Durante Internação em UTI, paciente necessitou de sedação contínua e suporte respiratório prolongado, evoluindo para extubação, porém, ainda com auxílio de traqueostomia respirando ar ambiente, já consciente e orientado. Após 18 dias de internação, houve melhora clínica, obtendo alta com encaminhamento para acompanhamento psiquiátrico. **Conclusão:** Este caso ilustra como a intoxicação aguda por metanfetamina pode evoluir com manifestações graves, exigindo um suporte clínico intensivo. O relato auxilia na compreensão das intoxicações, reforçando que o diagnóstico toxicológico célere, direcionando o manejo do paciente e a assistência fornecida pelo CIATox são importantes para redução da morbimortalidade associada ao uso de substâncias psicoativas.

Palavras-chave: metanfetamina; intoxicação; drogas de abuso.

Área temática: Eco-epidemiologia e controle de doenças.



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IMPACTO DA INATIVAÇÃO FOTODINÂMICA MEDIADA POR CURCUMINA NA BIOMASSA TOTAL DE BIOFILME IMATURO DE *Sporothrix brasiliensis*

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Introdução: A inativação fotodinâmica com curcumina (IFD-CUR) tem se mostrado eficaz contra células planctônicas de *Sporothrix brasiliensis*, mas pouco se sabe sobre a ação em biofilme. **Objetivo:** estudar a ação da IFD-CUR sobre a biomassa total do biofilme imaturo (após fase adesão) de *S. brasiliensis* na forma filamentosa. **Material e métodos:** inóculo (isolado 8678) foi ajustado em meio YPD ($2,4 \times 10^6$ conídios/mL) e incubado em placa de 24 poços/6 h/25 °C. Após, os poços foram lavados (2x) com tampão fosfato salino (PBS), e concentrações de CUR correspondentes a Concentração Fungicida Mínima (CFM; 0,78 µM), 2xCFM (1,56 µM), 4xCFM (3,125 µM) e 50 µM foram adicionadas. A CFM foi definida em estudos anteriores do grupo, como a menor concentração capaz de reduzir $\geq 3 \log_{10}$ nas unidades formadoras de colônia de células planctônicas. No controle positivo (C+, biofilme sem tratamento IFD-CUR), foi adicionada salina. A placa (inóculo + CUR) foi incubada a 25 °C/15 min, irradiada com LED azul (λ : 450 nm, 15 minutos; irradiância: 17,18 mW/cm²). Os poços foram lavados (1x/PBS) e adicionado YPD para nova incubação/72 h, formação do biofilme maduro. Ao término, os poços foram lavados (1x/PBS) e a biomassa total quantificada por cristal violeta. Os resultados foram expressos em absorbância absoluta, e comparados com C+. A análise foi realizada com Teste t de *Student* (Software Prism 8.0). **Resultados:** Todas [CUR] reduziram significativamente a biomassa total, quando comparado ao C+. As médias de absorbância foram <0,5 para os grupos tratados, enquanto o C+ apresentou média de 1,3. **Conclusão:** A IFD-CUR aplicada em estágio inicial de formação do biofilme de *S. brasiliensis* foi capaz de diminuir a formação de biomassa total (células fúngicas + matriz extracelular), mostrando indícios favoráveis em prevenir a evolução das lesões nos casos de esporotricose.

Palavras-chave: *Sporothrix*; biofilmes; matriz extracelular de substâncias poliméricas

Financiador (es): CAPES e CNPq

Área temática: Patógenos de interesse médico.



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MIELOMA MÚLTIPLO: DIAGNÓSTICO, ABORDAGENS TERAPÊUTICAS E TENDÊNCIAS FUTURAS

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Introdução: O Mieloma Múltiplo (MM) é caracterizado como uma neoplasia maligna que ocasiona a produção excessiva de plasmócitos advindos da medula óssea. Trata-se do segundo câncer hematológico mais comum do mundo e tem por característica acometer principalmente os idosos entre 60 e 75 anos. **Objetivo:** Identificar as principais dificuldades no diagnóstico do mieloma múltiplo, as principais perspectivas terapêuticas e olhares futuros para a doença. **Métodos:** Revisão da literatura a partir de levantamento de dados na base Pubmed, Scielo e Google Acadêmico, sendo utilizando os termos “Mieloma Múltiplo”, “Diagnóstico” e “Prognóstico”, incluindo estudos que apontassem: meios de diagnóstico, idade, métodos terapêuticos e evoluções esperadas. **Resultados:** O diagnóstico precoce do MM melhora o prognóstico do paciente e aumenta a sobrevida dos pacientes em geral, sendo incluídos exames como: hemograma, cálcio, eletroforese de proteínas (Proteína M), ressonância magnética e principalmente o padrão ouro: a biópsia de medula óssea. As manifestações clínicas do MM como anemia, fraqueza e dor óssea, muitas vezes atrasam o diagnóstico prévio da doença, tendo em vista que são sintomas inespecíficos e difíceis de serem relacionados apenas com uma patologia. O R-ISS (Sistema Internacional de Estadiamento Revisado) é o atual padrão de estratificação da doença em: estágio I, II e III, incorporando biomarcadores como: Beta-2 Microglobulina e Albumina. Algumas opções de tratamento para o MM são: inibidores de proteossomo, agentes imunomoduladores, quimioterapia com bortezomibe e dexametasona, e em alguns casos o transplante de medula óssea. Devido às terapias eficazes, a maioria dos pacientes com MM agora apresentam uma sobrevida prolongada em média de 5 anos. **Conclusão:** Conclui-se que o diagnóstico precoce do MM é fundamental para melhorar o prognóstico e a sobrevida dos pacientes. Avanços terapêuticos, como inibidores de proteossomo e transplante de medula óssea, têm ampliado a expectativa de vida, destacando a importância de estratégias diagnósticas e tratamentos eficazes.

Palavras-Chave: mieloma múltiplo; neoplasia; sobrevida.

Área Temática: Etiofisiopatologia de doenças humanas e animais.



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IMUNOPATOGÊNESE DE BIOFILMES POLIMICROBIANOS DE *Candida albicans* ASSOCIADOS A BACTÉRIAS EM INFECÇÕES NA OROFARINGE

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Introdução: *Candida albicans* é um fungo pleomórfico comum na microbiota humana, essencialmente oportunista e patogênico quando há comprometimento na resposta imune do hospedeiro. A candidíase orofaríngea é uma manifestação comum em pacientes imunossuprimidos e pode levar a infecções secundárias graves. *C. albicans* possui uma excelente capacidade adaptativa, resistente a alterações de pH, antimicrobianos e estresse oxidativo. Sua interação com bactérias da mucosa oral, que protegem o fungo e se beneficiam da adaptação dele, é frequentemente observada. Biofilmes polimicrobianos na orofaringe estão associados a bacteremias, periodontite, endocardite e autoimunidade.

Objetivo: Elucidar os mecanismos de imunopatogênese associados a biofilmes polimicrobianos de *Candida albicans* com bactérias Gram-positivas e negativas na cavidade oral, explorando a forma como essa cooperação afeta não só aos microrganismos envolvidos como também ao hospedeiro. **Métodos:** Foi realizada uma revisão sistemática de acordo com o protocolo PRISMA em cinco bases diferentes: PubMed, Scopus, Web of Science, Embase e Lilacs. **Resultados:** Os 8 artigos incluídos nesta revisão ressaltam as interações imunológicas que biofilmes mistos desempenham entre si e com o hospedeiro, sobretudo os biofilmes de *C. albicans* com: *Staphylococcus aureus*; *Streptococcus* sp.; *Porphyromonas gingivalis* e *Aggregatibacter actinomycetemcomitans*. A presença de *C. albicans* promove nicho para o crescimento de outros microrganismos, ao passo que estes promovem evasão da resposta imune, por diversos mecanismos. *C. albicans* em biofilmes mistos na orofaringe demonstrou maximizar infecções por agentes da microbiota oral, utilizando proteínas de adesão como ALS3 e HWP1, que facilitam sua interação com bactérias e hospedeiro; a resposta imunológica contra essas comunidades envolve receptores TLR2 e TLR4, com a participação da resposta Th17 e de IL-1 β . **Conclusão:** A imunopatogênese dos biofilmes polimicrobianos envolve interações complexas entre fungos, bactérias e o hospedeiro, resultando em modificações nas vias desses agentes. A persistência das infecções associadas aos biofilmes polimicrobianos pode ser explicada por esses mecanismos imunológicos.

Palavras-chave: *Candida albicans*; biofilme; imunidade.

Área temática: Etiofisiopatologia de doenças humanas e animais.



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EFEITOS DA MEDITAÇÃO TRANSCENDENTAL NAS VARIÁVEIS DE RISCO CARDIOVASCULARES

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Introdução: Problemas cardiovasculares causam cerca de 300 mil óbitos anuais, afetando toda a população. A meditação transcendental (MT) é uma técnica natural e sem esforço com potenciais efeitos sobre o sistema cardiovascular. **Objetivo:** Identificar os efeitos da MT nas variáveis de risco cardiovascular. **Material e métodos:** Este estudo transversal-longitudinal, de amostragem por conveniência, aprovado pelo COPEP sob número CAAE 77175724700000104 realizou coletas no Laboratório de Fisiologia do Exercício em dois momentos: imediatamente no início das práticas de MT (C1) e após três meses (C2). Os participantes assinaram o Termo de Consentimento Livre e Esclarecido. Foi realizada avaliação perimétrica medindo relação cintura-quadril (RCQ), aferição da pressão arterial e verificação de pulso em repouso, com manômetro digital. Para perfil metabólico realizou-se a dosagem de biomarcadores por método colorimétrico com sangue venoso coletado após jejum de 12 horas. Os resultados foram tabulados em planilha Excel e analisados estatisticamente no software Jamovi Open Now versão 2.6.44. **Resultados:** A amostra contou com 13 mulheres com idade média de 49,5 ($\pm$ 8,55). Apresentaram RCQ média de 0,810 na C1 e 0,793 no C2, não alterando a classificação de risco. O perfil lipídico apresentou redução dos valores médios de LDL (C1: 138,13 $\pm$ 41,18; C2: 111,96 $\pm$ 38,08); Triglicerídeos (C1: 112,27 $\pm$ 31,3; C2: 86,0 $\pm$ 31,82); e Colesterol total (C1: 197,37 $\pm$ 40,58; C2: 161,08 $\pm$ 41,42) e manutenção de HDL (C1: 36,65 $\pm$ 9,87; C2: 34,58 $\pm$ 9,32). **Conclusão:** Os resultados apresentam diminuição do perfil lipídico nos indivíduos assim como da RCQ, podendo indicar diminuição dos riscos cardiovasculares pelos praticantes de MT. Entretanto, há necessidade de mais estudos para a melhor compreensão dos efeitos da MT.

Palavras-chave: saúde coletiva; meditação transcendental; saúde cardiovascular.

Financiador (es): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Área temática: Eco-epidemiologia e controle de doenças.



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RESVERATROL COMO ALTERNATIVA ANTIFÚNGICA: AVALIAÇÃO *in vitro* SOBRE *Candida albicans* E IMPLICAÇÕES NA ESTÉTICA REGENERATIVA

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Introdução: O envelhecimento cutâneo é um processo fisiológico que compromete a integridade da pele. O Resveratrol, um polifenol natural, destaca-se por suas propriedades antioxidantes e anti-inflamatórias, apresentando-se como uma opção promissora para rejuvenescimento e cicatrização. Estudos recentes demonstram sua capacidade de modular processos celulares ligados ao estresse oxidativo e à inflamação. No entanto, a investigação sobre seus efeitos antifúngicos e sua aplicação na estética regenerativa ainda é limitada, evidenciando a necessidade de aprofundar o conhecimento sobre suas propriedades e mecanismos de ação. **Objetivo:** Avaliar *in vitro* o efeito antifúngico do Resveratrol sobre *Candida albicans*. **Material e métodos:** Foi utilizado o Resveratrol® (5mg/2mL) produzido pela PHD, utilizado para aplicações injetáveis. Para a avaliação da atividade antifúngica, foram utilizadas cepas de *Candida albicans* (ATCC 90028) reativadas em ágar Sabouraud Dextrose a 37°C por 24 horas. As células foram suspensas em meio RPMI 1640, ajustando a concentração para 3×10^6 células/mL. O resveratrol foi testado em concentrações de 1250 µg/mL a 2,4 µg/mL, obtidas por diluições seriadas de 1:2. As microplacas de 96 poços foram incubadas a 37°C por 48 horas. A Concentração Inibitória Mínima (CIM) foi determinada por leitura visual, enquanto a Concentração Fungicida Mínima (CFM) foi avaliada por cultura em ágar SDA. O fármaco padrão utilizado para comparação foi o fluconazol na concentração inicial de 64 µg/mL. **Resultados:** A CIM e CFM do resveratrol para *Candida albicans* foi de 625 µg/mL. **Conclusão:** O Resveratrol possui atividade antifúngica contra *Candida albicans* tanto na sua concentração comercial e quando diluída 1:4. Esse resultado corrobora a hipótese de que o Resveratrol pode inibir o crescimento e a formação de biofilmes, além disso os achados deste estudo abrem caminho para futuras investigações que possam esclarecer o uso do Resveratrol em protocolos de tratamento injetável estético e sua eficácia na prevenção de infecções fúngicas cutâneas.

Palavras-chave: resveratrol; *Candida albicans*; antifúngico.

Área temática: Patógenos de interesse médico.



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ATIVIDADE DA MOLÉCULA *HIT21* EM FRENTE A *Mycobacterium tuberculosis* E SUA INTERAÇÃO COM FÁRMACOS PADRÕES

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Introdução: A tuberculose (TB) é uma doença com alta incidência global, sendo um importante problema de saúde pública. É causada por bacilos do complexo *Mycobacterium tuberculosis* (Mtb) e transmitida através de aerossóis expelidos pela tosse ou fala de pessoas doentes. A TB exige um tratamento longo e rigoroso com uso diário de múltiplos medicamentos. Nesse contexto, a resistência aos antibióticos é um desafio, por comprometerem a eficácia do tratamento exigindo esquemas mais longos, caros e tóxicos, afetando a adesão do paciente e dificultando a eliminação da doença. Diante disso, torna-se essencial a busca por novas moléculas que possam ser opções terapêuticas contra TB. **Objetivo:** Investigar a atividade da molécula nova *Hit21* frente a Mtb, assim como seu efeito combinado com fármacos padrão. **Materiais e métodos:** A Concentração Inibitória Mínima (CIM) foi determinada em cepa de referência Mtb H₃₇Rv pelo método REMA (*Resazurin Microtiter Plate Assay*). A ação combinatória com fármacos padrão foi avaliada por REDCA (*Resazurin Drugs Combination Microtiter Assay*), e as interações classificadas segundo o Índice de Concentração Inibitória Fracionada (FICI) em sinérgicas ($\leq 0,5$), aditiva (0,5–4) ou antagonistas (>4). **Resultados:** A execução do REMA em triplicata demonstrou que a menor concentração da molécula *Hit21* capaz de inibir o crescimento bacteriano foi de 0,48 µg/mL. O REDCA foi realizado combinando o composto *Hit21* com Isoniazida (INH), Rifampicina (RIF) e Etambutol (EMB), resultando em valores de FICI de 0,375, 0,281 e 1,13, respectivamente. Assim, as combinações de *Hit21* com INH e RIF foram classificadas como sinérgicas, enquanto a combinação com EMB foi classificada como aditiva. **Conclusão:** Desta forma, a molécula apresentou atividade antimicrobiana relevante e efeito sinérgico com INH e RIF, sendo promissora para a continuidade dos estudos pré-clínicos como protótipo ao desenvolvimento de fármacos para compor o esquema de tratamento contra a tuberculose.

Palavras-chave: tuberculose; resistência bacteriana; *Mycobacterium tuberculosis*.

Área temática: Patógenos de interesse médico.



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HIDROGÉIS DE MATRIZ EXTRACELULAR DERIVADOS DE TECIDOS REPRODUTIVOS SUÍNOS ACELERAM A CICATRIZAÇÃO E REMODELAÇÃO TECIDUAL EM MODELO PRÉ-CLÍNICO MURINO

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Introdução: Hidrogéis derivados de matriz extracelular (MEC) têm emergido como biomateriais promissores para a medicina regenerativa devido à sua biocompatibilidade, complexidade estrutural e capacidade de fornecer sinais bioativos que modulam a cicatrização de feridas. Em especial, tecidos do trato reprodutivo, como matrizes placentária, uterina e ovariana, constituem fontes únicas para a geração desses hidrogéis, por serem naturalmente enriquecidos em moléculas bioativas e passarem por ciclos dinâmicos de remodelação, o que lhes confere potencial translacional diferenciado para a reparação tecidual. **Objetivo:** Avaliar a atividade cicatrizante de hidrogéis derivados de MECs ovariana (OvECM), ampular (AmpECM), uterina (UtECM) e placentária (PlaECM) em modelo pré-clínico de feridas excisionais. **Materiais e Métodos:** Utilizou-se modelo de ferida cutânea em ratos Wistar (70 dias, CEUA nº 9958021224), com duas incisões dorsais de 1 cm²: esquerda controle e direita tratada com hidrogel (n = 5/grupo/tempo; total = 60 animais). A cicatrização foi avaliada nos dias 3, 7 e 14, e as feridas coletadas após eutanásia para análise histopatológica por Hematoxilina e Eosina, Tricrômio de Masson e Picrosirius Red. **Resultados:** A avaliação macroscópica demonstrou que os hidrogéis de MEC favoreceram cicatrização mais rápida e eficiente, com redução progressiva do diâmetro das feridas, aceleração do fechamento e melhor qualidade do tecido recém-formado em comparação ao controle. Esses achados foram consistentes com as análises histológicas, que revelaram modulação do infiltrado inflamatório, estímulo à reepitelização, à angiogênese e à deposição de colágeno. Entre as formulações testadas, AmpECM e PlaECM apresentaram desempenho superior, evidenciando maior eficácia no reparo tecidual. **Conclusão:** Os hidrogéis de MEC aceleraram o fechamento das feridas, favoreceram a reepitelização, estimularam a atividade de fibroblastos, a angiogênese e a deposição de colágeno, promovendo remodelação tecidual mais organizada.

Palavras-chave: matriz extracelular; reparo tecidual; hidrogel.

Financiador (es): CNPq e FAPESP.

Área temática: Etiofisiopatologia de doenças humanas e animais.



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DESENVOLVIMENTO DE METODOLOGIA PARA ANÁLISE DE COCAÍNA EM MANCHAS SECAS DE SALIVA COLETADA EM PAPEL FILTRO

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Introdução: O uso abusivo de drogas psicoativas, em especial a cocaína, representa um desafio crescente para a saúde pública e para a toxicologia forense. Entre as matrizes alternativas disponíveis, o fluido oral se destaca por ser de fácil coleta, não invasivo e menos suscetível a adulterações. Nesse cenário, a técnica de *Dried Saliva Spot* (DSS) mostra-se promissora por demandar pequeno volume de amostra, facilitar armazenamento e transporte, além de reduzir custos e impacto ambiental. **Objetivo:** Desenvolver e avaliar metodologia para detecção de cocaína e metabólitos em fluido oral em mancha seca de saliva, associada a técnica de Cromatografia Líquida de Ultra Performance acoplado à espectrometria de massas. **Materiais e métodos:** Foram coletadas amostras negativas de fluido oral de voluntários, aplicadas em papel filtro (30 µL) e secas à temperatura ambiente. Após secagem, os discos foram submetidos à extração com solvente metanol/acetonitrila, em diferentes condições de tempo no banho ultrassônico (10, 20 e 30 min) e volumes de solvente (500, 750 e 1000 µL). A separação e identificação dos analitos (cocaína (COC), benzoilecgonina (BZE) e cocaetileno (CE)) foram realizadas por cromatografia líquida de ultra performance acoplada à espectrometria de massas (UPLC-MS). Projeto aprovado pelo COPEP/UEM sob parecer n. 6.956.864. **Resultados:** Observou-se maior extração de COC e BZE em 20 minutos no sonificador, com queda na detecção quando o tempo foi prolongado a 30 minutos, sugerindo degradação da BZE. Para o CE, o pico máximo ocorreu em 10 minutos. Em relação ao volume de solvente, 750 µL apresentou melhor equilíbrio entre recuperação dos analitos e consistência dos resultados. **Conclusão:** A técnica de DSS demonstrou potencial como ferramenta eficiente e prática para análise de cocaína em fluido oral, oferecendo vantagens frente a métodos convencionais e podendo futuramente ser aplicada em contextos clínicos e forense.

Palavras-chave: cocaína; fluido oral; mancha seca.

Área temática: Eco-epidemiologia e controle de doenças.



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RELATO DE CASO: TENTATIVA DE AUTOEXTERMÍNIO COM DELONGA DOS PROCEDIMENTOS DE DESCONTAMINAÇÃO

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Introdução: Os medicamentos estão entre as principais fontes de intoxicação mundial. Eles constituem a segunda causa de mortalidade relacionada às intoxicações, gerando grande impacto social e econômico. **Objetivo:** Relatar um caso de intoxicação polimedicamentosa em paciente de 21 anos que ingeriu doses indeterminadas de amitriptilina, dipirona, nimesulida e amoxicilina, com delonga nos procedimentos de descontaminação, que favoreceram um desfecho fatal. **Material e métodos:** Estudo de caso realizado por meio de análise da ficha de exposição humana registrada em um Centro de Assistência e Informação Toxicológica (CIATox). Projeto aprovado pelo Comitê de Ética em Pesquisa, parecer n. 6.940.629. **Resultados:** Caso de intoxicação exógena polimedicamentosa foi notificada ao CIATox regional em julho de 2025 com tempo estimado de exposição de 4h. Paciente apresentava asma, hipertensão arterial sistêmica não tratada e obesidade. Havia ingerido quantidade indeterminada de quatro medicamentos (amitriptilina, dipirona, nimesulida e amoxicilina) e apresentava Glasgow 5, RASS -3 e um episódio de vômito, em coma. Foram realizadas as orientações de descontaminação com administração de três doses de carvão ativado de 4/4h via sonda nasogástrica e demais medidas sintomáticas e de suporte. Entretanto, durante acompanhamento do caso, 22h após a notificação, o médico residente da UTI questionou se deveria realizar a segunda e terceira dose de carvão ativado, pois apenas a primeira tinha sido realizada. Infelizmente, foi informado que já não havia eficácia em seu uso. Paciente foi a óbito oito dias depois do acidente após complicações do quadro clínico. **Conclusão:** O acionamento imediato do CIATox e a execução rápida das medidas de descontaminação recomendadas são fundamentais para otimizar o manejo de intoxicações, especialmente em pacientes com múltiplas comorbidades. No caso descrito, o atraso em contatar o CIATox e em administrar o carvão ativado comprometeu o tratamento toxicológico, favorecendo o agravamento do quadro e culminando em desfecho fatal.

Palavras-chave: descontaminação; intoxicação; medicamentos.

Área temática: Eco-epidemiologia e controle de doenças.



INTERFACES DA BIOCÊNCIA: CÉLULAS, GENES E ALGORITMOS

10º International Meeting of Biosciences and Physiopathology
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RESPOSTA IMUNE ASSOCIADA A COAGULOPATIA NA COVID-19: REVISÃO DE REVISÕES SISTEMÁTICAS

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Introdução: o SARS-CoV-2, vírus que emergiu na China no final de 2019, desencadeou a pandemia de COVID-19. Em casos graves e críticos, a COVID-19 pode levar a eventos tromboembólicos sistêmicos, uma complicação com potencial para desfechos fatais.

Objetivo: avaliar a resposta imunológica frente a eventos tromboembólicos na evolução da COVID-19, para entender os mecanismos efetores e patológicos envolvidos.

Material e métodos: o estudo é uma revisão sistemática de revisões sistemáticas. A pesquisa seguiu as recomendações do PRISMA e o protocolo foi submetido na plataforma PROSPERO. As bases de dados PubMed, Embase, Scopus, Web of Science, Cochrane Library e BVS/Lilacs foram consultadas. A estratégia de busca foi estruturada em três blocos, adaptada para cada base: “SARS-CoV-2 OR COVID-19” AND “Vascular Diseases OR Ischemia OR Hemostasis” AND “Immunology OR Immune System OR Host-Pathogen Interactions OR Receptors, immunologic OR Cytokines OR Immune System Phenomena”.

A seleção e extração dos dados foi realizada em duplicata, com divergências resolvidas por consenso. A ferramenta AMSTAR-2 foi aplicada para avaliar a qualidade metodológica dos estudos. **Resultados:** inicialmente, recuperamos 1.097 publicações. Após remoção das duplicatas e critérios de elegibilidade, 7 artigos foram incluídos na revisão sistemática. Os principais eventos tromboembólicos analisados foram a embolia pulmonar, trombose venosa profunda e infarto agudo do miocárdio, que apresentaram associação com o aumento do risco de mortalidade. Os homens são os mais acometidos, na faixa etária de 50 a 65 anos. Foi identificado que o aumento da concentração sérica de anticorpos antifosfolipídicos, IL-6 e leucócitos pode elevar o risco de formação de trombos e tempestade de citocinas, sendo eles associados a resposta imune contra a infecção viral.

Conclusão: a COVID-19 desencadeia alterações importantes na resposta imune que aumentam a chance de eventos tromboembólicos, agravando o quadro clínico do paciente e possível óbito.

Palavras-chave: SARS-CoV-2; resposta imune; doenças vasculares.

Financiador: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

Área temática: Eco-epidemiologia e controle de doenças.



INTERFACES DA BIOCÊNCIA: CÉLULAS, GENES E ALGORITMOS

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***Tribulus terrestris* REDUZ MASSA CORPORAL DE RATOS WISTAR OBESOS E ALTERA A MASSA DE ÓRGÃOS DO SISTEMA REPRODUTOR MASCULINO**

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Introdução: O conceito DOHaD envolve pesquisas que investigam como insultos, em fases críticas do desenvolvimento, podem desencadear doenças como a obesidade, a qual afeta órgãos do sistema reprodutor masculino, levando à infertilidade. Pesquisas por tratamentos complementares aos convencionais, que podem reverter esta condição, são de extrema relevância. A *Tribulus terrestris* (TT), erva natural com propriedades antioxidantes e de melhora em parâmetros metabólicos, pode ser uma aliada no tratamento da obesidade e, conseqüentemente, da infertilidade masculina. **Objetivo:** Avaliar os efeitos da TT sobre a massa corporal e de órgãos do sistema reprodutor de ratos Wistar. **Material e métodos:** Foram utilizados 28 ratos Wistar machos (CEUA: 3494141024), com 30 dias de idade, distribuídos nos grupos (n = 8): Controle (CTL): alimentados com dieta comercial (DC); Controle + TT (CTL+TT): receberam DC e extrato de TT; Dieta rica em gordura (HFD): alimentados com HFD; Grupo HFD + TT: receberam HFD e extrato de TT. O período experimental foi de 30 dias e os animais receberam ração e água *ad libitum*. Os grupos CTL + TT e HFD + TT receberam o extrato de TT via gavagem na dosagem de 10 mg/kg. Foi utilizado o programa STASTISTICA e valores de $p > 0,05$ foram considerados significantes. **Resultados:** Durante 60 dias, observou-se aumento da massa corporal no grupo HFD, em comparação ao CTL, confirmando o quadro de obesidade. A TT reverteu o ganho de massa corporal nos animais obesos (HFD+TT) em comparação ao seu respectivo controle (HFD). A massa dos testículos e da próstata aumentou e a do epidídimo diminuiu no grupo HFD em comparação ao CTL. O uso da TT (CTL+TT) reduziu a massa da próstata em comparação ao CTL. **Conclusão:** A TT reduziu a massa corporal de ratos obesos e promoveu alterações na massa dos órgãos reprodutores, indicando sua influência metabólica e saúde reprodutiva.

Palavras-chave: obesidade; *Tribulus terrestris*; infertilidade.

Área temática: Etiofisiopatologia de doenças humanas e animais.



INTERFACES DA BIOCÊNCIA: CÉLULAS, GENES E ALGORITMOS

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AVALIAÇÃO DA CITOTOXICIDADE DE UMA SÉRIE DE TIOSSEMICARBAZONAS DERIVADAS DA ISONIAZIDA EM LINHAGEM CELULAR

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Introdução: A tuberculose (TB) é uma doença causada por bactérias do complexo *Mycobacterium tuberculosis* (*Mtb*) e representa um dos maiores problemas de saúde pública a nível mundial. Ademais, a TB tem um difícil esquema terapêutico que exige um longo período de tratamento de, no mínimo, 6 meses. Esse fato aliado aos efeitos colaterais que acometem o paciente agravam o problema de adesão ao tratamento, tornando evidente a necessidade de se desenvolver novas alternativas terapêuticas que sejam seletivas e eficazes. **Objetivo:** O presente estudo visou avaliar a citotoxicidade de tiossemicarbazonas derivadas da isoniazida em células J774A.1 pelo ensaio de redução do MTT. **Material e métodos:** As células foram cultivadas em meio Dulbecco's Modified Eagle's Medium (DMEM), tratadas com diferentes concentrações dos derivados (6,25-250 µg/mL), testadas quanto à viabilidade celular e posteriormente foi analisado índice de seletividade (IS) contra cepa padrão H37RV de *Mycobacterium tuberculosis* e a linhagem de coleção multirresistente 3614. A Concentração Inibitória Mínima (CIM) e a Concentração Citotóxica 50% (CC₅₀) foram utilizadas para o cálculo do índice de seletividade (IS). **Resultados:** Os valores de Concentração Citotóxica 50% (CC₅₀) ficaram entre 28,92 a 94,25 µg/mL. A TSC-5 apresentou a menor CC₅₀ (28,92 µg/mL) e maior IS (7,42), TSC-4 também apresentou a segunda maior seletividade (IS = 6,28), ao passo que a TSC-1, TSC-2 e principalmente a TSC-3 apresentaram perfis menos favoráveis, resultados observados em ambas as cepas. **Conclusão:** O composto TSC-5 mostrou-se o mais promissor entre os testados, uma vez que possui uma melhor combinação da concentração citotóxica e maior índice de seletividade. Além disso, o composto TSC-4 também deve ser considerado para investigações futuras através da otimização de suas propriedades.

Palavras-chave: *Mycobacterium tuberculosis*; citotoxicidade; J774A.1.

Financiador (es): Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Área temática: Patógenos de interesse médico.



INTERFACES DA BIOCÊNCIA: CÉLULAS, GENES E ALGORITMOS

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ASSOCIAÇÃO ENTRE A OCORRÊNCIA DE POLIMORFISMO NO GENE *GSTP1* EM TRABALHADORES EXPOSTOS A AGROTÓXICOS COM O USO DE EQUIPAMENTOS DE PROTEÇÃO INDIVIDUAL E A FORMAÇÃO DE MICRONÚCLEOS

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Introdução: A ocorrência de polimorfismo no gene *GSTP1* tem associação com aumento da susceptibilidade a danos genéticos. **Objetivo:** Avaliar a ocorrência dos alelos Ile-Ile; Ile-Val e Val-Val em trabalhadores expostos a agrotóxicos e associar com a frequência de micronúcleos (MN) e o uso de equipamentos de proteção individual (EPI). **Material e métodos:** Este estudo foi aprovado pelo COPEP/UEM e teve a participação de 27 agricultores do município de Marialva-PR, distribuídos em 2 grupos: aplicadores de agrotóxicos com EPI e aplicadores que não utilizam EPI. Amostras da mucosa bucal e de sangue foram coletadas para realização do teste do micronúcleo e da técnica da PCR-RFLP, respectivamente. **Resultados:** No grupo aplicador com EPI estão presentes os alelos Ile-Ile (n= 2; 14,29%); Ile-Val (n= 9; 64,28%) e Val-Val (n= 3; 21,43%), enquanto que no grupo aplicador sem EPI estes alelos apresentaram a seguinte distribuição: Ile-Ile (n= 4; 30,77%); Ile-Val (n= 7; 53,85%) e Val-Val (n= 2; 15,38%). No grupo aplicador com EPI foi possível observar média com desvio padrão e mediana dos MN com valores de 6,35±7,14 e 5. E no grupo aplicador sem EPI os valores foram 2,53±3,93 e 1. Esta diferença também foi observada entre os portadores dos alelos, conforme segue, em aplicadores com EPI e portadores do alelo Ile-Ile, Ile-Val e Val-Val a média foi de 11,00; 7,22 e 0,66 MN, respectivamente. Enquanto que aqueles aplicadores que não usam EPI média de MN para estes alelos foi 0,50; 3,14 e 4,50 respectivamente. **Conclusão:** Não houve relação entre o polimorfismo genético e uso de EPI com a formação de MN, sendo provável que a ocorrência de MN pode estar relacionada com características próprias dos agricultores, mas como trata-se de amostragem pequena e restrita ao município de Marialva-PR, portanto, há necessidade de estudos adicionais.

Palavras-chave: micronúcleo; equipamento de proteção individual; agrotóxicos.

Financiador (es): Fundação Araucária.

Área temática: Etiofisiopatologia de doenças humanas e animais.



INTERFACES DA BIOCÊNCIA: CÉLULAS, GENES E ALGORITMOS

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AVALIAÇÃO DE CÉLULAS CALICIFORMES NO ÍLEO DE CAMUNDONGOS INFECTADOS POR *Trypanosoma cruzi* ASSOCIADA A VESÍCULAS EXTRACELULARES

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Introdução: A Doença de Chagas causada pelo protozoário *Trypanosoma cruzi* afeta milhões de pessoas, principalmente na América Latina, sendo uma doença tropical negligenciada. O parasito produz vesículas extracelulares (VEs) que modulam a resposta imune e favorecem sua disseminação, o que contribui para complicações crônicas nos sistemas cardiovascular e digestório. Embora alterações no esôfago e cólon sejam bem descritas, pouco se sabe sobre os impactos no intestino delgado, órgão fundamental para resposta imune. **Objetivo:** Avaliar as células caliciformes (CC) do íleo de camundongos durante a infecção por *T. cruzi* associada a VEs. **Material e métodos:** O estudo foi aprovado pela CEUA/UEM (parecer nº 58644311022). Foram utilizados 12 camundongos machos Balb/c, distribuídos em 4 grupos ($n=3$). O grupo controle (GC) recebeu apenas solução salina via intravenosa pela veia peniana; O grupo infectado (GI) recebeu uma suspensão de 1×10^5 tripomastigotas de *T. cruzi* (cepa CL Brener). Os demais grupos receberam a suspensão de tripomastigotas juntamente com VEs obtidas da interação da cepa CL Brener ou de DM28 com culturas de célula C2C12 (GI-CLC2; GI-DmC2). Após 28 dias, coletou-se 1 cm do íleo para análise histológica com Alcian blue (AB) e ácido periódico de Schiff (PAS), nas quais foram quantificadas as CC para realização das análises estatísticas ($p < 0,05$). **Resultados:** Não foram observadas alterações nas CC coradas com AB. Contudo, observou-se um aumento significativo nos grupos GI-CLC2 ($55,84 \pm 8,41$ CC/ $0,96 \text{ mm}^2$) e GI-DmC2 ($46,69 \pm 1,68$ CC/ $0,96 \text{ mm}^2$) em comparação ao GC ($30,36 \pm 3,41$ CC/ $0,96 \text{ mm}^2$) e do GI-CLC2 quando comparado ao GI ($36,04 \pm 7,69$ CC/ $0,96 \text{ mm}^2$) na coloração de PAS. **Conclusão:** As VEs modulam a mucosa intestinal do íleo durante a infecção por *T. cruzi*, promovendo aumento de células caliciformes produtoras de mucinas neutras, o que pode promover a proteção epitelial intestinal.

Palavras-chave: doença de chagas; vesículas extracelulares; intestino delgado.

Área temática: Etiofisiopatologia de doenças humanas e animais.



INTERFACES DA BIOCIÊNCIA: CÉLULAS, GENES E ALGORITMOS

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ANÁLISE DOS ANOS DE 2013 A 2023 DE TUBERCULOSE NO ESTADO DO PARANÁ

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Introdução: a tuberculose (TB) é uma doença contagiosa sendo um problema de saúde pública mundial. No Brasil, no período de 2013 a 2023 foram registrados 1.015.565, estando entre os 22 países responsáveis por 82% dos casos da doença registrados no mundo, ocupando o 20° lugar. **Objetivo:** o trabalho descreve o perfil epidemiológico de tuberculose no estado do Paraná nos anos de 2013 a 2023, de uma avaliação demográfica que inclui gênero e faixa etária. **Material e métodos:** foram usados dados contidos na plataforma DataSus e método de análise estatística descritiva. **Resultado:** foram analisados 25.299 casos durante o ano de 2013 a 2023, na faixa etária de 15 a 64 anos. O estudo mostra maior predomínio de pacientes positivos do sexo masculino (71,40%), onde 49,08% tinham entre 20 e 39 anos, seguido da faixa etária de 40 a 59 anos (40,72%), e a menos contaminada foi a faixa etária entre 15 a 19 anos (4,01%). Já o feminino, apresentou 28,6%, tendo o maior predomínio de idades de 20 a 39 anos (50,11%) e 40 a 59 anos (36,87%), sendo entre a faixa etária de 60-64 anos (5,92%) os menos contaminados. **Conclusão:** com o presente estudo concluímos que no estado do Paraná, a TB tem maior predominância no sexo masculino (71,40%), afetando principalmente pessoas entre 20 a 39 anos de idade. Medidas de orientação e prevenção são importantes, para que esse número elevado de pacientes infectados diminua.

Palavras-chaves: tuberculose; epidemiologia; infecções.

Área temática: Eco-epidemiologia e controle e doenças.



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AVALIAÇÃO DO PERFIL EPIDEMIOLÓGICO DE PACIENTES PORTADORES DE RECORRÊNCIA NA HANSENÍASE NO NOROESTE DO PARANÁ, BRASIL

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Introdução: A hanseníase é uma doença infecciosa crônica causada pelo *Mycobacterium leprae* (*M. leprae*), que possui tropismo por macrófagos da pele, células de Schwann do Sistema Nervoso Periférico e macrófagos na pele, sendo o Brasil o segundo país com maior número absoluto de casos, ficando atrás da Índia. A recorrência da hanseníase é definida como aquela que ocorre em pacientes com novos sintomas clínicos, incluindo sinais e sintomas da doença ativa, após tratamento e diagnóstico de cura. **Objetivo:** O objetivo deste estudo foi observar o perfil epidemiológico de portadores da hanseníase, que apresentaram recorrência, no Noroeste do Paraná, Brasil. **Material e métodos:** Trata-se de um estudo transversal, descritivo, retrospectivo, baseado na revisão de prontuários de 30 pacientes com diagnóstico de hanseníase acompanhados e tratados no Consórcio Público Intermunicipal de Saúde do Setentrão Paranaense (CISAMUSEP) entre os anos de 2005 a 2021. A distribuição de frequência das variáveis foi analisada por meio de contagem direta, utilizando o *software* Microsoft Office Excel 365. O projeto de pesquisa foi aprovado pelos Comitês de Ética COPEP-UEM nº 2.424.046/2017 e CEP-SESA / HT nº. 400/201. **Resultados:** A faixa etária com maior frequência foi de 40 a 59 anos (50%), sexo feminino (53,3%) e autodeclarados brancos (66,7%). A classificação operacional predominante foi a multibacilar (90%), forma clínica virchowiana (46,7%). O nível de incapacidade na recaída mais observado foi o grau 1 (36,7%), esquema terapêutico inicial Poliquimioterapia/Multibacilar (PQT/MB) 24 doses (46,6%), com tempo decorrido do tratamento até a recaída de 77,2 meses. **Conclusão:** Os resultados mostraram maior prevalência de recorrência em pacientes com a classificação operacional multibacilar, forma clínica virchowiana e com grau 1 de incapacidade no momento da recorrência, indicando uma possível alta transmissibilidade da doença e também o diagnóstico tardio da mesma.

Palavras-chave: epidemiologia; *Mycobacterium-leprae*; recorrência.

Financiador (es): Laboratório de Imunogenética da Universidade Estadual de Maringá (Projeto nº1589/2017-CSD-UEM), CAPES e CNPq.

Área temática: Etiofisiopatologia de doenças humanas e animais.



INTERFACES DA BIOCIÊNCIA: CÉLULAS, GENES E ALGORITMOS

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VALIDAÇÃO DE PRIMERS PARA AMPLIFICAÇÃO DAS REGIÕES CODIFICANTES DO GENE *TP53* COMO PARTE DO DESENVOLVIMENTO DE UM PAINEL MULTIGÊNICO PARA LEUCEMIAS MIELOIDES

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Introdução: Mutações no gene *TP53* são comuns em pacientes neoplásicos com diversos tipos de cânceres, sendo potenciais marcadores moleculares para diagnóstico, estratificação de risco e suporte no direcionamento clínico. Este gene codifica a proteína p53, um fator de transcrição que regula o ciclo celular e o processo de reparo do DNA, além de direcionar a célula para apoptose ou senescência. As alterações importantes resultam em proteínas disfuncionais, inviabilizando a função de supressão tumoral. Pacientes com neoplasias mieloides portadores de alterações no *TP53* são mais resistentes ao tratamento e possuem menor sobrevida global. **Objetivo:** Desenvolver *primers* para sequenciamento de nova geração (NGS) através de validação por sequenciamento de Sanger. **Material e métodos:** Neste trabalho, conjuntos de *primers* desenhados para os éxons do *TP53* foram validados por PCR e sequenciamento dos fragmentos amplificados. Os *primers* foram desenhados manualmente seguindo parâmetros de qualidade, além de outros relacionados à montagem das bibliotecas NGS para 300 ciclos. Os procedimentos *in silico* foram realizados com *Primer-BLAST*, *OligoAnalyzer* e *DECIPHER*. Diversos ensaios de PCR foram feitos até a obtenção da receita otimizada. Os *amplicons* foram tratados com *ExoSap-IT* e sequenciados no aparelho 3500 XL Genetic Analyzer. Os resultados foram analisados com o pacote sangeranalyseR e os alinhamentos no software JalView. O projeto foi aprovado pelo Comitê de ética em pesquisa sob o parecer nº 2.364.250/2017. **Resultados:** resultados demonstraram que os *primers* foram eficientes para gerar *amplicons* dentro dos parâmetros necessários para posterior montagem de bibliotecas de NGS. Os *amplicons* foram gerados a partir de três amostras de DNA independentes obtidos de *buffy coat* humano. **Conclusão:** Os ensaios de sequenciamento demonstraram padrão de qualidade suficientes para construção das sequências consenso e alinhamento contra o gene *TP53*, cobrindo as regiões codificantes entre previamente designadas.

Palavras-chave: leucemia mieloide; TP53.

Área temática: Etiofisiopatologia de doenças humanas e animais.



INTERFACES DA BIOCIÊNCIA: CÉLULAS, GENES E ALGORITMOS

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FOTOATIVAÇÃO DE *SCAFFOLDS* IMPREGNADOS COM AZUL DE METILENO COMO ESTRATÉGIA ANTIFÚNGICA PARA *FUSARIUM SOLANI*

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Introdução: O azul de metileno (AM) é um corante sintético amplamente estudado por suas propriedades fotodinâmicas, atuando como fotossensibilizador com potencial antimicrobiano e antitumoral na Terapia Fotodinâmica. Contudo, sua forma em solução líquida apresenta limitações, como baixa retenção no local de aplicação. Nesse contexto, *scaffolds* impregnados com AM surgem como alternativa eficiente, pois suas propriedades reológicas e mecânicas, em contato com a temperatura corporal, favorecem maior adesão ao tratamento. **Objetivo:** Avaliar o efeito antifúngico da Inativação fotodinâmica (IFD) do AM+*scaffolds*, contra *Fusarium solani*. **Material e métodos:** Os *scaffolds* utilizados neste estudo são estruturas sólidas, em formato de fibras constituídos por copolímero F108 de 1%, ácido cítrico 20% e AM 0,03198 mg/mL. Foi avaliada a atividade antifúngica dos polímeros que constituem a solução dos *scaffolds* e também da estrutura dos *scaffolds*, ambos com a mesma concentração de AM (previamente estabelecida no grupo de pesquisa). O ensaio antifúngico foi conduzido com LED vermelho (660nm) e inóculo fúngico a 1×10^5 UFC/mL. Para os polímeros em solução foi realizada microdiluição em caldo, e para as estruturas dos *scaffolds*, a difusão em ágar. Após 15 minutos de incubação no escuro, a placa foi irradiada a 30 J/cm². A atividade antifúngica foi avaliada pelo halo de inibição para as nanofibras sólidas e pelo plaqueamento em triplicata em placas de SDA para as soluções dos *scaffolds*. **Resultados:** Nas soluções dos *scaffolds*, observou-se inibição completa do crescimento fúngico em comparação ao controle. A IFD+AM+*scaffold* teve atividade fungicida contra o *F. solani* resultando em um halo de inibição de 0,5 cm² após 48 horas de incubação. **Conclusão:** Os resultados deste estudo indicam que a IFD+scaffolds+AM é eficaz contra a espécie de *F. solani* e incentivam mais estudos para avaliar a atividade antifúngica contra infecções causadas por fungos do gênero *Fusarium*.

Palavras-chave: fototerapia; azul de metileno; scaffolds.

Área temática: Patógenos de interesse médico



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INATIVAÇÃO FOTODINÂMICA DE *Candida parapsilosis* MEDIADA POR AZUL DE METILENO

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Introdução: *Candida parapsilosis* é o principal agente etiológico causador de onicomicose nas mãos. Apesar de fazer parte da microbiota da pele, essa levedura pode ser oportunista quando há um desequilíbrio imune e/ou uma porta de entrada para o sítio de infecção. A Terapia Fotodinâmica (TFD) tem se mostrado uma opção terapêutica eficaz em casos de onicomicose, devido a facilidade de aplicação e atividade antifúngica.

Objetivo: Avaliar a atividade antifúngica do Azul de Metileno (AM) fotoativado sobre cepa padrão de *Candida parapsilosis*. **Material e métodos:** *Candida parapsilosis* ATCC 22019 foram reativadas, cultivadas em ágar Sabouraud dextrose (SDA) e incubadas por 24h à 30°C. A partir disso, foi preparado um inóculo de 2×10^6 Unidade Formadora de Colônias (UFC)/mL. Preparou-se duas placas de 96 poços - uma para controle escuro (AM-TFD) e outra para testar a fotoativação do AM (AM+TFD). O AM foi testado no intervalo das concentrações 78,12 a 0,15 µg/mL. Ambas placas foram incubadas por 30 minutos/30°C. Ao ensaio AM+TFD, foi aplicada a irradiação por 30 minutos com fluência de 50,34 J/cm², utilizando o LED vermelho (660nm). Em seguida, realizou-se o subcultivo para avaliar a Concentração Fungicida Mínima (CFM) de forma qualitativa e quantitativa (UFC). A análise estatística foi realizada com o programa GraphPad Prism 8.0, e comparado ao crescimento no controle positivo (*C. parapsilosis* na ausência de AM e luz). **Resultados:** O AM não mostrou atividade antifúngica na ausência da luz. Por outro lado, a fotoativação do AM nas condições testadas, mostrou ação fungicida, ou seja, eliminação completa do crescimento fúngico a partir de 4,88 µg/mL. **Conclusão:** O estudo mostrou que a aplicação da tecnologia AM+TFD tem ação fungicida contra *C. parapsilosis*, sendo os primeiros indícios promissores para aplicação desta terapêutica nos casos de onicomicose.

Palavras-chave: levedura; terapia fotodinâmica; onicomicose.

Financiador (es): Fundação Araucária

Área temática: Patógenos de interesse médico



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ISOLAMENTO SOCIAL E IMOBILIZAÇÃO EM CAMUNDONGOS JUVENIS: EFEITOS SEXO-DEPENDENTES NO CPFm E HIPOCAMPO

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Introdução: Na infância ocorre intensa neuroplasticidade, e determinadas experiências ambientais podem favorecer transtornos mentais. O estresse de isolamento social (EIS) e imobilização (EI) juvenil podem estar relacionados a alterações neuroanatômicas e comportamentais duradouras. **Objetivo:** Verificar os efeitos do Estresse de Isolamento Social (EIS), Estresse de Imobilização (EI) e EIS+EI, nos parâmetros neuroanatômicos no córtex pré-frontal medial (CPFm) e hipocampo dorsal. **Material e métodos:** Camundongos C57BL/6 (machos e fêmeas) foram mantidos em condição padrão de biotério, conforme CEUA/UEM (1281250722). Após desmame em 21 dias pós natal (P21), foram distribuídos em: Controle (C), EIS, EI e EIS+EI. O estresse ocorreu entre P21-P35. Em P40, os animais foram eutanasiados, os encéfalos coletados, seccionados (50 µm) e corados pela técnica de Nissl. A espessura do CPFm foi medida em Cg1 e Cg3 com régua milimetrada acoplada ao microscópio. O volume de Ca1, Ca3 e Giro denteado (GD) do hipocampo foi estimado pelo princípio de Cavalieri. Na análise estatística utilizou Two-way ANOVA. **Resultados:** Na espessura do CPFm, apenas no grupo EIS+EI_m houve redução em ambas sub-regiões Cg1 e Cg3. No hipocampo (n=6-9), apenas no grupo combinado EIS+EI houve aumento do volume, sendo que na região Ca3 ocorreu em ambos os sexos e em Ca1 apenas no macho. No GD, apenas o grupo EIS+EI apresentou aumento de volume, sendo específico para os machos. **Conclusão:** O estudo permite concluir que os efeitos do estresse foram sexo e tipo dependente. Em que curiosamente o EIS+EI mostrou ser redutivo no CPFm, e trófico no hipocampo.

Palavras-chave: estresse juvenil; privação social; estresse físico.

Financiador (es): CAPES, CNPq, UEM.

Área temática: Etiofisiopatologia de doenças humanas e animais.



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EFEITO DO TRATAMENTO COM γ -TERPINENO SOBRE CÉLULAS CALICIFORMES NO CÓLON DE CAMUNDONGOS EM UM MODELO DE SEPSE EXPERIMENTAL

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Introdução: O γ -terpineno é um composto pertencente a um grupo de moléculas com propriedades anti-inflamatórias, inibidoras de mediadores pró-inflamatórios, antibacterianas, antitumorais, apresentando em especial efeitos analgésicos, antimicrobianos e anti-inflamatórios. A sepse é um quadro de inflamação sistêmica, que leva à disfunção generalizada de múltiplos órgãos, sendo uma condição grave potencialmente fatal. Este estudo tem como objetivo investigar e analisar os efeitos da administração do γ -terpineno em um modelo experimental de sepse, focando nas células caliciformes do cólon, já que estudos realizados previamente abordam inflamações locais e agudas. **Objetivo:** Investigar os efeitos do γ -terpineno sobre células caliciformes do cólon sob condição de sepse induzida, considerando a resposta inflamatória colônica. **Material e métodos:** A sepse foi induzida utilizando o método de ligação e perfuração do ceco com os camundongos anestesiados. Uma hora antes do procedimento, os camundongos foram tratados com γ -terpineno nas doses de 50mg/kg ou 75mg/kg e realocados em quatro grupos (n=10), utilizando óleo de soja como veículo. Após 24 da indução, os camundongos foram submetidos a eutanásia e coletou-se um centímetro do cólon para análise histológica. As amostras foram processadas, coradas com Periodic Acid Schiff (PAS) e avaliadas em microscopia óptica. A quantificação celular foi submetida à análise estatística por ANOVA. **Resultados:** A contagem de células caliciformes não evidenciou diferenças relevantes entre os grupos tratados e controle. O γ -terpineno demonstrou efeito mínimo sobre a resposta inflamatória intestinal, não alterando significativamente o número de células caliciformes. **Conclusão:** Os achados sugerem que a sepse não modificou substancialmente a densidade de células caliciformes no cólon e que o tratamento com γ -terpineno não exerceu impacto relevante nesse parâmetro. Estudos adicionais são necessários para esclarecer se o composto apresenta efeitos em outros marcadores inflamatórios intestinais ou sistêmicos.

Palavras-chave: sepse; γ -terpineno; células caliciformes colônicas.

Área temática: Etiofisiopatologia de doenças humanas e animais.



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ALTERAÇÃO NO EIXO IMUNO-ENDÓCRINO PELO *Toxoplasma gondii* CAUSA PERDA NEURONAL NO PLEXO SUBMUCOSO DO ÍLEO DE RATOS *WISTAR*

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Introdução: Os seres humanos são hospedeiros intermediários do *Toxoplasma gondii*. Estes podem ser infectados por oocistos deste parasita encontrados na água ou em verduras mal lavadas. Na mucosa intestinal o *T. gondii* entra em contato com as células enteroendócrinas (CEs) e com o plexo submucoso, como os neurônios e a glia entérica. As CEs e a glia entérica fazem parte do eixo imuno-endócrino responsáveis por liberar citocinas e quimiocinas ocasionando inflamação intestinal. **Objetivo:** Avaliar as alterações do *T. gondii* sobre o eixo imuno-endócrino e os neurônios entéricos do plexo submucoso do íleo de ratos *Wistar*. **Material e métodos:** O protocolo experimental foi aprovado pelo CEUA (013/2013). Quinze ratos machos *Wistar* (60 dias de idade) foram aleatoriamente distribuídos em 3 grupos (n=5): não infectado (GC) e, com 24 (GI24h) e 72 horas (GI72h) de infecção com a inoculação de 5000 oocistos de *T. gondii* esporulados (cepa Me-49, genótipo II). Segmentos diferentes do íleo foram utilizados para a técnica de imunofluorescência para marcação dos neurônios (HuC/D) e células da glia (S100) e, para a técnica de Grimelius para evidenciar as CEs. Os resultados obtidos foram submetidos à análise de variância *One-way ANOVA*, seguida de Tukey, com nível de significância de 5%. **Resultados:** Os neurônios do plexo submucoso obtiveram uma perda significativa a partir de 24 horas de infecção ($p<0,05$) que permaneceram assim até 72hs. Já, a glia entérica e as CEs obtiveram um aumento significativo nos grupos GI24h ($p<0,05$) e GI72h ($p<0,05$). O aumento das células presentes no eixo imunoendócrino e secreção das citocinas pró-inflamatórias geram uma inflamação local, ocasionando uma perda neuronal neste plexo. **Conclusão:** A alteração do eixo imuno-endócrino pelo *T.gondii* causa uma perda neuronal do plexo submucoso do íleo de ratos *Wistar*.

Palavras-chave: toxoplasmose; células enteroendócrinas; glia entérica.

Área temática: Etiofisiopatologia de doenças humanas e animais.



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ASSOCIAÇÃO DE NANOPARTÍCULAS DE SELÊNIO E CARVACROL: NOVA PERSPECTIVA NO CONTROLE DE *Staphylococcus aureus*

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Introdução: *Staphylococcus aureus* é um patógeno relevante pela sua resistência antimicrobiana e por causar intoxicações alimentares. O carvacrol apresenta ação antimicrobiana, mas é limitado pelo sabor residual, enquanto as nanopartículas de selênio (SeNPs), também são eficazes, mas tóxicas em altas doses. A associação desses compostos pode potencializar a atividade antimicrobiana e ampliar a eficácia contra diferentes bactérias. **Objetivos:** Avaliar a atividade antimicrobiana da associação entre SeNPs e carvacrol contra *S. aureus*. **Material e métodos:** A concentração inibitória mínima (CIM) do carvacrol e das SeNPs, frente a *S. aureus* ATCC 29213, *S. aureus* ATCC 43300 (MRSA) e *S. aureus* FRI S6 (cepa toxigênica), foi determinada pelo método de microdiluição em caldo, segundo o *Clinical Laboratory Standard Institute* (CLSI). Para determinar a concentração bactericida mínima (CBM), alíquotas de 10 µL dos poços sem crescimento, foram semeadas em Ágar Triptona de Soja (TSA), considerando-se como CBM a menor concentração sem crescimento bacteriano. O método *checkerboard*, com leitura em fluorímetro, avaliou a combinação dos compostos, seguido do cálculo do índice de concentração inibitória fracionada (FICI). Os *softwares* Prism e Combenefit representaram os resultados em gráficos. **Resultados:** Para *S. aureus* ATCC 29213, a CIM de SeNPs e carvacrol, foi 2 mM e 312 µg/mL, respectivamente, com CBM 4 mM (SeNPs) e 625 µg/mL (carvacrol). Para *S. aureus* ATCC 43300, SeNPs apresentou CIM e CBM 0,5 mM, já o carvacrol, demonstrou CIM 312 µg/mL e CBM 625 µg/mL. A CIM e a CBM de SeNPs contra *S. aureus* FRI S6 foi 1 mM, enquanto o carvacrol apresentou CIM 312 µg/mL e CBM 625 µg/mL. Os valores de FICI foram 0,3 (*S. aureus* ATCC 29213), 0,2 (*S. aureus* ATCC 43300) e 0,3 (*S. aureus* FRI S6), demonstrando efeito sinérgico (FICI ≤ 0,5) contra as cepas testadas. **Conclusão:** A combinação de SeNPs e carvacrol mostrou-se altamente eficaz e sinérgica contra *S. aureus*, inclusive em cepas resistentes e toxigênicas.

Palavras-chave: *Staphylococcus aureus*; carvacrol; nanopartículas de selênio.

Área temática: Patógenos de interesse médico.



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CARACTERIZAÇÃO ESTRUTURAL DA PROTEÍNA RECOMBINANTE TIORREDOXINA REDUTASE DE *Paracoccidioides lutzii*

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Introdução: O sistema tiorredoxina é composto pelas proteínas tiorredoxina e tiorredoxina redutase (TRR1), responsáveis pelo controle do estresse oxidativo e homeostase redox garantindo a sobrevivência do patógeno. Assim, tornam-se alvo para pesquisas de novos medicamentos. A TRR1 é uma flavoenzima com estrutura monomérica e homodimérica de peso molecular 37,983 kDa, essencial para várias espécies de fungos. **Objetivo:** Caracterizar a estrutura tridimensional da TRR1 do fungo patogênico *Paracoccidioides lutzii*. **Material e métodos:** A massa molar foi determinada por gel filtração (SEC) e espalhamento de luz multiangular (MALS). A proteína alvo foi purificada por cromatografia de afinidade (HisTrap de alto desempenho – Cytiva) seguida por cromatografia por exclusão de tamanho (SEC, sigla em inglês) no aparelho ÄKTA Purifier (GE Healthcare) acoplado a coluna HiloLoad 16/600 Superdex 200, pré-equilibrada com tampão (25 mM NaH₂PO₄, 150 mM NaCl, pH7.4), sendo este também a fase móvel com fluxo de 1 mL/min, a 25 °C. **Resultados:** Dois picos principais foram obtidos após análise das frações coletadas no SEC, sendo o primeiro aproximadamente em 63 mL (menor intensidade) e o segundo, aproximadamente 75 mL (maior intensidade). Os resultados do MALS corroboraram com o SEC, apresentando dois estados oligoméricos: com a formação de agregados em hexâmero (235±8 kDa) em menor quantidade e de dímeros (86±3 kDa), em maior quantidade. **Conclusão:** A TRR1 do *P. lutzii* foi obtida na conformação dimérica e com peso molecular compatível com a literatura. Assim, este estudo poderá contribuir com a resolução experimental da estrutura tridimensional das proteínas do sistema tiorredoxina, sendo fundamental para o desenvolvimento de novos antifúngicos mais seletivos.

Palavras-chave: tiorredoxina redutase; antifúngicos; gel filtração.

Financiador (es): CNPq/CAPES, FAPDF.

Área temática: Patógenos de interesse médico.



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EFEITO DO ÓLEO DE CAPIVARA (*Hydrochoerus hydrochaeris*) SOBRE O MODELO EXPERIMENTAL DE ASMA ALÉRGICA EM CAMUNDONGOS C57BL/6

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Introdução: A asma é uma condição respiratória crônica marcada por inflamação das vias aéreas e representa uma preocupação global com impacto significativo em milhões de pessoas. Apesar do uso comum de corticóides e broncodilatadores, o controle da asma é desafiador, com potenciais efeitos colaterais, motivando busca por novas estratégias terapêuticas, como o uso de ácido graxo poli-insaturado ômega-3. **Objetivo:** Avaliar se o uso terapêutico do óleo de capivara pode reverter ou atenuar as mudanças inflamatórias provocadas no modelo experimental de asma alérgica. **Material e métodos:** Camundongos C57BL/6 machos (CEUA/UERJ: N°003/2024) foram sensibilizados com ovalbumina nos dias 0, 7 e 14, seguido por desafios com ovalbumina ou Salina em aerossol nos dias 22, 24, 26 e 28. No 21° dia a contar da primeira sensibilização, os animais foram tratados com óleo de capivara ou água filtrada por gavagem orogástrica durante 7 dias, com tratamento realizado 2 horas antes do desafio. No 29° dia os animais foram eutanasiados, foi realizado o lavado broncoalveolar, os pulmões foram perfundidos com solução salina 0,9% e os lobos foram separados para análise histológica (H&E, PAS e gomori). Os resultados foram expressos como média $\pm$ erro padrão da média e analisados através de análise de variância (ANOVA), seguida de teste de comparação múltipla de "Holm-Sidak", com significância $P < 0,05$. **Resultados:** O tratamento com óleo de capivara promoveu a redução do infiltrado inflamatório nas regiões peribronquiolares e a diminuição do número total de leucócitos no lavado broncoalveolar e na corrente sanguínea dos animais sensibilizados com ovalbumina. O óleo de capivara também atenuou a secreção de muco e a deposição de colágeno no pulmão, sugerindo efeito positivo sobre o remodelamento tecidual. Grupos controle e controle tratados não apresentaram alterações significativas. **Conclusão:** O óleo de capivara mostrou potencial terapêutico para reduzir inflamação e remodelamento pulmonar em modelo murino de asma.

Palavras-chave: Alergia; inflamação; PUFA's.

Financiador (es): Fundação Carlos Chagas Filho de Amparo à Pesquisa do Rio de Janeiro (FAPERJ); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Financiadora de Estudos e Projetos (FINEP); Universidade do Estado do Rio de Janeiro (UERJ).

Área temática: Etiofisiopatologia de doenças humanas e animais.



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INTOXICAÇÃO AGUDA POR ABUSO DE SUBSTÂNCIAS PSICOATIVAS: RELATO DE CASO

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Introdução: O processo de intoxicação pode ser caracterizado como uma manifestação clínica, que apresenta sinais e sintomas causados por efeitos nocivos no organismo do indivíduo em decorrência de interações com agentes tóxicos. **Objetivo:** Relatar um caso de intoxicação por uso concomitante de bebida alcoólica e substâncias psicoativas. **Material e métodos:** Estudo de caso realizado por meio de análise da ficha de exposição humana notificada a um Centro de Assistência e Informação Toxicológica (CIATox). Projeto aprovado pelo Comitê de Ética em Pesquisa, sob parecer n. 6.940.629. **Resultados:** Paciente do sexo masculino, 20 anos, socorrido pelo SAMU em estado de torpor e encaminhado para serviço de saúde hospitalar. Segundo a notificação ao CIATox, o paciente ingeriu bebida alcoólica, mas evoluiu para depressão do Sistema Nervoso Central (SNC) profunda após uso de lança-perfume. Relatou-se que o paciente era usuário de cocaína e crack. Na admissão hospitalar, o paciente foi entubado com escala de Glasgow 5 e permanecia com sonda de drenagem aberta. Mantinha sedação com benzodiazepínicos e hidratação endovenosa contínua. Após transferência para UTI, houve episódio de hipoglicemia, o qual foi corrigido. Paciente evoluiu para melhora clínica com extubação e retomada da consciência. O mesmo relatou ingerir um copo de bebida misturado com uma “bala rosa” no fundo e após não possuía mais lembranças. No segundo dia de internação, paciente evoluiu com períodos de agitação psicomotora e ansiedade pela alta hospitalar, por fim, evadiu-se do hospital. **Conclusão:** A intoxicação pela combinação de álcool e substâncias psicoativas pode provocar depressão profunda do SNC, exigindo suporte intensivo e orientação especializada dos CIATox. Protocolos ágeis de diagnóstico e tratamento, aliados a ações preventivas para jovens, como a redução de danos e apoio psicossocial são essenciais para reduzir riscos e evitar a evasão hospitalar.

Palavras-chave: intoxicação; drogas ilícitas; relatos de casos.

Área temática: Eco-epidemiologia e controle de doenças.



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AVALIAÇÃO *in vitro* DA INTERAÇÃO ENTRE ITRACONAZOL E 2-ETIL-1-HEXANOL FRENTE A *Candida albicans* ATCC 90028

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Introdução: *Candida albicans* é um fungo oportunista capaz de causar infecções superficiais e sistêmicas, com diferentes graus de severidade. Apresenta diversos mecanismos de virulência e patogenicidade, sobretudo a resistência a antifúngicos. Trabalhos anteriores já demonstraram a capacidade de algumas cepas de *C. albicans* em apresentar resistência, principalmente a classe dos azólicos. Diante disso, a busca por novas substâncias com potencial antifúngico ou que atuem em associação com fármacos convencionais contra *C. albicans* é de grande relevância. Nesse contexto, moléculas de *quorum sensing* (MQS) têm sido relatadas na literatura por seu potencial antimicrobiano. Entre elas, 2-etil-1-hexanol (2-EH), produzida por *Fusarium oxysporum*, na qual sua atividade antifúngica já foi demonstrada. Assim, investigar o caráter antifúngico de 2-EH e sua interação com antifúngicos convencionais frente *C. albicans*, apresenta importância científica. **Objetivo:** Avaliar *in vitro* a interação entre 2-EH e ITC frente *C. albicans* ATCC 90028 e verificar se há sinergismo, adição ou antagonismo. **Material e métodos:** A interação foi avaliada pela técnica de *checkerboard*, com interpretação baseada nos valores do índice de concentração inibitória fracionária (ICIF). **Resultados:** 2-EH e ITC apresentaram efeito sinérgico frente ao isolado de *C. albicans* com o valor de ICIF = 0,0688 ($\leq 0,5$). A concentração inibitória mínima (CIM) de ITC na combinação foi de 0,0625 µg/ml, representando uma redução de 128 vezes em relação ao fármaco isolado (8 µg/ml). Para 2-EH, a CIM na associação foi de 0,0016 g/ml, 16 vezes menor, quando comparado a molécula isolada (0,0260 g/ml). **Conclusão:** 2-EH apresentou potencial modulador da atividade antifúngica do ITC contra *C. albicans*, evidenciado pela interação sinérgica significativa e pela redução expressiva da CIM dos dois compostos em combinação.

Palavras-chave: sinergismo; 2-etil-1-hexanol; itraconazol.

Financiador (es): Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e CAPES.

Área temática: Eco-epidemiologia e controle de doenças.



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INTERFACES DA HOMEOPATIA MÉDICA E A BIOCIÊNCIAS: UMA REVISÃO INTEGRATIVA.

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Introdução: Com mais de 200 anos de existência, a homeopatia continua a gerar controvérsias, onde o debate persiste em razão da inconsistência e fragilidade das evidências. **Objetivo:** Analisar, por meio de uma revisão integrativa, resultados científicos disponíveis sobre efeitos biológicos e moleculares da homeopatia. **Material e métodos:** Com rigor e transparência metodológica, esta revisão integrativa sintetizou criticamente evidências e debates atuais sobre a relação entre homeopatia e biociências. A busca em banco de dados científicos, resultando na seleção e análise de 27 artigos para identificar evidências fundamentadas em estudos *in vitro*, criando um panorama completo sobre o tema. **Resultados:** De acordo com a literatura científica, as preparações homeopáticas não são biologicamente inativas. No entanto, alguns estudos *in vitro* com *Apis mellifica* (doi:10.1016/j.homp.2014.01.003) e *Drosera rotundifolia*, utilizando tintura-mãe e diluições (10–6 M a 10–17 M) ou potências (3C, 5C, 7C e 3x), indicaram efeitos biológicos como: modulação da expressão gênica, indução de respostas horméticas (efeito benéfico em baixas doses), regulação da resposta inflamatória, e promoção do reparo tecidual e cicatrização de feridas. Os ensaios clínicos mostraram resultados favoráveis, com a melhoria na qualidade de vida em casos de rinite alérgica sazonal e diminuição da dor na osteoartrite do joelho, no qual o uso do gel homeopático, comparado ao piroxicam, resultou em maior redução da dor e menos efeitos colaterais (doi:10.1093/rheumatology/39.7.714). O estudo sobre câncer de mama indica que o benefício não residiu na prescrição, mas sim no aspecto comportamental, no qual o ritual diário de medicação oferece aos pacientes controle e empoderamento (doi:10.1177/15347354241233302). **Conclusão:** A homeopatia apresenta um potencial terapêutico, influencia a expressão gênica e a hormese, sugerindo uma fundamentação biológica para seu efeito. No entanto, as evidências clínicas ainda não são conclusivas. Essa discrepância entre a promessa molecular e os benefícios clínicos requer uma investigação mais rigorosa, transparente e reproduzível.

Palavras-chave: homeopatia; biociências; revisão integrativa.

Financiador (es): Instituto de Homeopatia Benoit Mure.

Área temática: Etiofisiopatologia de doenças humanas e animais.



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RELAÇÃO ENTRE ESPÉCIES REATIVAS DE OXIGÊNIO E MORTE CELULAR DE *Trichophyton rubrum* APÓS TERAPIA FOTODINÂMICA MEDIADA POR Hyp-P123

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Introdução: A combinação da hipericina com o plurônico P123® (Hyp-P123) favorece a permeação da Hyp nas células de *Trichophyton rubrum* atuando como fotossensibilizador na terapia fotodinâmica (TFD). A fotoativação da Hyp-P123 possibilita reações bioquímicas responsáveis pela morte celular por meio da geração de espécies reativas de oxigênio (EROs). **Objetivo:** Estudar a liberação de EROs em cepas de *T. rubrum* expostas à TFD+Hyp-P123. **Material e métodos:** Cepas de *T. rubrum* (TR0022 e TR0870) foram ajustados a 1×10^5 conídios/mL e submetidos a concentrações inibitórias e fungicidas mínimas (CIM e CFM) de Hyp-P123+TFD obtidas em estudos anteriores do grupo de pesquisa. Para isso, as cepas foram incubadas 2h à 25°C, com as devidas concentrações de Hyp-P123 e posteriormente irradiadas a 37,8 J/cm². A liberação de EROS foi avaliada em 5, 15, 30, 60 e 120 minutos após a TFD, utilizando 10 µM de H2DCFDA (diacetato de 2',7'-diclorodihidrofluoresceína). A fluorescência foi captada pelo citômetro BD FACSCalibur (Becton-Dickinson, CA, EUA), utilizando filtros FL1 (530/30nm) e FL2 (585/42), 10.000 eventos. Como controle positivo para EROS foi utilizado H₂O₂, e controle negativo, fungo sem exposição a TFD+Hyp-P123. **Resultados:** A liberação de EROS foi linearmente aumentando após TR0022 ser submetido a CIM da TFD+Hyp123, passando de 23% (5') para 60% (120'). Por outro lado, a cepa TR0870, manteve uma média constante de EROS, com liberação média de 13% até o último tempo avaliado. Valores comparados ao controle negativo. **Conclusão:** Trabalhos anteriores mostram que TR0022 era mais sensível à ação da TFD+Hyp-P123 do que TR0870. Neste estudo, mostramos que a baixa detecção de EROS em TR0870 pode ser oriunda de uma detoxificação realizada por essa cepa, tornando-a menos sensível à aplicação da TFD nas concentrações testadas.

Palavras-chave: terapia fotodinâmica; espécies reativas de oxigênio; citometria de fluxo.

Financiador: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Área temática: Etiofisiopatologia de doenças humanas e animais.



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UTILIZAÇÃO DE ESPÉCIES DE *Bacillus* NO CONTROLE BIOLÓGICO DE *Candida albicans*

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Introdução: O gênero *Candida* compreende aproximadamente 200 espécies de leveduras oportunistas. *C. albicans* é uma levedura dimorfa, ou seja, apresenta forma de levedura, importante para a colonização e disseminação, e forma filamentosa importante para a invasão dos tecidos e danos às células hospedeiras. Devido ao aumento de resistência microbiana, estudos visando a aplicação de antimicrobianos alternativos têm ganhado destaque. Bactérias do gênero *Bacillus* podem produzir compostos bioativos com potencial antimicrobiano de importância biotecnológica representando uma nova alternativa terapêutica contra infecções fúngicas. **Objetivo:** O objetivo deste trabalho foi verificar a ação fungicida, contra *Candida albicans* cepa ATCC90028, de metabólitos secundários produzidos por *Bacillus* spp. e analisar as possíveis alterações morfológicas nas células fúngicas, após contato com as bactérias. **Material e métodos:** Foi realizada a cultura pareada entre oito espécies de *Bacillus* e a levedura, em meio de cultura batata dextrose ágar (BDA). Para verificação de mudanças no padrão de filamentação das leveduras sob a ação das bactérias foi realizado microcultivo em ágar Fubá acrescido de TWEEN 80. A análise dos resultados foi realizada por microscopia eletrônica de varredura. **Resultados:** Foram observadas variações relacionadas à presença de blastoconídios, hifas e pseudohifas. Ademais, foi possível observar alterações morfológicas associadas à deformação da membrana e na parede celular reduzindo o volume citoplasmático e levando à formação de células fantasmas (*Ghost cells*). Os resultados obtidos demonstraram a ação fungicida de metabólitos secundários produzidos pelas cepas de *Bacillus* utilizadas contra *C. albicans*. **Conclusão:** Os resultados obtidos demonstraram a ação fungicida de metabólitos secundários produzidos pelas cepas de *Bacillus* utilizadas contra *C. albicans*. Além disso, as análises por microscopia possibilitaram a observação de alterações morfológicas na levedura, confirmando a ação antifúngica desses compostos sobre a estrutura celular.

Palavras-chave: biossurfactantes; *Candida* spp.; *Bacillus* spp.

Área temática: Patógenos de interesse médico.



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ADMINISTRAÇÃO DE METFORMINA DURANTE A ADOLESCÊNCIA E SEUS EFEITOS A LONGO PRAZO

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Introdução: a puberdade é uma fase crítica de plasticidade metabólica, segundo o conceito DOHaD, uma janela de oportunidade para intervenções que podem impactar a saúde no ciclo vital de animais. **Objetivo:** avaliar se o tratamento com metformina na adolescência atenua parâmetros metabólicos alterados pela sobrenutrição neonatal.

Materiais e Métodos: os procedimentos experimentais foram aprovados pelo comitê de ética da Universidade estadual de Maringá (CEUA/4718150623). Durante a lactação, ratos Wistar machos foram distribuídos em grupos Ninhada Normal (NL) e Ninhada Reduzida (SL). Entre 30 e 60 dias, receberam metformina (300 mg/kg, via água) ou apenas água filtrada, formando os grupos NL-C, NL-M, SL-C e SL-M. Foram avaliados peso corporal, consumo alimentar, ingestão hídrica e peso de gorduras (retroperitoneal, perigonadal e mesentérica) até os 120 dias. **Resultados:** Animais SL apresentaram maior peso corporal, consumo alimentar e acúmulo de tecido adiposo em relação aos NL; o grupo SL reproduziu características de obesidade induzida por sobrenutrição neonatal. O tratamento durante a puberdade reduziu significativamente o ganho de peso corporal, diminuiu o consumo alimentar e reduziu o acúmulo de gordura em animais SL ($p < 0,05-0,01$), sem alterar a ingestão hídrica e sem efeitos relevantes nos animais NL. A metformina durante a puberdade foi eficaz em reduzir peso corporal e adiposidade apenas em animais SL, sugerindo que os efeitos benéficos de intervenções nesta janela ficam evidentes na presença de distúrbios metabólicos. A redução do consumo alimentar observada pode indicar influência do fármaco em mecanismos de saciedade durante períodos plásticos do desenvolvimento, reforçando o potencial da puberdade para intervenções benéficas à saúde. **Conclusão:** o tratamento com metformina durante a puberdade atenuou os efeitos da sobrenutrição neonatal, ressaltando a puberdade como uma janela crítica para intervenções que visem atenuar a obesidade e suas complicações no ciclo vital de mamíferos.

Palavras-chave: obesidade; programação metabólica; DOHaD.

Financiador (es): CNPq, CAPES, JBS, ISPAM.

Área temática: Etiofisiopatologia de doenças humanas e animais.



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FATORES DE RISCO AUMENTADOS PARA GESTANTES COM COVID-19, DE ACORDO COM VARIÁVEIS EPIDEMIOLÓGICAS E CLÍNICAS: UMA REVISÃO SISTEMÁTICA DE REVISÕES

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Introdução: revisões sistemáticas e metanálises relatando fatores de risco epidemiológicos, clínicos e laboratoriais para a COVID-19 em mulheres grávidas estão disponíveis, mas não há nenhuma revisão sistemática de revisões ou metanálises sobre esse tema. **Objetivo:** investigar os fatores de risco e complicações em gestantes com COVID-19 por meio de uma revisão sistemática de revisões sistemáticas com metanálise. **Material e métodos:** O estudo seguiu as recomendações da declaração PRISMA e aplicou o checklist AMSTAR-2 para análise de viés. Foram realizadas buscas nas bases de dados PubMed, Embase, Scopus, Web of Science, Cochrane Library e BVS/Lilacs, incluindo estudos publicados entre 01 dezembro de 2019 e 30 de abril de 2025. A estratégia de busca foi estruturada em três blocos de pesquisa: COVID-19 OR SARS-CoV-2 AND Pregnant women OR Pregnancy AND Pregnancy complication OR Risk factor. As publicações selecionadas relataram variáveis epidemiológicas e clínicas de gestantes. As variáveis preditoras e de desfecho foram resumidas de acordo com medidas de efeito (risco relativo, *odds ratio*) ou proporção, com intervalo de confiança de 95%. **Resultados:** foram incluídas 10 revisões sistemáticas e metanálises, envolvendo 428 artigos originais e 2.395.733 gestantes com COVID-19. A idade média das mulheres foi de 31 anos, com idade gestacional entre 30 e 40 semanas. Diabetes mellitus, diabetes gestacional, hipertensão, doença cardiovascular, obesidade, tabagismo, doença respiratória crônica (incluindo asma) e pré-eclâmpsia apresentam maior risco de gravidade para COVID-19. Para pacientes admitidas em unidade de terapia intensiva, o baixo peso antes da gravidez apresentou risco aumentado para ventilação mecânica e óbito relacionado à gravidez. A mortalidade foi particularmente elevada entre gestantes com diabetes mellitus, hipertensão e doenças cardiovasculares. **Conclusão:** gestantes com COVID-19 e comorbidades pré-existentes apresentam risco significativamente maior de morbidade e mortalidade materna. As principais complicações associadas foram descolamento de placenta, pré-eclâmpsia, eclâmpsia e distúrbios hipertensivos da gravidez.

Palavras-chave: SARS-CoV-2; fator de risco; gravidez.

Financiador: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

Área temática: Eco-epidemiologia e controle de doenças.



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ANÁLISE DE ASSOCIAÇÃO ENTRE O POLIMORFISMO *rs8192917* (Q48R) DO GENE DA GRANZIMA B E O PROGNÓSTICO DA COVID-19

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Introdução: a apoptose induzida por granzimas e perforinas é a principal via empregada pelos linfócitos T CD8+ e células NK no combate às infecções virais, sendo a granzima B uma enzima capaz de induzir a morte celular por diversas vias. O vírus SARS-CoV-2, causador da COVID-19, é um patógeno de elevada transmissibilidade e taxa de mutação, capaz de desencadear quadros sintomáticos de diversos níveis de gravidade. Alguns polimorfismos do gene da granzima B (*GZMB*), como o *rs8192917* (Q48R), afetam sua estrutura e podem prejudicar sua função. **Objetivo:** analisar a frequência genotípica e alélica do polimorfismo *rs8192917* em pacientes infectados pelo vírus da COVID-19. Verificar seu potencial como marcador genético de prognóstico da doença, analisando estatisticamente a relação entre o polimorfismo e os parâmetros clínicos dos pacientes.

Material e métodos: este trabalho foi aprovado pelo Comitê de Ética em Pesquisa com Seres Humanos da Universidade Estadual de Maringá (pareceres 4.357.896/2020 e 4.508.676/2020). Foram genotipadas 200 amostras de pacientes com COVID-19, por qPCR. Os resultados obtidos foram analisados por regressão logística binária e ordinal (nível de significância adotado: 5%) ajustada para fatores confundidores como idade, sexo e comorbidades. **Resultados:** não foi encontrada associação significativa entre o polimorfismo Q48R e a necessidade de internação do paciente em Unidade de Terapia Intensiva (modelo genotípico, CT: $p=0,698$; modelo dominante: $p=0,946$) e o desfecho da doença (modelo genotípico, $p=0,524$; modelo dominante: $p=0,575$). O polimorfismo também não foi associado à gravidade da doença, no entanto, uma tendência de associação entre o genótipo CT ($p=0,08$) e pior gravidade da doença foi observada.

Conclusão: os resultados obtidos não sugerem associação entre o polimorfismo *rs8192917* e o prognóstico da COVID-19. Se faz pertinente, no entanto, a realização de estudos mais abrangentes sobre o tema.

Palavras-chave: gene *GZMB*; polimorfismo de nucleotídeo único; COVID-19.

Área temática: patógenos de interesse médico.



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OTIMIZAÇÃO DE METODOLOGIA PARA ANÁLISE DE ANFETAMINAS EM AMOSTRAS DE *DRIED URINE SPOT*

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Introdução: o impacto do consumo de anfetaminas (AFs) nas análises toxicológicas é significativo, uma vez que essas substâncias exigem métodos eficazes para detecção dos inúmeros derivados existentes. As AFs são predominantemente excretadas pela urina, tornando-a uma matriz adequada para análise, com uma coleta simples e não invasiva. A metodologia de *Dried Urine Spot* (DUS) surge como alternativa para facilitar o armazenamento e transporte, com menor risco de contaminação quando comparada às amostras biológicas líquidas. **Objetivo:** otimizar metodologia para detecção de AFs e seus produtos de biotransformação em amostras de urina seca, utilizando Cromatografia Líquida de Ultra Performance acoplada à espectrometria de massas (UPLC-MS). **Materiais e métodos:** amostras negativas de urina (30 µL) obtidas de voluntários foram aplicadas em papel filtro, secas por 2 horas à temperatura ambiente, recortadas e colocadas em tubo Eppendorf. A otimização da extração foi realizada com diferentes volumes de solvente metanol/acetonitrila (500, 750 e 1000 µL) e condições de tempo no banho ultrassônico (10, 20 e 30 minutos). A identificação e quantificação de anfetamina, metanfetamina e MDMA foi realizada pela UPLC-MS. Projeto aprovado pelo COPEP/UEM sob parecer n. 6.956.864. **Resultados:** a extração de anfetamina e MDMA apresentou melhor rendimento após 20 minutos em banho ultrassônico, havendo redução na detecção dessas substâncias quando o tempo foi estendido para 30 minutos. Em contrapartida, a metanfetamina demonstrou maior eficiência de extração no tempo de 30 minutos. Quanto ao volume de solvente empregado, observou-se que a anfetamina e o MDMA apresentaram maior eficiência de extração com 750 µL, ao passo que a metanfetamina apresentou melhor detecção com 1000 µL. **Conclusão:** a técnica de DUS demonstrou ser uma alternativa eficiente para as análises toxicológicas de AFs com melhor volume de extração em 750 µL de solvente e 20 minutos de banho ultrassônico.

Palavras-chave: anfetamínicos; urina; mancha seca.

Área temática: eco-epidemiologia e controle de doenças



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HIDRÓXI-PIRROLONAS COMO CANDIDATOS ANTIVIRAIS: UMA ABORDAGEM TEÓRICO-EXPERIMENTAL CONTRA O HERPESVÍRUS HUMANO TIPO 1

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Introdução: A infecção pelo vírus Herpes simples tipo 1 (HSV-1) constitui um importante problema de saúde global, agravado pelo surgimento de cepas resistentes aos tratamentos convencionais, como o aciclovir, o que reforça a necessidade de novos agentes terapêuticos. Este estudo investiga as hidróxi-pirrolonas, por sua semelhança estrutural a derivados pirrólicos, já amplamente estudados por diversas atividades biológicas, incluindo efeitos antivirais. **Objetivo:** Avaliar o potencial antiviral de dez hidróxi-pirrolonas inéditas (JP1-10) contra o vírus Herpes simples tipo 1 (HSV-1) por meio de uma abordagem combinando análises experimentais e teóricas. **Material e métodos:** Inicialmente, os compostos JP1-10 foram submetidos a análises *in silico* de parâmetros ADMET e à Regra dos Cinco de Lipinski. Em seguida, foram conduzidos ensaios *in vitro* em células Vero para determinação da citotoxicidade (CC₅₀) e da atividade antiviral frente ao HSV-1. Por fim, foram submetidos a simulações de *docking* molecular, visando avaliar a afinidade de ligação com a timidina quinase (TK) viral. **Resultados:** As análises *in silico* indicaram que a maioria dos compostos possuem perfis farmacocinéticos favoráveis. Nos ensaios *in vitro*, os compostos JP6 e JP9 se destacaram pela baixa citotoxicidade e por apresentarem atividade antiviral moderada e dose-dependente, embora não tenham atingido 50% de inibição nas concentrações testadas. Diante desse desempenho, apenas esses dois derivados foram selecionados para o *docking* molecular, que revelou que o composto JP6 possui afinidade de ligação similar a inibidores conhecidos da enzima TK viral, sugerindo um possível mecanismo de ação, enquanto o JP9 apresentou baixa afinidade, indicando um alvo alternativo. **Conclusão:** O estudo demonstrou o potencial das hidróxi-pirrolonas como candidatos anti-HSV-1. O derivado JP6 destacou-se por sua baixa toxicidade, atividade antiviral moderada e possível mecanismo de ação via inibição da Timidina Quinase. Esses achados contribuem para o avanço no desenvolvimento de agentes antivirais inovadores e justificam investigações futuras mais aprofundadas.

Palavras-chave: antiviral; hidróxi-pirrolonas; HSV-1.

Área temática: Eco-epidemiologia e controle de doenças.



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CARACTERIZAÇÃO ESPECTROSCÓPICA E PERMEÇÃO DO EXTRATO DE *Muyocopron* sp.

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Introdução: A crescente resistência dos microrganismos aos fármacos convencionais incentiva a busca por novos compostos bioativos com potencial antimicrobiano. Fungos endofíticos destacam-se nesse contexto por produzirem metabólitos únicos com atividades biológicas específicas. As moléculas produzidas pelo fungo *Muyocopron* sp., isolado como endofítico da orquídea *Cattleya purpurata*, são promissoras como antifúngicos, porém não se sabe as suas características físico-química e de permeação, dados estes relevantes para aplicações no tratamento de onicomicoses. **Objetivo:** Caracterizar o extrato de *Muyocopron* sp. e avaliar sua permeação em unhas humanas. **Materiais e métodos:** O extrato foi caracterizado por espectroscopia fotoacústica (PAS), espectroscopia na região do ultravioleta-visível (UV-Vis) e espectroscopia no infravermelho com transformada de Fourier via reflectância total atenuada (FTIR-ATR). A capacidade de permeação em unha humana, foi realizada por PAS no intervalo espectral de 300-600 nm após 2 horas de aplicação do extrato na unha. O estudo foi aprovado pelo Comitê Permanente de Ética em Pesquisa com seres humanos (COPEP), número CAA: 31702520.4.0000.0104. Para a caracterização, o FTIR-ATR registrou os espectros entre 4000-400 cm^{-1} , com resolução de 4 cm^{-1} e 128 varreduras e as demais técnicas, no intervalo de 300 a 800 nm. **Resultados:** As técnicas PAS e UV-Vis mostraram que o extrato de *Muyocopron* sp. apresenta uma banda larga de absorção até aproximadamente 550 nm, além de uma banda adicional em 670nm, relacionada a presença de clorofila. Por FTIR-ATR, observaram-se bandas entre 3100–2800 cm^{-1} (–CH) e 1800–1700 cm^{-1} (–C=O). Na análise de permeação, detectou-se o aumento de absorção nas superfícies dorsal e ventral das unhas, indicando a propagação do extrato através da lâmina ungueal. **Conclusão:** O extrato de *Muyocopron* sp. foi caracterizado por diferentes técnicas espectroscópicas e demonstrou capacidade de permeação em unhas humanas, sugerindo potencial para desenvolvimento terapêutico contra onicomicoses.

Palavras-chave: fungo endofítico; onicomicose; caracterização espectroscópica.

Financiador (es): CAPES.

Área temática: Eco-epidemiologia e controle de doenças.



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PADRONIZAÇÃO DE PRIMERS PARA AMPLIFICAÇÃO DO GENE *TET2*

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Introdução: As neoplasias mieloproliferativas (MPNs) compreendem um grupo de patologias hematológicas caracterizadas por expansão clonal de células tronco-hematopoiéticas alteradas. O seu diagnóstico é facilitado pela presença ou ausência de determinadas mutações gênicas, como a mutação no gene *TET2*. Este gene está localizado no cromossomo 4q24 possuindo 11 éxons, sendo sua principal função o processo de desmetilação do DNA, quando a enzima *TET2* catalisa a conversão de 5-metilcitosina em 5-hidroximetilcitosina no DNA. Além disso, o *TET2* é responsável por atuar na renovação das células tronco-hematopoiéticas. Quando existem mutações no gene, ocorre um acúmulo de metilação do DNA, alterando sua expressão e favorecendo o surgimento de neoplasias. A maioria das mutações neste gene é de origem somática.

Objetivo: Padronizar e validar pares de primers para amplificação gênica de regiões codificantes do gene, atingindo os critérios de reprodutibilidade e sensibilidade. **Material e métodos:** Trata-se de um estudo experimental voltado à padronização e validação de primers específicos para o gene *TET2*, em que foram construídos primers F (Forward) e R (Reverse) específicos para *TET2*, por meio do GeneBank (NCBI), Primer-BLAST, Multiple Primer Analyzer e Oligo Analyzer. Posteriormente foi realizada a PCR (Reação em Cadeia de Polimerase) a fim de padronizar os mesmos, na qual os produtos amplificados foram analisados em eletroforese em gel de agarose e submetidos ao Sequenciamento de Sanger para confirmar a especificidade e integridade da amplificação gênica. Os experimentos foram realizados no Laboratório de Imunogenética da UEM após aprovação do CEP com o parecer nº 318.552. **Resultados:** Foram padronizados três pares de primers, 11.1, 11.2 e 11.3, os quais amplificaram a região de interesse, sendo alterada apenas a concentração de cloreto de magnésio entre os primers. **Conclusão:** A padronização dos primers mostrou-se eficiente para o gene *TET2*, permitindo reprodutibilidade e especificidade das regiões codificantes, contribuindo para análises genéticas de MPNs.

Palavras-chave: neoplasias mieloproliferativas; reação em cadeia de polimerase; *TET2*.

Financiador (es): Laboratório de Imunogenética da UEM (IMUNOGEN) e CNPq.

Área temática: Etiofisiopatologia de doenças humanas e animais.



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FOSFATO DE CÁLCIO BIFÁSICO DE OSSOS DE TILÁPIA: CARACTERIZAÇÃO QUÍMICA E BIOCOMPATIBILIDADE EM MODELO ANIMAL

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Introdução: A regeneração de defeitos ósseos ainda é um desafio. Em defeitos de tamanho crítico (DTC), o reparo natural do organismo é insuficiente, tornando necessário o uso de enxertos. Nesse contexto, as biocerâmicas à base de fosfato de cálcio (CaP) têm se destacado como substitutos ósseos promissores, pois podem ser obtidas de fontes naturais, constituindo alternativas abundantes e sustentáveis. O fosfato de cálcio bifásico (BCP), foco deste estudo, foi obtido a partir de ossos da tilápia (*Oreochromis niloticus*).

Objetivo: Caracterizar quimicamente o BCP por DRX e avaliar sua biocompatibilidade *in vivo* por meio de Microscopia Eletrônica de Varredura (MEV). **Materiais e métodos:** Foi utilizado difratômetro Shimadzu XRD 7000. Para o estudo *in vivo* em ratos Wistar (CEUA/UEM: 6263280422) foram criados DTCs circulares na calvária (8 mm de diâmetro e 0,8 mm de profundidade), com uma broca trefina. Os defeitos receberam em média, 0,012 g de BCP *in natura*. Após 90 dias foram eutanasiados. Para análise em MEV, foram utilizadas uma amostra de BCP puro e uma amostra do DTC contendo o enxerto.

Resultados: O DRX com refinamento de Rietveld revelou composição de 34% de β -TCP e 66% de hidroxiapatita (HAp). As imagens de MEV revelaram a porosidade natural do material *in natura*, essencial para migração de células e crescimento de vasos sanguíneos. A presença e organização de osteoblastos, bem como a matriz óssea diretamente na superfície do material, demonstram sua biocompatibilidade e capacidade de osteointegração. **Conclusão:** O fosfato de cálcio bifásico (BCP) obtido de ossos de tilápia é um material de enxerto ósseo promissor. Os resultados de DRX e MEV confirmam sua composição e a porosidade natural, demonstram sua biocompatibilidade e capacidade de osteointegração ao promover a adesão e organização de osteoblastos, o que o torna uma alternativa eficaz e sustentável para a regeneração de defeitos ósseos.

Palavras-chave: Regeneração óssea; biocompatibilidade; osteointegração.

Financiador (es): Finep, CAPES, Fundação Araucária.

Área temática: Etiofisiopatologia de doenças humanas e animais.



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AVALIAÇÃO DA ATIVIDADE DE DERIVADO DE SALICILIDRAZONAS E HÍBRIDOS DE 4-PIRIDINA-TIOSSEMICARBAZONAS EM *Mycobacterium abscessus*

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Introdução: As micobactérias não tuberculosas (MNT) de crescimento rápido, especialmente o complexo *Mycobacterium abscessus*, podem causar infecções pulmonares e de tecidos moles denominadas de micobacterioses e são caracterizadas pela alta resistência aos tratamentos disponíveis atualmente. Derivados de salicilidrazonas e híbridos de 4-piridina-tiossemicarbazonas, vêm sendo investigados por seu potencial antimicrobiano promissor em estudos *in vitro*. **Objetivos:** Diante do aumento da incidência de doenças causadas por MNT, da dificuldade de estabelecer um tratamento eficiente e da necessidade de buscar alternativas terapêuticas mais eficazes, este trabalho propôs avaliar a eficácia desses compostos contra *Mycobacterium abscessus* ATCC 19977. **Material e métodos:** Foi determinado a concentração inibitória mínima (CIM) para as substâncias, por meio do método *resazurin broth microdilution assay*, em triplicata, com repiques independentes. As microplacas foram incubadas a 30°C durante 3 dias e posteriormente, foi adicionado 30µL de solução de resazurina 0,01% que foram reincubadas por 24 horas. A CIM foi definida como a menor concentração da substância capaz de inibir a mudança de cor da resazurina de azul para uma coloração rósea, ou seja, inibição do crescimento bacteriano. **Resultados:** A CIM foi de 32 µg/mL para TSC 2, 512 µg/mL para TSC 3, 256 µg/mL para TSC 4, 16 µg/mL para TSC 5 e 128 µg/mL para SH-18. **Conclusão:** Com base nos resultados obtidos, observa-se que as substâncias testadas apresentaram atividade antimicrobiana variável contra *M. abscessus*. O composto TSC 5 demonstrou maior atividade antimicrobiana, com a menor CIM (16 µg/mL) seguido por TSC 2 (32 µg/mL), indicando um bom potencial inibitório. Em contrapartida, os compostos TSC 3, TSC 4 e SH-18 apresentaram CIMs um pouco elevadas, sugerindo uma atividade menos eficaz. Esses dados indicam que especialmente TSC 5 e TSC 2 podem ser explorados em estudos adicionais, visando o desenvolvimento de novas abordagens terapêuticas contra infecções causadas por *M. abscessus*.

Palavras-chave: micobactérias; antimicobacterianos; tratamento.

Financiador (es): Universidade Estadual de Maringá, Fundação Araucária (FA) e Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Área temática: Patógenos de interesse médico.



INTERFACES DA BIOCÊNCIA: CÉLULAS, GENES E ALGORITMOS

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DESEMPENHO DE DUAS METODOLOGIAS, EM AMOSTRAS EXTRAPULMONARES, PARA O DIAGNÓSTICO DA TUBERCULOSE

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Introdução: O diagnóstico da tuberculose extrapulmonar (TB-EP) é complexo devido à baixa carga bacilar e à diversidade de amostras. **Objetivos:** A validação do TRM GeneXpert® MTB/RIF para amostras extrapulmonares ainda não é padronizada, tornando essencial avaliar sua concordância com a cultura. **Material e métodos:** Estudo retrospectivo com 137 amostras EP processadas no Laboratório de Ensino e Pesquisa em Análises Clínicas (LEPAC/UEM) (jan/2021-mar/2023), submetidas ao TRM GeneXpert® MTB/RIF Ultra e cultura automatizada BACTEC® MGIT® 320. Dados de registros laboratoriais compararam TRM com baciloscopia e cultura para positividade e concordância. O estudo foi aprovado pelo Comitê de Ética em Pesquisa da UEM (parecer nº 6.198.443). **Resultados:** Foram analisadas 137 amostras EP. O TRM detectou quatro amostras positivas para o complexo *Mycobacterium tuberculosis*, sendo uma confirmada por cultura. Ao avaliar os resultados da cultura, padrão-ouro de diagnóstico, cinco amostras apresentaram crescimento microbiano: duas foram positivas para *M. tuberculosis*, uma para *M. avium* e duas para *Paracoccidioides* spp. Para análise de desempenho diagnóstico, foram considerados apenas os casos positivos para *M. tuberculosis*. Para a avaliação da sensibilidade, especificidade e valores preditivos do TRM, consideraram-se apenas as duas amostras com cultura positiva para *M. tuberculosis*. O TRM apresentou sensibilidade de 50,0%, especificidade de 97,8%, valor preditivo positivo (VPP) de 25,0% e valor preditivo negativo (VPN) de 99,2% em relação à cultura. Esse desempenho pode estar relacionado à natureza paucibacilar dessas amostras, nas quais a carga bacilar pode ser suficiente para detecção molecular, mas não para o crescimento em cultura. **Conclusão:** Apesar da praticidade do TRM e da baciloscopia, sua menor sensibilidade restringe o uso isolado desses métodos. A cultura, por sua vez, permanece fundamental para detectar bacilos viáveis e confirmar a infecção ativa, especialmente em casos com baixa carga bacilar.

Palavras-chave: técnicas de laboratório clínico; *Mycobacterium tuberculosis*; reação em cadeia da polimerase.

Financiador (es): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) (código de financiamento 001).

Área temática: Eco-epidemiologia e controle de doenças.



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ATIVIDADE ANTIMICROBIANA DO EXTRATO AQUOSO DO FUNGO BASIDIOMICETO *Macrocybe* sp.

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Introdução: Diante da problemática global do aumento de microrganismos resistentes à fármacos, estudos acerca de novas substâncias têm sido relevantes. Cogumelos do gênero *Macrocybe* são pouco estudados, mas apresentam potencial biotecnológico, especialmente quanto à atividade antimicrobiana. **Objetivo:** Avaliar o potencial antimicrobiano do extrato aquoso do cogumelo *Macrocybe* sp. contra bactérias, leveduras e fungos patogênicos. **Material e métodos:** O cogumelo foi coletado em Toledo-PR. O extrato foi preparado pelo método de sonicação de ponteira, em água destilada, utilizando 2,5 g de basíomas frescos. A atividade antimicrobiana foi avaliada contra as cepas ATCC de *Bacillus cereus* 10876, *Escherichia coli* O157:H7 43888, *Staphylococcus aureus* 6538, *Klebsiella pneumoniae* 13883, *Candida albicans* 90028 e *C. tropicalis* 750. *Aspergillus fumigatus* foi isolado de aspergilose canina e *Sporothrix schenckii*, isolado de um felino. Para as bactérias e *Candida* spp., foram determinadas a CIM, pela técnica de microdiluição em caldo, CBM e CFM. Para *S. schenckii* e *A. fumigatus*, foram determinados apenas valores em milímetros, dos halos de inibição. Para as bactérias foi seguido o NCCLS, CLSI 2003, M7-A6 e para as leveduras, CLSI, 2008, M27-A3. **Resultados:** No ensaio de microdiluição as CIMs observadas foram 0,0097 mg/mL para *K. pneumoniae*, 0,190 mg/mL para *S. aureus*, 1,560 mg/mL para *E. coli* e 3,125 mg/mL para *B. cereus*. Para *C. albicans*, a CIM não foi observada. Para *C. tropicalis*, a CIM foi de 12,5 mg/mL. No teste de poço-difusão foram observados halos de inibição de 30 mm para *K. pneumoniae*, 26 mm para *S. aureus*, 24 mm para *E. coli* e 19 mm para *B. cereus*. Dentre os fungos, 18 mm para *S. schenckii* e 14 mm para *A. fumigatus*. *Candida* spp. não foram inibidas. **Conclusão:** O extrato de *Macrocybe* sp. apresentou atividade antimicrobiana relevante frente a diferentes microrganismos patogênicos, reforçando seu potencial como fonte de novos agentes bioativos.

Palavras-chave: *Macrocybe* sp.; atividade antimicrobiana; microrganismos patogênicos.

Área temática: Patógenos de interesse médico.



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AVALIAÇÃO DA REGENERAÇÃO ÓSSEA INDUZIDA POR BIOCERÂMICA, DERIVADA DE OSSOS DE PEIXES, ATRAVÉS DA DENSIDADE CELULAR

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Introdução: O aumento da expectativa de vida torna as doenças ósseas um desafio global. A regeneração óssea com biomateriais exige integração de histologia, física médica e engenharia tecidual. Entre os mais estudados, os fosfatos de cálcio destacam-se por sua semelhança com a matriz mineral, biocompatíveis e osteoativos. Em especial, os bifásicos (BCPs) de origem em ossos de peixe surgem como alternativa sustentável e bioativa. O tecido ósseo maduro se caracteriza pela presença de osteócito em lacunas imersas na matriz mineralizada, que expressam marcadores como E11/gp38, CD44 e fimbrina. Essa célula apresenta potencial avaliativo em modelos de regeneração.

Objetivo: Avaliar o grau de regeneração óssea, pela densidade de lacunas de osteócito após enxerto de BCP, extraído de ossos de peixes, em defeitos de tamanho crítico (DTC) na calvária de ratos. **Materiais e Métodos:** Utilizaram-se ratos *Wistar* machos (CEUA/UEM - nº 6263280422), submetidos à anestesia seguida de craniotomia para criação de DTC (8 mm de diâmetro). Os animais foram divididos em dois grupos conforme o enxerto (n=5/grupo): Grupo BCP e Grupo Controle Negativo (CN), ausência de biomaterial. Após 90 dias, as calotas cranianas foram removidas, fixadas, descalcificadas, incluídas em parafina e coradas em H&E. A análise morfométrica foi realizada em microscópio *Olympus®-BX41TF*. A densidade de lacunas de osteócitos foi determinada em cortes semi-seriados, totalizando 9,16 mm²/animal, e os resultados foram avaliados estatisticamente por ANOVA (p<0,05). **Resultados:** A análise revelou diferença estatisticamente significativa entre as médias de densidade dos grupos (p<0,0001), sendo o grupo BCP com média superior ao CN. **Conclusão:** Neste estudo é possível concluir que, apesar da autorregeneração óssea (CN), o grupo BCP apresentou um maior grau de neoformação óssea ao conter a maior densidade de lacunas de osteócitos que caracterizam a presença de tecido ósseo maduro.

Palavras-chave: osteogênese; biomaterial; densidade de lacunas de osteócito.

Financiador (es): Finep, CNPq e Fundação Araucária.

Área temática: Etiofisiopatologia de doenças humanas e animais.



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INTOXICAÇÕES POR PRODUTOS DE USO DOMICILIAR: PANORAMA BRASILEIRO DOS ÚLTIMOS CINCO ANOS

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Introdução: As intoxicações por produtos de uso domiciliar têm aumentado, principalmente entre crianças. O fácil acesso, o manuseio indevido e as chamadas “misturinhas” de limpeza, especialmente quando armazenadas em frascos e locais inadequados, podem ocasionar intoxicações graves e até óbitos. **Objetivo:** Descrever o perfil epidemiológico das intoxicações por produtos de uso domiciliar no Brasil nos últimos cinco anos. **Material e métodos:** Estudo descritivo com base em dados secundários do Sistema de Informação de Agravos de Notificação, referentes às notificações de intoxicação exógena por produtos de uso domiciliar no período de 2020 a 2024, com a aplicação dos filtros de faixa etária, sexo, evolução, circunstâncias, e UF de residência. **Resultados:** No período analisado foram notificados 44.349 casos de intoxicação por produtos de uso domiciliar no Brasil. O estado com mais notificações foi São Paulo (27,0%; n = 11.997), seguido pelo Paraná (11,9%; n = 5.286) e Minas Gerais (11,4%; n = 5.072). O sexo feminino representou a maioria dos casos (56,3%; n = 25.004), enquanto os homens corresponderam a 43,6% (n = 19.337). A faixa etária mais acometida foi a de 1 a 4 anos (37,4%; n = 16.599). A principal circunstância registrada foi a acidental (66,3%; n = 29.427), seguida de tentativa de suicídio (21,0%; n = 9.316). Quanto à evolução, a maioria evoluiu para cura sem sequelas (81,2%; n = 36.041), mas houve óbitos decorrentes da intoxicação (0,3%; n = 166). **Conclusão:** A maioria dos casos ocorreu nas regiões Sul e Sudeste, afetando principalmente crianças do sexo feminino. Intoxicações acidentais e tentativas de suicídio foram as mais frequentes, e apesar da maioria dos casos evoluir sem sequelas, há registros de óbitos, evidenciando a necessidade de prevenção dessas intoxicações.

Palavras-chave: epidemiologia; intoxicação; domissanitários.

Financiador (es): Fundação Araucária - Programa Universidade Sem Fronteiras e CAPES

Área temática: Eco-epidemiologia e controle de doenças.



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INTOXICAÇÃO EXÓGENA ACIDENTAL EM CRIANÇAS: ANÁLISE EPIDEMIOLÓGICA NO BRASIL DOS ÚLTIMOS CINCO ANOS

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Introdução: As intoxicações exógenas acidentais constituem um importante agravo à saúde infantil no Brasil, sendo causa relevante de morbidade e hospitalização. Crianças apresentam maior vulnerabilidade devido à curiosidade, imaturidade física e mental e incoordenação motora, reforçando a necessidade de prevenção e capacitação de profissionais de saúde. **Objetivo:** Caracterizar o perfil epidemiológico das intoxicações acidentais em crianças registradas no Brasil entre 2020 e 2024. **Material e métodos:** Estudo descritivo baseado em dados do Sistema de Informação de Agravos de Notificação (SINAN), considerando agente tóxico, circunstância acidental, sexo, evolução e unidade federativa. **Resultados:** Foram registradas 81.835 ocorrências entre crianças de 0 a 14 anos, com tendência crescente: 2024 (23,9%, n = 19.564), 2023 (23,1%, n = 18.917), 2021 (18,1%, n = 14.827), 2020 (17,5%, n = 14.330) e 2022 (17,3%, n = 14.197). São Paulo concentrou mais registros (23,3%, n = 19.108), seguido por Minas Gerais (11,5%, n = 9.420) e Paraná (11,4%, n = 9.340). Medicamentos foram o principal agente (40,1%, n = 32.861), seguidos por produtos de uso domiciliar (23,6%, n = 19.343) e produtos químicos (5,1%, n = 4.182). Predomínio do sexo masculino (52,7%, n = 43.153) sobre o feminino (47,3%, n = 38.672). A maioria evoluiu para cura sem sequelas (82%, n = 67.117), com 98 óbitos (0,1%) e 12.289 registros ignorados ou em branco (15,0%), evidenciando subnotificação. **Conclusão:** As intoxicações acidentais em crianças no Brasil são crescentes, concentrando-se em medicamentos, produtos domésticos e químicos. Vigilância eficaz, capacitação profissional e ações educativas dirigidas a cuidadores são essenciais para reduzir riscos e proteger a população infantil.

Palavras-chave: intoxicação acidental; crianças; vigilância em saúde.

Financiador (es): Fundação Araucária - Programa Universidade Sem Fronteiras e CAPES.

Área temática: Eco-epidemiologia e controle de doenças.



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***Candida albicans* E *Trichosporon asahii* EM INFECÇÕES DO TRATO URINÁRIO: POTENCIAL DE VIRULÊNCIA E IMPACTO DOS BIOFILMES MISTOS**

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Introdução: As infecções do trato urinário (ITU) estão entre as doenças infecciosas mais comuns no mundo todo, com cerca de 150 milhões de casos anuais, sendo uma das principais infecções associadas à assistência em saúde. Embora as bactérias sejam os agentes etiológicos mais frequentes, observa-se aumento na incidência de ITUs causadas por fungos oportunistas, como as leveduras, principalmente, acometendo pacientes imunocomprometidos e em uso de dispositivos médicos. Além disso, é preciso um olhar atento sobre as infecções mistas. **Objetivo:** Apresentar uma síntese sobre a importância clínica de *Candida albicans* e *Trichosporon asahii* como agentes de ITU, destacando seu potencial de virulência e a relação com a formação de biofilmes, enfatizando biofilmes mistos. **Material e métodos:** Este estudo consiste em uma revisão de literatura com buscas realizadas nas bases PubMed, SciELO, BVS e Scopus, em março de 2025. Utilizou-se as estratégias: ("*Candida albicans* OR *Trichosporon asahii*") AND ("Mixed fungal biofilm") AND ("Urinary infection"). **Resultados:** Foram encontrados 18.644 artigos, dos quais 14 foram selecionados para o estudo. *C. albicans* e *T. asahii* são fungos com alto potencial de virulência e capacidade de formar biofilmes, o que é associado à resistência antimicrobiana e infecções persistentes, especialmente em pacientes em uso de dispositivos médicos. Biofilmes mistos têm sido altamente responsabilizados por infecções humanas (65% a 80%), representando um grave desafio à saúde pública. **Conclusão:** Ambas as leveduras são importantes agentes oportunistas de ITU, com destaque para sua adaptação ao hospedeiro e capacidade de formar biofilmes, especialmente sobre dispositivos médicos. A alta prevalência de biofilmes mistos evidencia a necessidade de atenção às infecções polimicrobianas, a estratégias preventivas, controle de infecções hospitalares e desenvolvimento de terapias mais eficazes para reduzir a morbidade associada a essas infecções.

Palavras-chave: biofilmes mistos; infecções urinárias; leveduras.

Área temática: Patógenos de interesse médico.



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CONTROLE DE FITOPATÓGENOS ATRAVÉS DE EXTRATO DE LIPOPEPTÍDEOS PRODUZIDOS POR *Bacillus pumilus*

Mariana Aparecida de Carvalho Lima¹; Juliet Nathalia Lizarazo Maldonado²; Bianca Campagnolo Martinazzo³; Patrícia Louise da Silva Ferreira⁴; Luísa Renata Mutelstad Hugen⁵; Fabio Rogério Rosado⁶; Adriana Fiorini Rosado⁷

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Introdução: Fungos fitopatogênicos representam grande ameaça a culturas de importância econômica, afetando qualidade e quantidade. Micotoxinas trazem riscos à saúde animal e humana pela bioacumulação, além da resistência cruzada a antifúngicos e efeitos sobre microrganismos não alvos. Bactérias do gênero *Bacillus* produzem diversos metabólitos secundários com aplicações biotecnológicas; entre eles, biossurfactantes, compostos anfipáticos com atividade antiviral, antibacteriana e antifúngica. Dentre estes, os lipopeptídeos apresentam grande potencial no controle de fitopatógenos, surgindo como alternativa complementar aos fungicidas sintéticos. **Objetivo:** O objetivo do trabalho foi desenvolver e validar uma metodologia para extração de lipopeptídeos produzidos por *Bacillus pumilus* e comprovar sua atividade antifúngica contra fitopatógenos. **Material e métodos:** Os microrganismos utilizados pertencem ao Núcleo Experimental de Micologia Aplicada (NEMA). A extração dos lipopeptídeos iniciou-se com o preparo de um pré-inóculo de *B. pumilus* em meio Luria Bertani (30 °C, 24 h, 120 rpm). Uma alíquota de 100 mL, ajustada para densidade óptica 0,05 a 620 nm, foi transferida para meio mínimo com sais e cultivada por 72 h (30 °C, 200 rpm). Após fermentação, o cultivo foi centrifugado (20 min, 4.000 rpm), e o sobrenadante acidificado até pH 2,0 com HCl por *overnight*. O precipitado obtido após nova centrifugação foi extraído com clorofórmio:metanol (2:1 v/v), evaporado a 40 °C e ressuspensionado em PBS. Para o ensaio, 200 µL do extrato foram aplicados em placa de ágar dextrose batata, seguido da inoculação de um *plug* de 5 mm do fungo e incubação a 28 °C por 5 dias. A análise dos resultados foi realizada por microscopia eletrônica de varredura. **Resultados:** A metodologia possibilitou verificar atividade fungicida contra fitopatógenos, onde verificou-se diminuição do crescimento e alterações da morfologia. **Conclusão:** Conclui-se que o extrato de lipopeptídeos de *B. pumilus* apresenta potencial de controle de fitopatógenos, e surge como alternativa complementar ao uso de fungicidas sintéticos.

Palavras-chave: lipopeptídeos; fitopatógenos; fungicida.

Área temática: Eco-epidemiologia e controle de doenças.



INTERFACES DA BIOCÊNCIA: CÉLULAS, GENES E ALGORITMOS

10^o International Meeting of Biosciences and Physiopathology
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CARACTERIZAÇÃO DE UM NOVO ALELO HLA IDENTIFICADO PELO LABORATÓRIO DE IMUNOGENÉTICA DA UEM ATRAVÉS DA TÉCNICA DE SEQUENCIAMENTO DE NOVA GERAÇÃO (NGS)

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Introdução: As moléculas do sistema *Human Leukocyte Antigen* (HLA) de classe II estão envolvidas na apresentação de antígenos e no cenário dos transplantes, as moléculas HLA alogênicas podem desencadear respostas imunes contra as células do enxerto, causando rejeição. **Objetivo:** Neste resumo, descrevemos o segundo alelo HLA-DQB1 identificado no Laboratório de Imunogenética da Universidade Estadual de Maringá (UEM). **Material e métodos:** O alelo foi identificado em um doador brasileiro do Registro Nacional de Doadores Voluntários de Medula Óssea (REDOME), que assinou o termo de consentimento livre e esclarecido. O DNA genômico foi isolado a partir dos leucócitos periféricos, utilizando o kit *QIAamp DNA Blood Mini*, conforme as instruções do fabricante. A tipificação de HLA foi realizada utilizando o kit *AllType FASTplex NGS 11 Loci* (One Lambda) na plataforma *Ion Torrent*. Os dados de sequenciamento foram analisados pelo software *TypeStream Visual 3.1* (One Lambda) e o banco de dados IPD-IMGT/HLA versão 3.57. Para confirmação, o sequenciamento adicional foi conduzido na plataforma *Illumina*. **Resultados:** A tipificação HLA completa do doador foi A*01:01:01; A*11:01:01; B*18:01:01; B*55:02:01; C*01:02:01; C*12:03:01; DRB1*04:05:01; DRB1*15:01:01; DRB4*01:01P; DRB5*01:01P; DQA1*01:02:01; DQA1*03:03:01; DQB1*04:NOVO; DQB1*06:02:01; DPA1*01:03:01; DPA1*02:02:02; DPB1*04:01:01; DPB1*05:01:01. O novo alelo primeiramente identificado como DQB1*04:NOVO, difere do DQB1*04:01:01:03, apresentando uma substituição de A>G no códon 33.2 do éxon 2, resultando em uma mudança de aminoácido de Asparagina (AAC) para Serina (AGC). O alelo foi submetido ao GenBank (número de acesso PQ963842) e ao IPD-IMGT/HLA (submissão HWS10100366). O nome DQB1*04:107 foi oficialmente atribuído pelo Comitê de Nomenclatura da OMS para os Fatores do Sistema HLA em março de 2025. **Conclusão:** A identificação do novo alelo DQB1*04:107 amplia o conhecimento sobre a diversidade genética do sistema HLA e demonstra a eficácia do sequenciamento de nova geração na caracterização de variantes alélicas.

Palavras-chave: novo alelo; HLA; análise de sequenciamento.

Área temática: Etiofisiopatologia de doenças humanas e animais.



INTERFACES DA BIOCIÊNCIA: CÉLULAS, GENES E ALGORITMOS

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PADRONIZAÇÃO DA ANFOTERICINA B COMO COMPOSTO DE REFERÊNCIA EXPERIMENTAL CONTRA *Leishmania amazonensis*

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Introdução: As leishmanioses, doenças tropicais negligenciadas causadas por protozoários do gênero *Leishmania*, constituem um grande desafio devido aos efeitos adversos causados pelos tratamentos, bem como a crescente resistência dos parasitos. Evidencia-se a necessidade do estudo de novos fármacos, utilizando drogas de primeira linha padronizadas como importantes referências para o desenvolvimento experimental seguro e eficiente. **Objetivos:** Obter a concentração inibitória em 50% (IC₅₀) da Anfotericina B USP (AmB) contra *Leishmania amazonensis* (LLA) para que seja possível sua padronização como droga de referência em ensaios. **Material e métodos:** Promastigotas de LLA foram incubados a 26°C durante 24h com concentrações decrescentes de AmB a partir de 10 ug/mL. Posteriormente a avaliação da viabilidade dos parasitos foi realizada pelo método XTT, no qual parasitos viáveis convertem XTT (amarelo) em formazan (laranja), passível de quantificação por espectrofotometria a 450/620 nm. **Resultados:** A AmB apresentou uma IC₅₀ de 0,0598± 0,012 ug/mL contra promastigotas de LLA. **Conclusão:** Conclui-se que a AmB testada demonstra uma atividade leishmanicida considerável, em baixas concentrações, contra formas promastigotas de LLA em experimentos *in vitro*, o que viabiliza a sua utilização como droga de referência experimental.

Palavras-chave: leishmaniose; anfotericina B; padronização.

Financiador (es): CNPq - Código 001.

Área temática: Eco-epidemiologia e controle de doenças.



INTERFACES DA BIOCIÊNCIA: CÉLULAS, GENES E ALGORITMOS

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PREDIÇÃO DO SUCESSO TERAPÊUTICO EM FUMANTES UTILIZANDO ALGORITMOS DE APRENDIZADO DE MÁQUINA

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Introdução: O tabagismo é um grave problema de saúde pública, associado a elevada mortalidade e baixa adesão aos tratamentos. O aprendizado de máquina (AM) surge como ferramenta promissora para prever o sucesso terapêutico, integrando variáveis clínicas e comportamentais. **Objetivo:** Avaliar o potencial de algoritmos de AM na predição do sucesso terapêutico em fumantes atendidos em um centro especializado, considerando variáveis clínicas e comportamentais relevantes para identificar fatores determinantes da abstinência e subsidiar estratégias personalizadas. **Materiais e métodos:** Estudo retrospectivo aprovado pelo Comitê de Ética da Universidade Estadual de Maringá (parecer nº 468.857/2021). Foram analisadas variáveis com diferenças significativas no teste qui-quadrado entre abstinentes e não abstinentes: término do tratamento, frequência de participação, motivação, uso de medicamentos, bupropiona e reposição de nicotina. A amostra incluiu 798 pacientes atendidos entre 2006 e 2017. Os dados foram divididos em treino (80%) e teste (20%). Avaliaram-se sete algoritmos de AM: árvore de decisão, floresta aleatória, regressão logística, C50, Naive Bayes linear generalizado (NBglm), k-vizinhos mais próximos (KNN) e gradiente de impulso. O desempenho foi mensurado por acurácia, precisão, especificidade, sensibilidade e área sob a curva ROC. **Resultados:** O NBglm apresentou melhor equilíbrio, com acurácia de $0,607 \pm 0,009$, sensibilidade de $0,634 \pm 0,006$ e maior área sob a curva ROC ($0,566 \pm 0,006$). A regressão logística destacou-se pela elevada precisão ($0,913 \pm 0,109$), mas baixa especificidade ($0,109 \pm 0,139$). O KNN obteve precisão de $0,875 \pm 0,125$, porém especificidade limitada ($0,086 \pm 0,117$). A árvore de decisão apontou a frequência como principal determinante (100%), seguida pelo término do tratamento (65,9%) e uso de fármacos, especialmente bupropiona (36,6%) e reposição de nicotina (21,1%). A motivação não apresentou relevância preditiva. **Conclusão:** Os algoritmos de AM mostraram aplicabilidade na predição do sucesso terapêutico em fumantes brasileiros, destacando adesão ao tratamento e uso de fármacos como fatores-chave. Tais achados reforçam a importância de políticas que priorizem continuidade terapêutica e acesso a medicamentos.

Palavras-chave: adesão; fármacos; tabagistas.

Financiador: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Área temática: Eco-epidemiologia e controle de doenças.



INTERFACES DA BIOCÊNCIA: CÉLULAS, GENES E ALGORITMOS

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MACROMORFOLOGIA DE FUNGOS DE IMPORTÂNCIA MÉDICA EM DIFERENTES MEIOS DE CULTURA

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Introdução: O padrão ouro no diagnóstico laboratorial de agentes fúngicos baseia-se nos achados macromorfológicos e micromorfológicos. No contexto laboratorial, há padronização no uso de meios de cultura para este tipo de investigação, sendo o Ágar Sabouraud Dextrose (SDA), o mais utilizado devido à sua eficácia no crescimento da maioria dos fungos patogênicos. **Objetivo:** Comparar as características macromorfológicas de fungos dermatófitos (*Microsporum* spp. e *Trichophyton rubrum*) e não dermatófitos (*Fusarium* spp.) cultivados em diferentes meios de cultura utilizados rotineiramente no laboratório de Micologia Médica. **Material e métodos:** Fragmentos de colônia de cada fungo foram inoculados em placas de Petri contendo três meios de cultura: SDA, Ágar Batata Dextrose (PDA) e Ágar Mycosel. As colônias foram avaliadas e registradas diariamente por até 15 dias. Os critérios analisados incluíram crescimento, aspecto e coloração da colônia. **Resultados:** Em relação à macromorfologia, *Fusarium* spp. apresentou colônia de aspecto cotonoso, borda irregular, relevo plano, inicialmente branca a rosada, evoluindo para tonalidade lilás no 4^o dia, sendo mais evidente a partir do 7^o dia. Já *T. rubrum* apresentou colônia de aspecto cotonoso branco, com borda bem definida e relevo plano, enquanto *Microsporum* spp. apresentou colônia de aspecto pulverulento, coloração branco-creme, borda irregular e relevo apiculado. O SDA mostrou-se o meio mais adequado para *Fusarium* spp., por possibilitar a observação nítida da pigmentação característica, bem como a distinção clara entre a coloração da colônia e a da borda. Para *Microsporum* spp., em ambos os meios testados, a morfologia foi semelhante. Já *T. rubrum*, o SDA foi o único que apresentou pigmentação significativa no meio com coloração da borda distinta da pigmentação da colônia no meio. **Conclusão:** A escolha adequada do meio influencia diretamente na velocidade de crescimento, o aspecto e coloração da colônia e a pigmentação no meio cultivado, elementos que constituem parâmetros essenciais na diferenciação entre espécies.

Palavras-chave: dermatófitos; fungos filamentosos; morfologia.

Área temática: Patógenos de interesse médico.



INTERFACES DA BIOCÊNCIA: CÉLULAS, GENES E ALGORITMOS

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AVALIAÇÃO DA ATIVIDADE ANTIBACTERIANA DE NANOPARTÍCULAS DE PRATA BIOGÊNICAS CONTRA BACTÉRIAS DE ORIGEM ALIMENTAR

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Introdução: Nanopartículas sintetizadas por meio de rotas biogênicas surgiram como uma estratégia sustentável e inovadora para aplicações biomédicas. Entre elas, as nanopartículas de prata têm atraído atenção significativa devido às suas diversas propriedades físico-químicas únicas e alto potencial antibacteriano. **Objetivo:** Este estudo avaliou e comparou a atividade antibacteriana de nanopartículas de prata biogênicas produzidas a partir do extrato de Angico-Branco (*Anadenanthera colubrina*) (BioAgNP-AB) e do extrato de catuaba (*Trichilia catigua*) (BioAgNP-C) contra diferentes bactérias de importância em alimentos. **Material e métodos:** As concentrações inibitórias e bactericidas mínimas das nanopartículas de prata foram determinadas segundo *Clinical and Laboratory Standards Institute*. As bactérias utilizadas foram *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028 e *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778 e *Aeromonas hydrophila* ATCC 7966. As concentrações testadas variaram de 500 a 0,97 µM. Após 24 h de incubação a 35 °C, as CIMs foram definidas como as menores concentrações que inibiu o crescimento bacteriano visível. **Resultados:** A CIM da BioAgNP-AB foi de 31,25 µM para *E. coli*, 250 µM para *S. aureus*, 62,5 µM para *Salmonella*, 62,5 µM para *B. cereus* e 15,62 µM para *A. hydrophila*. Para BioAgNP-C a CIM foi de 62,5 µM para *E. coli*, 62,5 µM para *S. aureus*, 125 µM para *Salmonella*, 62,5 µM para *B. cereus* e 62,5 µM para *A. hydrophila*. **Conclusão:** Os resultados obtidos demonstraram valores de CIM semelhantes entre as duas nanopartículas avaliadas, indicando que tanto a BioAgNP-AB quanto a BioAgNP-C apresentam atividade antibacteriana contra diferentes bactérias. Esses achados reforçam que a síntese biogênica configura uma estratégia promissora e sustentável para a produção de nanopartículas com potencial aplicação antimicrobiana em patógenos de interesse em alimentos.

Palavras-chave: patógenos alimentares; atividade antimicrobiana; nanopartículas metálicas.

Financiador (es): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Capes ou CAPES/CNPq - código 001

Área temática: Patógenos de interesse médico.



INTERFACES DA BIOCIÊNCIA: CÉLULAS, GENES E ALGORITMOS

10º International Meeting of Biosciences and Physiopathology
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RELAÇÃO ENTRE ANSIEDADE, ESTRESSE E TAXA METABÓLICA BASAL EM INDIVÍDUOS PRATICANTES DE MEDITAÇÃO TRANSCENDENTAL NA UNIVERSIDADE ESTADUAL DE MARINGÁ

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Introdução: A meditação transcendental (MT) é uma técnica simples, natural e sem esforço que possui efeitos sobre o sistema nervoso, podendo influenciar a percepção do indivíduo quanto ao estresse e ansiedade. **Objetivo:** Identificar o comportamento da taxa metabólica basal (TMB) em relação aos sintomas de ansiedade e estresse em praticantes de MT. **Material e métodos:** Estudo de coorte transversal-longitudinal, aprovado pelo COPEP 77175724700000104. Os participantes assinaram o TCLE, responderam a Escala de Percepção de Estresse (EPS-10) e Ansiedade de Back, e foram submetidos à bioimpedância, em três momentos: antes da MT, após três e seis meses de prática. Os resultados foram tabulados em Excel, analisados no software Jamovi Open Now versão 2.6.44. **Resultados:** Participaram 21 indivíduos, 14 mulheres (66.7%) e 7 homens

(33.3%), com idade média de $41,6 \pm 18,1$ anos, 15,9% relataram fumar e ingerir álcool.

Esses padrões permaneceram inalterados nas três coletas ($p=0,62$). Quanto à prática de atividade física, 63,5% dos participantes eram ativos, sem alteração no período. Quanto à ansiedade, 39,7% dos participantes apresentaram ansiedade mínima e 20,6% ansiedade leve. Houve variação significativa entre as coletas, na segunda e terceira coletas predominou a ansiedade mínima (66,7% e 52,4%), acompanhada de um aumento da ansiedade leve na terceira coleta 42,9% ($p<0,01$). No estresse autorreferido, 38,1% que relataram estresse alto no início, reduzindo para 9,5% após 6 meses, o estresse médio, não teve alteração significativa (47,6% vs 42,9%) e o estresse baixo foi de 14,3% para 47,6% ($p<0,01$). Quanto à composição corporal, 46,0% dos participantes apresentaram massa magra normal e 38,1% sobrepeso. Observou-se discreto aumento do sobrepeso na terceira coleta ($p=0,051$), sem alteração da TMB (início $1424,7 \pm 254,1$ kcal; final

$1467,8 \pm 264,6$ kcal). **Conclusão:** A prática de MT esteve associada a redução significativa dos sintomas de estresse e ansiedade, mas sem alteração na TMB no período analisado.

Palavras-chave: saúde mental; meditação transcendental; metabolismo.

Financiador (es): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Área temática: Eco-epidemiologia e controle de doenças.



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TUBERCULOSE NO BRASIL: EFEITOS DA PANDEMIA DE COVID-19 SOBRE INCIDÊNCIA E DESFECHOS CLÍNICOS (2017-2024)

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Introdução: A tuberculose (TB), causada por *Mycobacterium tuberculosis*, segue como grave problema de saúde pública no Brasil. A pandemia de Covid-19 sobrecarregou os serviços de saúde, impactando notificações, dificultando o controle da doença e comprometendo metas de eliminação da TB. **Objetivo:** Descrever os efeitos da pandemia de Covid-19 sobre as notificações, taxas de incidência e desfechos da TB no Brasil (2017-2024), comparando os períodos pré-pandêmico, pandêmico e pós-pandêmico. **Material e métodos:** Estudo ecológico com dados do SINAN sobre TB no Brasil (2017-2024). Analisaram-se casos, incidência (100 mil hab.) e desfechos (cura, abandono, óbito), agrupados em períodos pré-pandêmico (2017-2019), pandêmico (2020-2022) e pós-pandêmico (2023-2024). A análise foi descritiva, com cálculos de médias, desvio padrão e coeficiente de variação. **Resultados:** Foram registrados 783.984 casos no Brasil entre 2017 e 2024, média anual de 97.998 (DP = 9292,6; CV = 9,5%), com pico em 2024 (111.521). As taxas médias foram 45,6/100 mil hab. (pré-pandêmico), 44,7/100 mil hab. (pandêmico) e 52,3/100 mil hab. (pós-pandêmico). A média de notificações caiu discretamente do pré-pandêmico para o pandêmico (-0,3%) e aumentou expressivamente do pandêmico para o pós-pandêmico (+18,2%). O desfecho “cura” foi o mais frequente, representando 68%, 64% e 50%, respectivamente, nos períodos (redução possivelmente influenciada por dados de 2024 em encerramento). Os desfechos “abandono” (13%, 15,6% e 14%) e “óbito” (3,6%, 4,2% e 4,2%) mantiveram-se estáveis nos períodos analisados. **Conclusão:** Apesar do crescente número de casos registrados no período pré-pandêmico, identificou-se redução nas notificações nos anos da pandemia e retomada posterior, padrão que sugere influência das medidas relacionadas à Covid-19 e limitações no acesso aos serviços de saúde. O cenário atual de aumento dos casos traz alerta e reforça a importância do monitoramento epidemiológico rumo às metas de eliminação da doença.

Palavras-chave: tuberculose; covid-19; epidemiologia.

Área temática: Eco-epidemiologia e controle de doenças.



INTERFACES DA BIOCIÊNCIA: CÉLULAS, GENES E ALGORITMOS

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IDENTIFICAÇÃO DE ESPÉCIES DE ARBOVÍRUS NO MUNICÍPIO DE MARINGÁ, ESTADO DO PARANÁ

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Introdução: A dengue é uma arbovirose causada por um vírus pertencente à família *Flaviviridae*, transmitida aos humanos pela picada do mosquito do gênero *Aedes* sp. O vírus é classificado em quatro sorotipos diferentes (DENV-1, DENV-2, DENV-3 e DENV-4), que circulam no Brasil e compartilham em torno de 65% de similaridade na sequência de aminoácidos. No ano de 2024, houve predomínio de casos gerados pelos DENV-1 e DENV-2 e estima-se que, atualmente, entre 2,5 e 3 bilhões de pessoas já vivem em condições de risco em países endêmicos. **Objetivo:** Identificar a espécie do gênero *Aedes* sp. capturados no município de Maringá, classificando por intermédio de suas diferenças morfológicas. **Material e métodos:** Foram capturados ovos dos mosquitos através de armadilhas do tipo ovitrampa, colocadas em locais estratégicos no município de Maringá. As palhetas contendo os ovos foram imersas em água até sua eclosão, quando a larva evolui próximo ao estado de pupa, é transferida para um pote adaptado para seu próximo estado evolutivo, o mosquito adulto. Cinco dias após o último mosquito nascido, foram armazenados em freezer a -80°C. A identificação entre as espécies *Aedes aegypti* e *Aedes albopictus* foi realizada com os mosquitos congelados, utilizando critérios morfológicos visíveis a olho nu, com auxílio de pinça e lupa convencional. **Resultados:** Das 90 palhetas espalhadas pela cidade, obteve-se um total de 57 mosquitos após o cultivo dos ovos contidos nelas, sendo 100% deles *Aedes aegypti*. **Conclusão:** As palhetas cultivadas apresentaram baixa prevalência de mosquitos desenvolvidos, podendo ser explicado pela presença de ovos inférteis, palhetas velhas e presença de larvicida em algumas delas. Apesar disso, a única espécie que foi observada foi o *Aedes aegypti*, favorecendo estudos com o uso do mosquito vetor causador da dengue.

Palavras-chave: dengue; mosquito; ovitrampa.

Área temática: Eco-epidemiologia e controle de doenças.



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ASSOCIAÇÃO ENTRE OBESIDADE E O RISCO DE DOENÇAS CARDIOVASCULARES EM AGRICULTORES DA REGIÃO NORTE PARANAENSE

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Introdução: Estima-se que aproximadamente 67,5% das mortes associadas ao índice de massa corporal (IMC) elevado sejam atribuídas às doenças cardiovasculares (DCV). O aumento de casos de DCV gera impacto nos custos para a saúde com internações e tratamentos, e contribui para a redução da produtividade e aposentadorias precoces por invalidez. No meio rural, a modernização e as mudanças nos hábitos alimentares e de vida têm contribuído para maior exposição dos trabalhadores ao risco de DCV. **Material e métodos:** Este estudo foi realizado com a participação voluntária de 237 agricultores da região do norte do Paraná que assinaram o Termo de Consentimento Livre e Esclarecido (TCLE) (CAAE: 31596620.9.0000.0104). Foram coletadas medidas antropométricas para estimar o risco cardiovascular. A análise estatística foi realizada no software Open Epi Mobile, com cálculo de odds ratio (OR), intervalos de confiança de 95% e nível de significância de 5%. **Resultados:** Entre as mulheres (n=77), 43 (55,8%) não apresentaram risco para doenças cardiovasculares, 8 (10,4%) apresentaram risco elevado e 24 (31,2%) risco muito elevado. Entre os homens (n=157), 52 (33,1%) não apresentaram risco, 34 (21,6%) risco elevado e 63 (40,1%) risco muito elevado. No agrupamento de risco elevado e muito elevado, 32 mulheres (41,6%) e 97 homens (61,8%) foram classificados em risco. As odds foram de 0,74 para mulheres e 1,86 para homens, resultando em OR de 2,5, indicando que os homens tiveram aproximadamente duas vezes e meia mais chance de desenvolver risco cardiovascular associado à obesidade. **Conclusão:** O estudo evidenciou elevada incidência de risco cardiovascular entre agricultores avaliados, com associação significativa entre obesidade e acúmulo de gordura corporal. As medidas antropométricas mostraram-se ferramentas eficazes e acessíveis para a identificação precoce de indivíduos com risco de desenvolver DCV, porém, deve ser associada a exames laboratoriais e à análise dos hábitos de vida e alimentares para uma abordagem mais completa.

Palavras-chave: obesidade; risco cardiovascular; agricultores.

Financiador (es): Fundação Araucária.

Área temática: Etiofisiopatologia de doenças humanas e animais.



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CONSUMO DE AGROTÓXICOS E INCIDÊNCIA DE LINFOMA: EVIDÊNCIAS NA REGIÃO SUL DO BRASIL (2013–2023)

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Introdução: O uso crescente de agrotóxicos, associado à exposição ocupacional, tem sido implicado na carcinogênese e no aumento da incidência de linfomas. **Objetivo:** Avaliar a tendência temporal da incidência de linfomas nas regiões do Brasil e sua relação com o consumo de agrotóxicos. **Métodos:** Trata-se de um estudo retrospectivo, abrangendo o período de 2013 a 2023, com dados provenientes do DATASUS, IBAMA e IBGE. A taxa de incidência de linfomas foi calculada por 100 mil habitantes, enquanto o consumo de agrotóxicos foi avaliado em toneladas anuais e ajustado pela área plantada (t/ha). Foram realizadas análises estatísticas descritivas, testes de proporções, qui-quadrado, Kruskal-Wallis, correlação de Spearman e regressão linear, utilizando o software R Core Team. **Resultados:** A taxa de incidência de linfomas no Brasil aumentou de 2,68/100 mil habitantes em 2013 para 4,45 em 2023, concomitantemente ao crescimento do consumo de agrotóxicos, de 495.800 para 899.000 toneladas. A correlação de Spearman foi elevada ($p = 0,888$), e a regressão linear indicou associação significativa (coeficiente = $4,23 \times 10^{-6}$; $p = 0,00127$). A região Sul apresentou a maior taxa de incidência de linfomas (68,2/100 mil habitantes), além do maior consumo de agrotóxicos ajustado à população e da maior intensidade de aplicação por hectare. Os municípios com maiores taxas são principalmente da região Norte Central do Paraná sendo Faxinal (73,4/100 mil hab.), Porecatu (68,8), Rolândia (67,0), Maringá (62,5), Apucarana (59,2) e Paranavaí (54,3). **Conclusão:** O aumento da incidência de linfomas no Brasil acompanha o crescimento do consumo de agrotóxicos, sendo mais acentuado no Norte Central do Paraná, onde a agropecuária intensiva e mecanizada intensifica a exposição populacional. Esses achados reforçam a necessidade de ações de vigilância epidemiológica, monitoramento ambiental e formulação de políticas regionais de prevenção na saúde pública.

Palavras-chave: linfoma; agrotóxicos; epidemiologia.

Área temática: Etiofisiopatologia de doenças humanas e animais



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VARIÁVEIS EPIDEMIOLÓGICAS ASSOCIADAS A ASPECTOS DE TRANSTORNO OPOSITOR DESAFIANTE INFORMADOS POR PAIS: UM ESTUDO TRANSVERSAL

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Introdução: transtorno Opositor Desafiante (TOD) é associado ao enfrentamento contra regras, figuras de autoridade e comportamento vingativo. Considerando que profissionais de saúde utilizam diversas fontes para avaliação do comportamento infantil para guiar avaliações e intervenções, interessa associar a percepção de cuidadores em relação às características do TOD e às variáveis epidemiológicas e de hábitos da criança. **Objetivo:** estabelecer correlações entre variáveis epidemiológicas e a percepção de cuidadores sobre características associadas ao TOD. **Material e métodos:** o estudo, observacional transversal, foi aprovado pelo Comitê Permanente de Ética em Pesquisa com Seres Humanos (COPEP) da UEM (código CAAE: 70683423.6.0000.0104). Foram avaliadas 152 crianças de 6 a 11 anos (somente uma delas com diagnóstico prévio de TOD) matriculadas no Colégio de Aplicação Pedagógica (CAP/UEM) a partir de questionários respondidos em 2023 por seus responsáveis. A variável dependente derivou da resposta a 13 perguntas, associadas somente durante a análise estatística à apresentação de sinais de TOD, conforme a quinta edição do Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-V). Foram realizados testes de Bartlett e de Shapiro-Wilk, e escolhidos os métodos estatísticos: Spearman, T de Student, T de Welch, Kruskal-Wallis e U de Mann-Whitney. **Resultados:** a presença de animais em casa ($p < 0,01$), acompanhamento médico especializado ($p < 0,02$), consumir água de torneira ($p < 0,04$), dificuldade para fazer atividades na pandemia ($p < 0,005$), possuir doença prévia ($p < 0,02$) e usar algum medicamento ($p < 0,015$) apresentaram relevância estatística em relação à sintomatologia do TOD. **Conclusão:** além da observação clínica, as relações entre questões relacionadas aos hábitos de vida, higiene, alimentação e comportamento desafiador/agressivo precisam ser mais estudadas para diagnósticos e intervenções mais precisas.

Palavras-chave: transtorno opositor desafiante; saúde mental; hábitos de vida.

Financiador: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Área temática: Eco-epidemiologia e controle de doenças.



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ESTUDO DA EXPRESSÃO NEURONAL DE CALRETININA E PGP9.5 NOS PLEXOS NERVOSOS MIOENTÉRICOS DE CAMUNDONGOS *BALB/C* DURANTE A DOENÇA DE CHAGAS

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Introdução: A doença de Chagas, causada pelo protozoário *Trypanosoma cruzi*, é uma importante doença tropical negligenciada. Suas formas digestivas associam-se à perda de neurônios do Sistema Nervoso Entérico, especialmente no plexo mioentérico. Vesículas extracelulares (VES), liberadas pelo parasito e células hospedeiras, modulam a resposta imune e interferem na integridade neuronal, embora seus efeitos não estejam totalmente esclarecidos. **Objetivo:** Avaliar os efeitos da infecção por *T. cruzi* (cepa CL Brener), isolada ou associada a VEs de diferentes origens celulares (Caco-2; C2C12) e cepas (CL Brener; Dm28c), sobre a expressão de PGP9.5 e calretinina no jejuno de camundongos *BALB/c*. Esses marcadores permitem avaliar a densidade neuronal, compreendendo melhor a neuropatologia digestiva da doença de Chagas. **Material e métodos:** Aprovado pela CEUA/UEM (protocolo nº 5864311022), o estudo utilizou 30 camundongos *BALB/c* em seis grupos. Após 30 dias da infecção, o jejuno foi processado para imunomarcagem com anti-PGP9.5 e anti-calretinina. Obtivemos 32 imagens por animal, com contagem manual dos neurônios. A análise estatística inclui teste de Shapiro-Wilk, seguido de ANOVA de uma via e pós-teste de Tukey ($p < 0,05$). **Resultados:** A marcação para anti-calretinina não demonstrou alterações significativas, sugerindo preservação dessa população neuronal, o que pode sugerir maior resistência dessas células por fatores neuroprotetores. Já a imunomarcagem com anti-PGP9.5, apresentou aumento da densidade neuronal em animais infectados apenas com CL Brener, possivelmente reflexo de mecanismos compensatórios. Em contraste, grupos com VES apresentaram redução acentuada da população neuronal, indicando que a redução de neurônios positivos para o PGP9.5 nos grupos com VES pode ser resultado de dano neuronal, especialmente nos estágios crônicos da infecção. **Conclusão:** Assim, acreditamos que as VES agravam a perda neuronal induzida por *T. cruzi*, enquanto neurônios calretinina-positivos demonstraram maior resistência, destacando a relevância dos mediadores extracelulares na neuropatologia da doença de Chagas.

Palavras-chave: doença de chagas; vesículas extracelulares; sistema nervoso entérico.

Área temática: Patógeno de interesse médico.



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POTENCIAL INIBIÇÃO DA PATOGENICIDADE DE *Candida spp.* POR SOBRENADANTES DE *Lactocaseibacillus rhamnosus*: UMA ATUALIZAÇÃO

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Introdução: As espécies de *Candida*, responsáveis por infecções mucosas e sistêmicas, são frequentemente isoladas em infecções associadas à assistência à saúde e por isso, um dos agentes mais importantes mundialmente. A formação de biofilme atua como um protetor contra agentes antifúngicos, aumentando a resistência das células fúngicas e levando a infecções de difícil resolução. O desenvolvimento dessa resistência aumenta a necessidade de tratamentos alternativos. A utilização de espécies probióticas de *Lactocaseibacillus rhamnosus* e seus metabólitos tem recebido atenção por prevenir e tratar essas infecções, sendo uma alternativa promissora. **Objetivo:** Apresentar evidências relacionadas aos efeitos antifúngicos de probióticos e do seu potencial uso como terapia alternativa contra infecções por *Candida spp.* **Material e métodos:** Revisão de literatura realizada a partir da busca nas bases de dados PubMed Central (PMC) e Scopus, usando operadores booleanos "AND" e "OR" associados com os descritores: "*Lactocaseibacillus rhamnosus*" AND "*Candida*" OR "supernatant AND candidíase". Foram selecionados artigos em inglês, publicados no período entre 2020 e 2024, associados ao objetivo da pesquisa e envolvendo estudos *in vitro* e *in vivo*. **Resultados:** Foi identificado que os sobrenadantes livres de células obtidas de *L. rhamnosus* podem interferir no potencial patogênico de *Candida*, inibindo significativamente o crescimento *in vitro* de seu biofilme. Sugere-se que outros exometabólitos além do ácido láctico produzidos pelas cepas, podem ser responsáveis pelo efeito, como a superprodução de inosina, uma molécula descrita por suas propriedades antioxidantes, anti-inflamatórias, anti-infecciosas e neuroprotetoras, podendo ser importante na atividade probiótica. **Conclusão:** O presente estudo demonstra o potencial de *L. rhamnosus* como agente terapêutico no tratamento da candidíase e como alternativa aos medicamentos convencionais. Porém, sugerem-se novos estudos em busca da melhor compreensão do mecanismo de secreção de metabólitos sobre a formação de biofilme fúngico.

Palavras-chave: biofilme; candidíase; probiótico.

Área temática: Patógenos de interesse médico.



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SUSCETIBILIDADE À INFECÇÃO POR *Aspergillus* EM PACIENTES IMUNOSSUPRIMIDOS PELO HIV: UMA SÉRIE DE CASOS

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Introdução: *Aspergillus* é um fungo ubíquo que pode provocar desde manifestações alérgicas até formas invasivas graves, especialmente em pacientes imunocomprometidos, como aqueles que vivem com HIV, os quais apresentam maior risco devido às falhas da resposta imunológica. A neutropenia e a disfunção da imunidade celular agravam esse quadro, favorecendo a progressão da infecção enquanto muitos indivíduos ainda evoluem com imunossupressão avançada mesmo com a terapia antirretroviral disponível, sobretudo em contextos de baixa renda, o que eleva a morbimortalidade associada. Assim, estratégias diagnósticas rápidas e terapêuticas eficazes tornam-se fundamentais no manejo clínico. **Objetivo:** Explorar uma série de casos que mostram a relação entre a imunossupressão causada pelo HIV e a suscetibilidade à infecção por *Aspergillus*, destacando seus impactos clínicos. **Material e métodos:** Foi realizada uma revisão de literatura com busca realizada nas bases BVS, Embase, PubMed, Scopus e Web of Science, em março de 2025. Utilizou-se a estratégia: (“*Aspergillus* OR *Aspergillosis*”) AND (“HIV OR AIDS”) AND (“Immunosuppression OR Immunocompromised”). Foram incluídos artigos entre 2014 e 2024 e apenas relatos de casos. **Resultados:** A revisão identificou 1984 artigos e foram selecionados 17 relatos de casos, destacando a relação entre imunossupressão pelo HIV e maior suscetibilidade à infecção por *Aspergillus*, especialmente no trato respiratório. Os pulmões foram os sítios mais afetados, mas também ocorreram infecções no sistema nervoso, intestino, medula espinhal, pele, unhas e olhos. Predominaram casos em homens, com média etária de 38 anos. Apesar dos avanços terapêuticos, a aspergilose mantém elevada mortalidade em imunocomprometidos, com 9 óbitos relatados. Combinar exames laboratoriais, histopatológicos e radiológicos, mostraram-se essenciais para detecção precoce e definição de tratamento adequado nos casos de sobrevivência. **Conclusão:** A imunossupressão pelo HIV aumenta a vulnerabilidade à infecção por *Aspergillus*, favorecendo evolução grave e alta mortalidade. Diagnóstico precoce e terapias eficazes são essenciais para controlar a doença e melhorar prognósticos.

Palavras-chave: micose invasiva; HIV/AIDS; imunossupressão.

Área temática: Patógenos de interesse médico



INTERFACES DA BIOCIÊNCIA: CÉLULAS, GENES E ALGORITMOS

10º International Meeting of Biosciences and Physiopathology
14º Simpósio de Biociências Aplicadas à Farmácia

EPIDEMIOLOGIA DAS INFECÇÕES DO TRATO URINÁRIO POR LEVEDURAS EM PACIENTES ATENDIDOS PELO LABORATÓRIO DE ENSINO E PESQUISA E ANÁLISES CLÍNICAS EM MARINGÁ DE 2024 A 2025

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Introdução: Infecções urinárias correspondem à invasão microbiana do trato urogenital, desencadeando resposta inflamatória. Entre os fatores de risco, destacam-se idade avançada, alterações prostáticas, menopausa e maior predisposição no sexo feminino. Embora geralmente de origem bacteriana, podem ser causadas por fungos, principalmente leveduras do gênero *Candida*, como *C. albicans* e *C. tropicalis*. Esses microrganismos integram a microbiota humana, mas em determinadas condições tornam-se patogênicos, ocasionando desde infecções superficiais até quadros sistêmicos.

Objetivo: Identificar a distribuição epidemiológica de leveduras em amostras de urina positivas analisadas no laboratório de micologia médica - LEPAC - UEM. **Material e métodos:** A partir do livro de registro pertencente ao laboratório (comitê de ética nº 2,748,843), foram coletados dados como: local de origem da amostra, idade, sexo do paciente e a espécie fúngica identificada, relativos ao período de agosto de 2024 à agosto de 2025. Os dados foram formatados e analisados utilizando o *software* Microsoft Excel.

Resultados: Das 49 amostras avaliadas, provindas do livro de registro do laboratório, 31 foram provenientes de pacientes do sexo feminino e 18 do sexo masculino. A média etária foi de 68 anos, variando de 20 a 96 anos. Quanto à etiologia, 22 casos (45%) foram causados por *C. albicans*, 15 (31%) por *Nakaseomyces glabratus* (anteriormente *C. glabrata*), 6 (12%) por *C. tropicalis*, 2 (4%) por *Pichia kudriavzevii* (anteriormente *C. krusei*) e 3 (6%) por *Trichosporon* spp. Em um caso (2%), ocorreu coinfeção por *C. albicans* e *C. glabrata*. Aproximadamente 75% dos episódios ocorreram em idosos (≥ 60 anos), sendo 42% mulheres. **Conclusão:** Conclui-se que *C. albicans* foi a espécie mais prevalente, em concordância com estudos brasileiros. Entretanto, observou-se um aumento da frequência de leveduras *Candida* não *Candida albicans*, com destaque para *C. glabrata*. De modo geral, as características epidemiológicas identificadas mostraram-se compatíveis com o perfil nacional.

Palavras-chave: infecção urinária; levedura; epidemiologia.

Área temática: Etiofisiopatologia de doenças humanas e animais.



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ESTUDO SOBRE DIFERENTES FONTES DE NUTRIÇÃO NA FORMAÇÃO DE BIOFILMES MICROBIANOS

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Introdução: Biofilmes microbianos são um desafio clínico devido à resistência aumentada aos antimicrobianos. As estratégias terapêuticas anti-biofilme são inicialmente avaliadas *in vitro*, porém não há consenso sobre o meio de cultura mais adequado. **Objetivo:** Comparar a formação de biofilmes de *Candida albicans* e *Staphylococcus aureus* nos meios TSB e RPMI. **Material e métodos:** *C. albicans* SC5314 foi cultivada em YPD (35 °C, 110 rpm, 16–18 h); o inóculo ajustado para 4×10^6 células/mL. *S. aureus* foi cultivado em TSB (35 °C, 110 rpm, 16–18 h), com inóculo DO=0,006. Biofilmes foram montados em placas de 24 poços: 100 µL de cada microrganismo (MO) + 200 µL de TSB ou RPMI + 100 µL de salina. Após adesão (90 min, 35 °C, 120 rpm), os poços foram lavados e incubados por 24h. A biomassa total foi analisada por cristal violeta e a viabilidade celular por Unidades Formadoras de Colônias/mL (UFC/mL). A análise estatística foi realizada no software GraphPad Prism 8, utilizando ANOVA (*one-way ANOVA*), considerando $p < 0,05$ nível de significância. **Resultados:** O biofilme formado em RPMI produziu maior quantidade de biomassa total (matriz extracelular + células), $p < 0,05$, *C. albicans* (DO=3,9) e *S. aureus* (DO=0,15), quando comparado com TSB (DO=1,7 e DO=0,09, respectivamente). Por outro lado, a viabilidade celular foi maior em TSB para ambos MO testados. *C. albicans* foi 2 Log mais viável em RPMI, e *S. aureus* 1 Log superior, $p < 0,05$. **Conclusão:** A discrepância entre os meios pode estar relacionada aos constituintes nutricionais, visto que o RPMI apresenta composição mais definida que o TSB. Esse fator pode estimular os MO a produzirem matriz extracelular (DNA, proteínas, carboidratos, etc.). Os resultados indicam que, dependendo da aplicabilidade terapêutica, a escolha do meio de cultura pode impactar a viabilidade técnica.

Palavras-chave: biofilme; *Candida albicans*; *Staphylococcus aureus*.

Área temática: Patógenos de interesse médico.



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INFLUÊNCIA DA PRÁTICA DE MEDITAÇÃO TRANSCENDENTAL NAS CONDIÇÕES GERAIS DE SAÚDE DOS SEUS PRATICANTES

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Introdução: Fatores de risco modificáveis influenciam a saúde cardiovascular. A meditação transcendental (MT) possibilita a redução do estresse-ansiedade e melhoras no sono. **Objetivo:** Identificar influências da prática de meditação transcendental nas condições gerais de saúde dos seus praticantes. **Material e métodos:** Estudo transversal–longitudinal, aprovado pelo COPEP (n.o. 77175724700000104), com coletas no início, 3 e 6 meses de prática. Participantes preencheram: Termo de Consentimento Livre e Esclarecido, questionário de saúde, “Escala de Percepção de Estresse (EPS-10)”, “Qualidade de sono de Pittsburg (PSQUI-PT), Questionário de atividade física (IPAQ). O perfil metabólico foi obtido por sangue em jejum e a dosagem de biomarcadores por colorimetria. Os resultados foram tabulados em planilha e analisados no Jamovi, versão 2.6.44, com correlação e teste de contingência (CC). **Resultados:** 22 indivíduos participaram: 68,2% mulheres e 31,8% homens, com idades médias de 46,7 (DP=18,9) e 32,3 anos (DP=9,93). Observou-se manutenção do número de fumantes e consumidores de álcool (CC: 0.00; 0.08). Na prática de atividade física, houve leve aumento seguido de redução entre mulheres e diminuição da frequência entre homens (p-Valor: 0.05; CC: 0.4). Melhora nos níveis de estresse, com maior proporção de indivíduos em estresse baixo após três e seis meses (IC: 0.0). Entre mulheres dobrou o número de casos de sono satisfatório no sexto mês, entre homens houve redução de cerca de 17% no sono insatisfatório (CC: 0.2). As médias das variáveis metabólicas apresentaram redução para triglicerídeos nos dois gêneros (F: 143>115, M: 119>79.8), discreta redução de glicose na população feminina (101>94), diminuição de TGO (F: 24.1>21.3; M: 29>19.3) e TGP (F: 16.6>13.1; M: 24>19), redução de creatinina na população masculina (1.55>1.15), aumento de níveis de proteínas para ambos (Fem: 7.11>7.15 e 7.44>11.1). **Conclusão:** Observou-se melhoria do sono, nível de estresse, triglicerídeos e marcadores hepáticos dos praticantes de MT. Ressalta-se a necessidade de mais estudos.

Palavras-chave: Saúde coletiva; meditação transcendental; metabolismo.

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Área temática: Eco-epidemiologia e controle de doenças.