baumannii in Brazil, combinations of β-lactams with next-generation β-lactamase inhibitors and tetracycline derivatives have emerged as promising antimicrobials.

Objective: To evaluate the *in vitro* activity of new antimicrobials against MDR isolates obtained from patients hospitalized in ICUs of six hospitals in the metropolitan region of São Paulo.

Methods: A total of 121 isolates of *K. pneumoniae* and 27 of *A. baumannii*, prospectively collected from patients hospitalized in six ICUs of the metropolitan region of São Paulo between 08/01/2023 and 10/31/2024, resistant to carbapenems, were analyzed. MICs for polymyxin B/E (POL), omadacycline (OMC), and eravacycline (ERV) were determined by broth microdilution (Sensititre, Thermo Fisher). For *K. pneumoniae*, 118 isolates without evidence of metallo-β-lactamase production were additionally tested for ceftazidime-avibactam (CZA), imipenem-relebactam (IMR), and meropenem-vaborbactam (MEV). Resistance genes of 55 *K. pneumoniae* and 21 *A. baumannii* were correlated with phenotypes.

Results: The susceptibility rates of *K. pneumoniae* to CZA, IMR, and MEV were 87.29%, 74.58%, and 82.20%, respectively. Among the 59 isolates resistant to amikacin (AMI) and POL, these rates were 96.91%, 75.86%, and 82.76%. Applying FDA breakpoints, 6 isolates (4.96%) were resistant to OMC (MIC > 8 mg/L) and 12 (9.92%) were intermediate (8 mg/L). Even among AMI/POL-resistant isolates, OMC susceptibility was 91.53%. For ERV, the MIC_{90} was 1 mg/L in the overall group and 2 mg/L among resistant isolates. Of the 55 genomes analyzed, 92.73% carried blaKPC-2, 3.64% blaNDM-1, and 1.82% both. Two isolates with blaNDM-1 showed favorable MICs for ERV (0.5 mg/L) and OMC (≤ 4 mg/L). In *A. baumannii*, MIC_{50}/MIC_{90} values were 2/8 mg/L for OMC and 0.5/2 mg/L for ERV. Among the 10 isolates resistant to POL, OMC showed an increased MIC_{90} to 8 mg/L, but ERV maintained a profile similar to the overall group.

Conclusion: *K. pneumoniae* strains showed excellent activity to CZA, IMR, and MEV. MDR *K. pneumoniae* and *A. baumannii* strains were also susceptible to OMC and ERV, including subgroups resistant to POL, reinforcing the potential of these drugs as therapeutic options in highly resistant settings such as Brazilian ICUs.

Keywords: Microbial resistance, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, New antimicrobials, β-lactamase inhibitors.

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IN VITRO ACTIVITY OF AZTREONAM-AVIBACTAM AGAINST CLINICAL ISOLATES OF ENTEROBACTERALES COLLECTED IN BRAZIL FOR THE ATLAS SURVEILLANCE PROGRAM, 2018–2023

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Introduction: Aztreonam-avibactam (ATM-AVI) was developed for the treatment of infections caused by Enterobacterales, particularly carbapenem-resistant isolates that produce metallo-β-lactamases (MBLs). Aztreonam is stable to hydrolysis by MBLs, while avibactam inhibits class A and C β-lactamases, which may also be co-produced with MBLs that inactivate aztreonam. In this study, the *in vitro* activity of ATM-AVI was evaluated against clinical isolates of Enterobacterales, both MBL producers and non-producers, collected as part of the global ATLAS surveillance program.

Methods: A total of 3151 non-duplicate Enterobacterales isolates collected from different infection sites in Brazilian hospitals between 2018 and 2023 were evaluated. Susceptibility testing was performed using broth microdilution. Antimicrobial susceptibility profiles were interpreted according to BrCAST (2025) breakpoints, including the established breakpoint for ATM-AVI of > 4 mg/L. Carbapenemase-encoding genes were investigated by PCR followed by sequencing.

Results: The majority of Enterobacterales species evaluated were *Klebsiella pneumoniae* (36.0%; n = 1137), followed by *Escherichia coli* (31.6%; n = 998) and *Enterobacter* sp. (12.6%; n = 399). Overall, ATM-AVI inhibited 100% of isolates (MIC50/90, 0.06/0.25 mg/L), including those producing carbapenemases (588/960 isolates tested [61.2%]). Overall susceptibility to colistin (MIC50/90, 0.25/>8 mg/L), meropenem (MIC50/90, ≤ 0.06/> 16 mg/L), cefepime (MIC50/90, ≤ 0.125/> 32 mg/L), and aztreonam (MIC50/90, 0.25/> 64 mg/L) was 85.7%, 80.6%, 58.4%, and 56.9%, respectively. Among the 960 isolates tested for carbapenemases, 119 (12.4%) were positive for NDM-1 and 492 (51.2%) for KPC. Twenty-five isolates showed coproduction of two carbapenemases, 24 with KPC-2+NDM-1 and one with KPC-2+IMP-1. All 492 KPC-positive isolates were 100% and 94.72% susceptible to ATM-AVI and ceftazidime-avibactam, respectively.

Conclusion: ATM-AVI demonstrated excellent *in vitro* activity, inhibiting 100% of Enterobacterales isolates, both carbapenemase producers and non-producers, including MBLs. Antibiotics that retain activity against MBL-producing bacteria represent an urgent medical need, especially given the rising prevalence of severe infections caused by these difficult-to-treat microorganisms.

Keywords: Metallo-β-lactamases, Carbapenemases, Enterobacterales, *Klebsiella pneumoniae*, Bacterial resistance.

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MICROBIAL ACTIVITY OF POMEGRANATE LEAF EXTRACT AGAINST RESISTANT *STAPHYLOCOCCUS AUREUS*

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Introduction: Bacterial resistance is a considerable public health risk, with projections of up to 10 million deaths per year by 2050. Resistant *Staphylococcus aureus* is one of the main multidrug-resistant hospital pathogens. Due to the low efficacy of many antimicrobials, there is growing interest in natural alternatives, such as pomegranate leaf extract (*Punica granatum*), which is rich in antimicrobial compounds such as punicalagin, ellagic acid, and tannins.

Objective: To evaluate the antimicrobial activity of the hydroalcoholic extract of pomegranate leaf (HPE) against one sensitive strain (*S. aureus* ATCC 33591) and nine resistant strains (ATCC 25904 and eight clinical strains – Ethics approval: CAAE 32572820.6.0000.5133).

Methods: Leaves of *P. granatum* were collected, macerated in hydroalcoholic solution (50%), filtered, evaporated, and lyophilized. The eight clinical strains were tested for susceptibility with 18 antimicrobials. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays were performed using broth microdilution in Mueller-Hinton broth, with HPE concentrations ranging from 1000 to 7.8 μg/mL. Azithromycin (40 μg/mL) was used as a control. Inocula (5×10^4 CFU/well) were prepared according to the 0.5 McFarland standard. Plates were incubated at 37°C for 24 h, and the MIC was defined as the lowest concentration visually inhibiting growth. For the MBC, 10 μL from wells without growth were plated on BHI agar.

Results: All tested strains were resistant (R) to benzylpenicillin and oxacillin, and most were resistant to clindamycin and erythromycin. Some showed intermediate susceptibility or resistance to rifampicin. Half of the strains were resistant to levofloxacin. Only one strain was resistant to trimethoprim/sulfamethoxazole, nitrofurantoin, and ciprofloxacin. HPE showed a mean MIC of 500 μg/mL with activity against four strains, including three resistant to at least one antimicrobial. It demonstrated microbiostatic action for four strains at the tested concentrations.

Conclusion: The data indicate the promising potential of HPE as a microbiostatic agent against *S. aureus*, reinforcing the importance of investigating higher concentrations and the development of natural therapeutic formulations against resistant strains.

Keywords: Antimicrobial activity, *Staphylococcus aureus*, Phytotherapy, *Punica granatum*, Bacterial resistance.

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EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF CORDIA VERBENACEA ESSENTIAL OIL AGAINST DIFFERENT CLINICALLY RELEVANT PATHOGENS

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Introduction/Objective: The growing resistance to conventional antimicrobials represents a global public health challenge, encouraging the search for effective and safe therapies. In this context, essential oils have stood out due to their antimicrobial activity, with reduced microbial resistance during their use against pathogens. This study aimed to evaluate the antimicrobial activity of *Cordia verbenacea* essential oil (EO) against clinically relevant pathogens.

Methods: The antimicrobial activity was assessed through Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC) assays of the EO. The microbial strains included *Acinetobacter baumannii* (ATCC 19606); *Candida albicans* (INCQS 40175); *Candida tropicalis* (ATCC 750); *Enterococcus faecalis* (ATCC 51299); *Enterococcus faecium* (ATCC 6569); *Klebsiella pneumoniae* (ATCC 700603 and BAA 2814); *Pseudomonas aeruginosa*(INCQS 2742); *Staphylococcus aureus* (ATCC 29213 and ATCC 33591); *Streptococcus mutans* (ATCC 25175); *Streptococcus oralis* (ATCC 10557). MIC and MMC values were determined by the broth microdilution method in Mueller-Hinton broth (MH), following CLSI (2022) guidelines, with visual evaluation of microbial growth and confirmation of microbicidal activity on Brain Heart Infusion (BHI) agar. Tested concentrations ranged from 30 to 0.23 mg/mL, and bacterial and fungal inocula were standardized by McFarland scale at 0.5×10^5 CFU/well and 0.5×10^3 CFU/well, respectively, with incubation under conditions specific for each microorganism.

Results: After MIC testing, it was observed that the EO showed inhibitory activity at 30 mg/mL for *S. aureus* (ATCC 33591), 25 $\pm$ 8.66 mg/mL for *E. faecium*, 20 $\pm$ 8.66 mg/mL for *A. baumannii* and *C. tropicalis*, 15 mg/mL for *C. albicans*and *S. aureus* (ATCC 29213), 15 $\pm$ 7.5 mg/mL for *E. faecalis*, 0.5 $\pm$ 0.42 mg/mL for *S. mutans*, and greater than 30 mg/mL for the remaining strains. The MMC assay showed that its activity was microbiostatic.

Conclusion: The EO demonstrated inhibitory activity at concentrations ranging from 30 to 0.5 mg/mL against the tested microorganisms, but no activity against *K. pneumoniae* and *P. aeruginosa* at the tested concentrations. Thus, EO represents a promising natural alternative for the control of clinically relevant pathogens, especially in light of the advance of microbial resistance.

Keywords: Essential oil, *Cordia verbenacea*, Antimicrobial activity.

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