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Original Article

Relationship between antimicrobial use and the highest number of multidrug-resistant-*Pseudomonas aeruginosa:* a 10-year study

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Abstract

Introduction: Multi-drug-resistant (MDR) *Pseudomonas aeruginosa* is a dangerous pathogen causing nosocomial infection, particularly in low- and middle-income countries like Brazil. This retrospective study at a Brazilian university hospital examined the relationship between antimicrobial use and MDR-*P. aeruginosa*.

Methodology: Data was collected from 358 patients with non-repetitive *P. aeruginosa* infections from 2009 to 2019. Antibiotic use was measured in grams and expressed as defined daily dose (DDD) per 1000 patient-days for meropenem, imipenem, polymyxin, and tigecycline.

Results: Extensively drug-resistant (XDR) *P. aeruginosa* occurred in 36.1%, and MDR in 32.6% of cases. Risk factors for XDR infection were hospitalization prior to infection (OR = 0.9901), intensive care unit (ICU) admission (OR = 0.4766), previous antibiotic use (OR = 1.4417), and use of cefepime (OR = 0.3883). Over the ten-year period, utilization of the monitored antibiotics increased, and there was a positive correlation between the rise in MDR-*P. aeruginosa* and the consumption of ceftriaxone, imipenem, meropenem, and polymyxin B. The 30-day mortality rate was 40.0% for all patients and 41.0% for those infected with XDR-*P. aeruginosa*.

Conclusions: This study highlights the negative impact of the indiscriminate use of antimicrobials, which has led to a significant increase in multidrug-resistant *P. aeruginosa* strains in hospitals.

Key words: antimicrobial; DDD; multi-resistance; XDR-*P. aeruginosa*; healthcare-associated infection.

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Introduction

Antimicrobial multi-resistance (AMR) has been a global cause of concern, particularly in developing countries like Brazil [1,2]. There is an extensive body of literature on the incidences of AMR and its effects on hospital stay length, healthcare costs, and mortality rates; particularly those associated with phenotypes that are more adapted to hospital environments [3,6].

This situation worsens because antibiotic therapy is commonly overused indiscriminately in hospitals, which renders the available therapeutic arsenal ineffective in treating certain infectious diseases [7]. If left unchecked, we may lose the ability to control this resistance, resulting in the emergence of much more lethal pathogens in the near future.

In this study, we aimed to investigate the relationship between antimicrobial consumption and the highest number of isolates of multi-drug-resistant (MDR) *Pseudomonas aeruginosa*, and identified the risk factors that predispose hospitalized patients with MDR-*P. aeruginosa* to develop infections caused by these microorganisms.

Methodology

Patients, study design, and data collection

The methodological approach used in our study, including the data analysis steps, are shown in Figure 1. A decade-long observational study was undertaken to identify patients afflicted with *Pseudomonas aeruginosa* infections at Uberlandia University Hospital—a tertiary-care university hospital in Brazil with 506 beds—situated in the southeastern region of the country. All strains were procured from the Microbiology Laboratory of the Clinical Hospital at the Federal University of Uberlandia (HC-UFU). We analyzed the risk factors among patients infected with the first occurrence of carbapenem-resistant or carbapenem-sensitive *P. aeruginosa*, to identify predictors of mortality and assess the impact of overuse of antimicrobial therapy on the outcomes of patients with these infections.

Additionally, we evaluated secondary outcomes; underlying conditions such as diabetes mellitus; bacteremia sources; age; period of hospitalization; admission to intensive care unit (ICU); and use of invasive medical devices such as central venous line, urinary catheter, tracheostomy, hemodialysis, and surgical drain during hospitalization. The clinical, and epidemiological characteristics of each patient included in the study were recovered from clinical records. Only patients with *P. aeruginosa* infections acquired through healthcare-related measures and microbiologically confirmed were included; cases where medical records were unavailable or lacked essential information were excluded.

Definitions

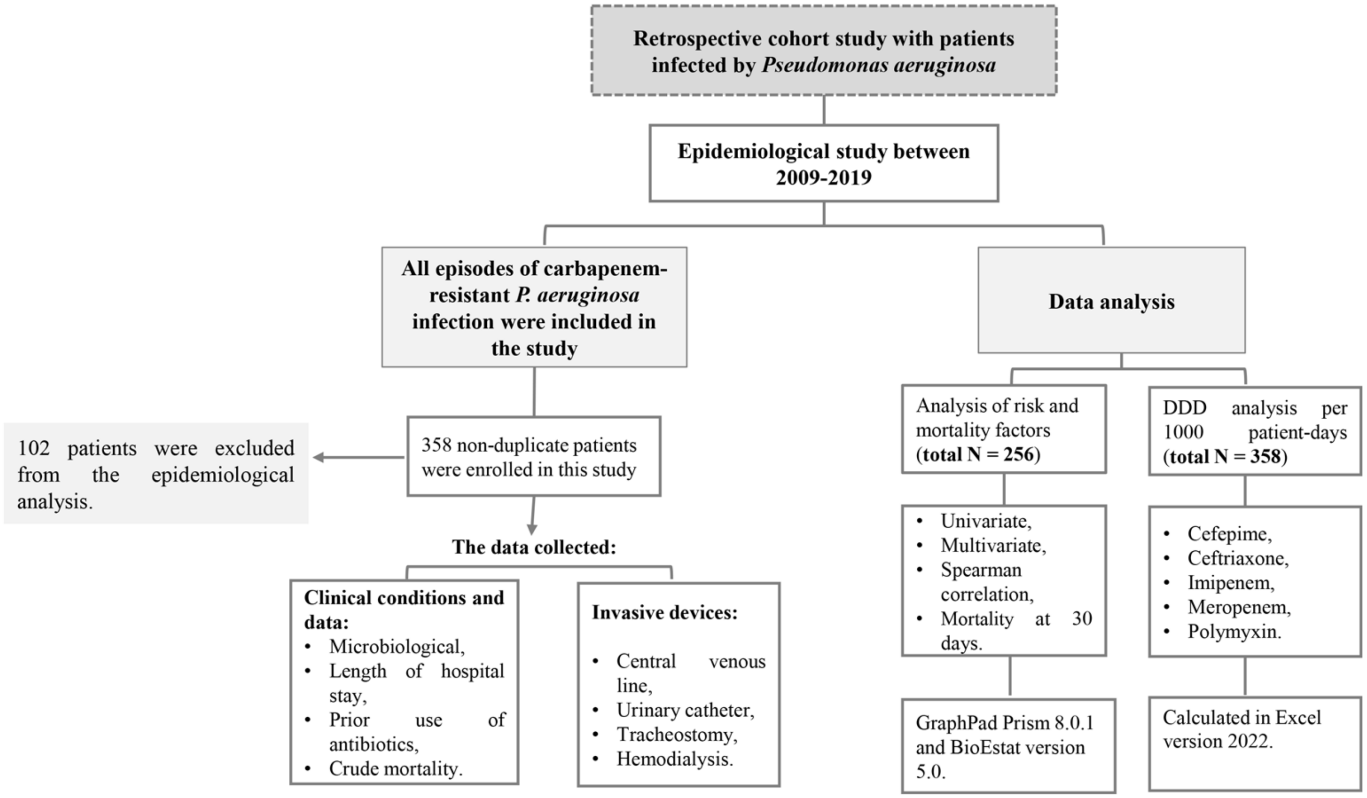
Healthcare-associated infections (HAIs) are characterized as infections acquired by patients after their admission to a healthcare facility. These infections may occur during the hospital stay or after discharge, and they are associated with hospitalization or medical procedures performed during that time [8]. The phenotypic resistance of the isolates was classified according to Magiorakos *et al.* [9], MDR was defined as acquired non-susceptibility to at least one agent belonging to three or more antimicrobial categories, and extensively drug-resistant (XDR) was defined as resistance to at least one antimicrobial agent from all mentioned categories, with the possibility of excluding up to two categories [9]. Additionally, prior antibiotic use was taken into consideration when the patient had been treated with any antibiotic for at least 72 hours within a 30-day period preceding the microbiological infection diagnosis [3].

Microbiological identification and susceptibility test

Microbial identification and antimicrobial susceptibility testing were carried out using a VITEK II system (VITEK II, bioMérieux, Uberlandia, Brazil). The Clinical and Laboratory Standard Institute guidelines [10] were followed to ensure quality control.

The antimicrobial susceptibility assessment encompassed an array of antimicrobial agents, including aminoglycosides (gentamicin, amikacin), carbapenems (imipenem, meropenem, ertapenem), cephalosporin (cefazolin, ceftriaxone), glycopeptides (vancomycin, teicoplanin), rifampicin, fluoroquinolone (ciprofloxacin), polymyxins (E and B), and penicillins (oxacillin) partnered with β-lactamase inhibitors (piperacillin-tazobactam, tetracyclines, ampicillin-sulbactam). Isolates with intermediate susceptibility were classified as resistant in accordance with the local antibiotic policy.

Figure 1. Flowchart of the methodological approach used for data collection and analysis.



Defined daily dose (DDD) of antimicrobial per 1000 patient-days

Antimicrobial consumption was quantified in terms of defined daily doses (DDD) per 1000 patient-days for the individuals encompassed within the cohort categorization of the study. The DDD values used for this metric were previously sourced from the hospital's pharmacy. To calculate the density of antimicrobial use per 1000 patient-days, a subset of antibiotics administered predominantly throughout the duration of the study was chosen: namely carbapenems (imipenem, meropenem), cephalosporin (cefepime, ceftriaxone), glycylcycline (tigecycline), and polymyxins (polymyxin B). The derivation of the utilization density per patient-days was executed according to the following formula:

$$Infections\ per\ 1000\ patient\ days = \frac{Number\ of\ infections}{Total\ number\ of\ patient\ days} \times 100$$

*Patient-day = P×B×O

Where: P = period of observation in days; B = beds available in the unit; O = occupancy rate in the period considered (%).

Defined daily dose of antimicrobial (DDD) per 1000 patient-days

$$DDD = \frac{Antibiotic\ consumption\ in\ grams}{Defined\ daily\ dose}\ [11]$$

$$DDD\ per\ 100\ patient\ days = \frac{DDD}{Total\ number\ of\ patient\ days} \times 100$$

Statistical analysis

Fisher's exact test was employed to compare discrete variables, whereas the Student's *t*-test was used to compare two quantitative variables. p values less than 0.05 were considered statistically significant. Pearson's correlation coefficient test was utilized to examine the correlation between antibiotic consumption and bacterial resistance rate. The epidemiological data were analyzed using GraphPad Prism 8.0.1 (La Jolla, California, United States) and BioEstat 5.0 (Tefé, Amazonas, Brazil).

Table 1. Comparison of risk factors in patients with the first episode of infection with *Pseudomonas aeruginosa* XDR, MDR, and sensitive isolates.

Characteristics	XDR n = 95	MDR n = 86	Sensitive n = 82	XDR		MDR	
				Univariate *p**/OR** (CI-95%)	Multivariate *p*/OR (CI-95%)	Univariate *p*/OR (CI-95%)	Multivariate *p*/OR (CI-95%)
Ages (Years) mean ± SD***	52.7 ± 23.3	56.6 ± 19.8	47.0 ± 26.7	0.1906 (-13.15–1.802)	-	0.0368/2832 (0.000–15.00	-
Hospitalization prior to infection mean ± SD	39.4 ± 41.8	33.6 ± 27.5	29.9 ± 39.9	**0.0151***/1980 (-1400–1.00)	**0.0089***/0.9901 (0.98–1.00)	**0.0151***/3863 (14.00–1.000)	**< 0.0001**/0.8176 (0.77–0.87)
ICU**** admission	60 (63.1)	59 (68.6)	36 (43.9)	**0.0016***/2.679 (1.454–4.8660)	**0.0144***/04766 (0.26–0.86)	**0.0004***/3.027 (1.622–5.789)	0.5301/0.7304 (0.27–1.95
Surgery	50 (52.6)	35 (40.6)	30 (36.5)	**0.0353***/1.926 (1.053–3.451)	0.1224/1.4460 (0.90–2.31)	0.6360/1.190 (0.6514–1.535)	-
Previous antibiotic use	82 (86.3)	76 (88.3)	56 (68.2)	**0.0059***/2.929 (1.377–6.009)	**0.0443***/1.4417 (0.20–0.98)	0.0023/3529 (1.627–7.702)	-
Cephalosporin (3rd generation)	43 (45.2)	32 (37.2)	27 (32.9)	0.1230/1.684 (0.9038–3.082)	-	0.6286/1.207 (0.6446–2.294)	-
Cefepime	31 (32.6)	27 (31.3)	13 (15.8)	**0.0141***/2.571 (1.259–5.146)	**0.0004***/0.3883 (023–0.65)	**0.0189***/2.464 (1.162–5.055)	0.4148/0.5883 (0.16–2.11)
Carbapenems	60 (63.1)	46 (53.4)	37 (45.1)	**0.0229***/2.085 (1.140–3.720)	0.1085/1.3907 (0.93–2.08)	0.2853/1.399 (0.7527–2.505)	-
Fluoroquinolones	10 (10.5)	14 (16.2)	7 (8.5)	0.7995/1.261 (0.4818–3.271)	-	0.1633/2.083 (0.8462–5.296)	-
Aminoglycosides	14 (14.7)	8 (9.3)	2 (2.4)	**0.0068***/6.914 (1.737–31.19)	0.1316/2.1042 (0.80–5.53)	0.0999/4.103 (0.9509–19.56)	-
Polymyxin	26 (27.3)	18 (20.9)	8 (9.7)	**0.0038***/3.486 (1.507–7.777)	0.3313/07371 (0.40–1.36)	0.0554/2.449 (1.036–5.780)	-
Invasive devices							
Mechanical ventilation	71 (74.7)	52 (60.4)	38 (46.3)	**0.0002***/3.425 (1.778–6.262)	0.8937/0.9558 (0.49–1.85)	0.0885/1.771 (0.9469–3.218)	-
Central venous catheter	80 (84.2)	62 (72.0)	61 (74.3)	0.1342/1.836 (0.8947–3.955)	-	0.8618/0.8893 (0.4469–1.739)	-
Tracheostomy	53 (55.7)	36 (41.8)	27 (32.9)	**0.0026***/2.571 (1.386–4.731)	0.1837/1.4457 (084–2.49)	0.2660/1.467 (0.7963–2.763)	-
Hemodialysis	20 (21.0)	15 (17.4)	19 (23.1)	0.8559/0.8842 (0.4229–1.764)	-	0.4429/0.7005 (0.3169–1.472)	-
Crude mortality	52 (54.7)	36 (41.8)	43 (52.4)	0.6536/1.152 (0.6360–2.093)	-	0.2161/0.6530 (0.3626–1.217)	-
Mortality at 30 days	39 (41.0)	26 (30.2)	31 (39.0)	0.7581/1.146 (0.6182–2.051)	-	0.3307/0.7129 (0.3696–1.350)	-

*Statistically significant < 0.05; **OR: odds ratio; CI: confidence interval; ***SD: standard deviation; ICU****: intensive care unit; SD: standard deviation; MDR: multi-drug resistant; XDR: extensively drug-resistant. The values in bold indicate statistically significant differences.

Ethical approval

The study was approved by the Research Ethics Committee for Human Participants of the Federal University of Uberlandia (Approval No. 2.527.621).

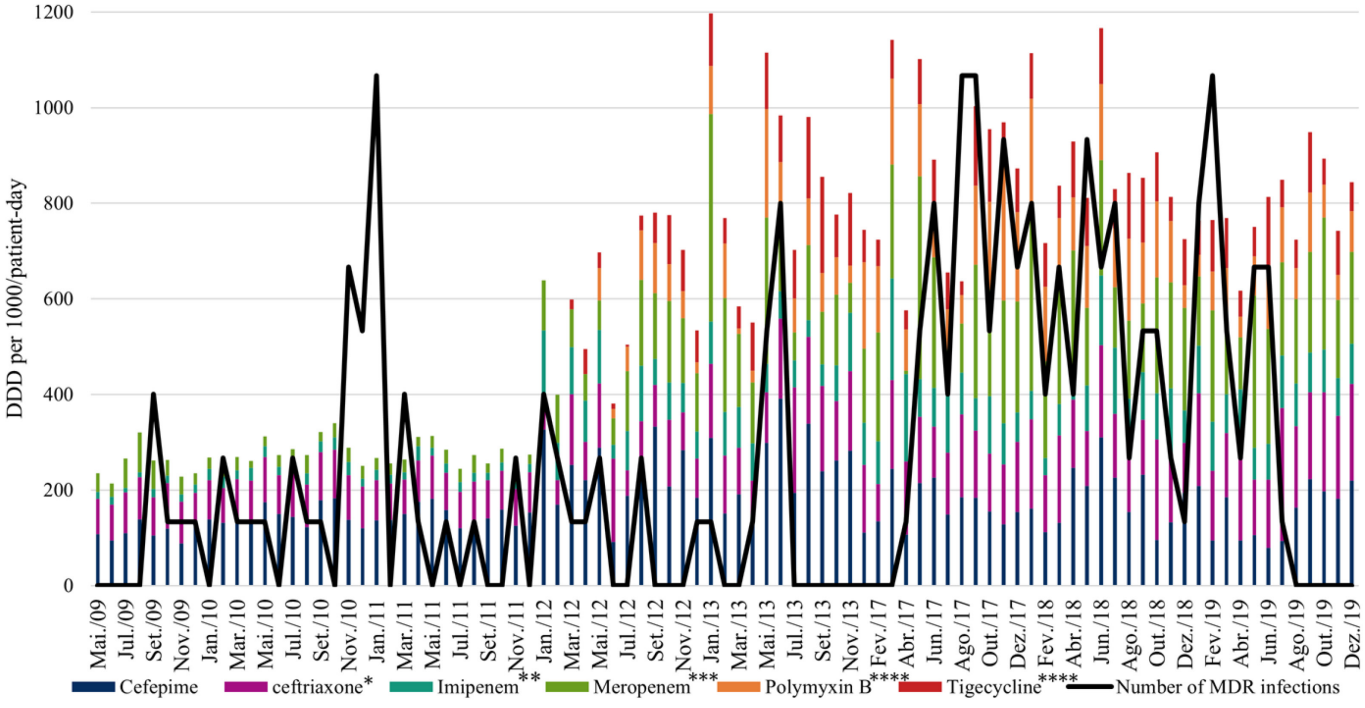
Results

A total of 358 non-duplicate patients with *P. aeruginosa* infections at the university hospital were enrolled in this study. Among these patients, 102 did not meet the inclusion criteria for the evaluated characteristics, and, therefore were excluded from the epidemiological analysis (Figure 1). Based on the results of an antimicrobial multi-resistance testing, extensively drug-resistant (XDR) *P. aeruginosa* was identified in 95 (36.1%) cases, while multidrug-resistant (MDR) was detected in 86 (32.6%) cases. These data were compared to those of non-resistant (sensitive) *P. aeruginosa*. Univariate and independent risk factors, as well as mortality associated with resistant *P. aeruginosa* infections, were summarized in Table 1.

Previous use of antibiotics (81.3%), ICU admission (58.9%), and central venous catheter use (58.9%) were common among the patients. Hospitalization prior to infection ($p = 0.0089$, OR = 0.9901), ICU admission ($p = 0.0144$, OR = 0.4766), previous antibiotic use ($p = 0.0443$, OR = 1.4417), and prior use of cefepime ($p = 0.0004$, OR = 0.3883) were identified as independent risk factors associated with XDR infection in the entire dataset. Subsequently, our multiple regression analysis revealed only hospitalization prior to infection ($p \leq 0.0001$, OR = 0.8176) as an independent variable associated with the development of MDR-*P. aeruginosa*. The overall 30-day mortality rate was 96 (40.0%) for all patients and 39 (41.0%) in patients with XDR-*P. aeruginosa* infections. Moreover, high crude mortality and mortality at 30 days were observed in all evaluated groups (Table 1).

The relation between the DDD of an antimicrobial per 1000 patient-days and the number of patients with resistant *P. aeruginosa* per 1000 patient-days is presented in Figure 2. Although the consumption of antimicrobials varied during the study period, the use of ceftriaxone, meropenem, and polymyxin B was particularly high. A positive correlation was observed between the increase in carbapenem-resistant *P. aeruginosa* (CRPA) isolates and the consumption of ceftriaxone (r = 0.3490, $p = 0.0007$), imipenem (r = 0.321, $p = 0.0019$), meropenem (r = 0.4604, $p = \leq 0.0001$), polymyxin B (r = 0.5284, $p = < 0.0001$), and tigecycline (r = 0.3913, $p = 0.0001$).

Figure 2. Relationship between the defined daily dose (DDD) of antimicrobials per 1000 patient-days and the number of patients with carbapenem resistant-*Pseudomonas aeruginosa.*



Spearman correlation test was performed and was considered statistically significant when the p ≤ 0.05. *p = 0.0007, r = 0.3490; **p = 0.0019, r = 0.3211; *** p ≤ 0.0001, r = 0.4604; ****p ≤ 0.0001, r = 0.5284; *****p = 0.0001, r = 0.3913.

Previous use of antibiotics in the MDR strain group is depicted in Figure 3A, while Figure 3B shows the entire group of patients. The most frequently observed combinations were 3rd and 4th generation cephalosporins and carbapenems, with a total of 56 combinations observed in all patients included in the MDR group (Figure 3A), and 146 combinations in all patients in the study (Figure 3B). The simultaneous use of the four antibiotics mentioned in the previous paragraph resulted in fewer combinations, with only 3 out of 174 patients presenting this type of combination in the MDR group (Figure 3A), and 9 out of 358 combinations in all patients (Figure 3B). Although there was a high use of polymyxin in both groups, the MDR group showed combinations of polymixin, carbapenem, and 3rd–4th cephalosporins in 9 patients. In this group, 9 out of 9 (100%) infected patients died within 30 days of diagnosis. Most patients were treated with prior therapy using meropenem (30.4%), followed by cefepime (29.5%).

Discussion

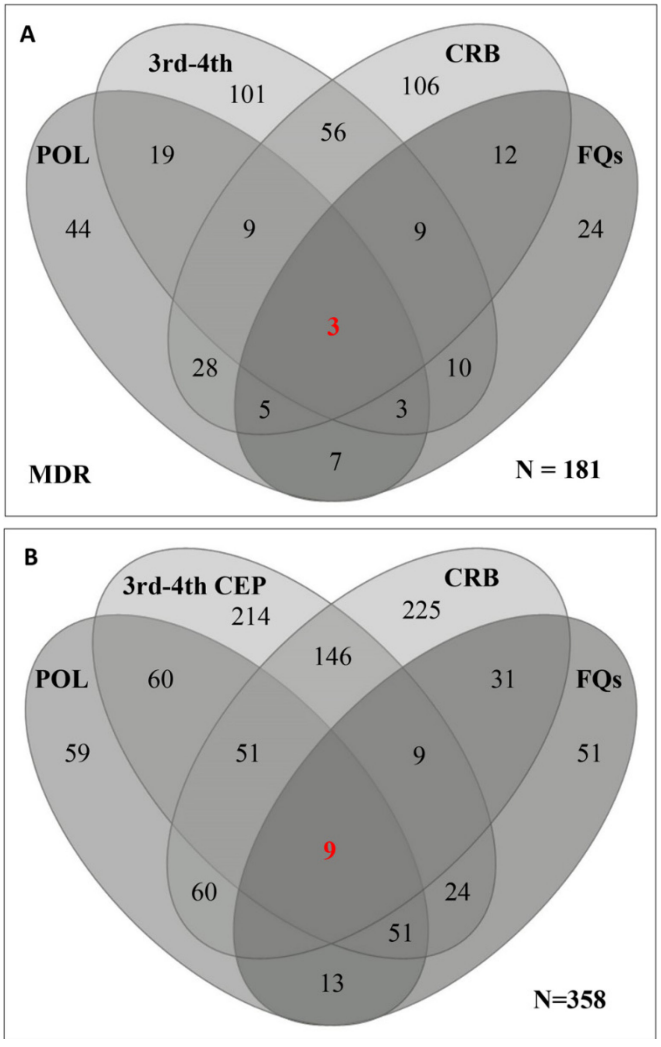
The spread of resistance, especially among Gram-negative pathogens, is a pressing concern. Despite significant advances in health, diagnosis, and treatment in recent years, it is crucial to understand the true extent of resistance in countries like Brazil. In this study, we reported antimicrobial consumption in DDD among patients with MDR-*P. aeruginosa* infections and we found alarming data indicating significant carbapenem and polymyxin use during the study period.

In our study, the defined DDD of antimicrobials was high, surpassing levels seen in other countries and exhibiting a rising trend over the years [4,12]. We observed that 50% of CRPA were MDR, a finding consistent with data from a multicenter study in Brazil that reported 48.7% of these strains as MDR [13]. The relation between antimicrobial consumption and patients with CRPA isolates was significant, with a positive correlation. This emphasizes that inappropriate and excessive use of antibiotics, especially broad-spectrum antibiotics, increases the number of microorganisms with MDR and XDR phenotypes.

Additionally, we identified concerning levels of MDR and XDR *P. aeruginosa* strains, with several independent risk factors. The findings in this study confirmed most of the risk factors mentioned in the scientific literature regarding infections caused by antimicrobial-resistant *P. aeruginosa*, which include previous overuse of antimicrobials, particularly cefepime, as well as extended hospital stays and admission to an ICU [4,14,15]. Furthermore, our study also revealed alarming rates of concomitant antibiotic use. The majority of our patients used more than two classes of antibiotics, with over two antibiotics per class; and with a significant emphasis on the use of polymyxins concomitantly with 3rd- and 4th-generation cephalosporins and carbapenems. We also observed worrying crude mortality and 30-day mortality rates.

Since these infections are highly avoidable, prevention and control strategies should be consistently advocated. The data from this study emphasizes the importance of monitoring antimicrobial usage, the ongoing need to promote sustained improvements in best preventive practices, and the training of healthcare professionals. These efforts can lead to a reduction in the risk of the infections and their adverse

Figure 3. Venn diagram displays of previous use of antibiotics. **A:** Prior use of the most used antibiotics in the multi-drug resistant (MDR) group; and **B:** Prior use of the most used antibiotics in all patients.



3rd-4th CEP, 3rd- and 4th- generation cephalosporins; CRB = carbapenems; FQs = fluoroquinolones; POL = polymyxins.

consequences, particularly in resource-limited countries. Approaches like these, when coupled with data obtained from epidemiological studies, are effective in reducing infection rates, as has been well documented in scientific literature.

Conclusions

We provided perspective on the current threatening, exacerbated, and indiscriminate use of antimicrobials in patients with MDR-*P. aeruginosa*. Furthermore, the epidemiological data obtained showed that previous indiscriminate use of antimicrobials, and intrinsic risk factors, may be associated not only with the escalation of MDR strains in hospital environments, but also with an unfavorable prognosis for patients infected with *P. aeruginosa* strains exhibiting antimicrobial resistance. Therefore, it is crucial to establish appropriate antimicrobial stewardship programs and implement efficient infection control measures to minimize the emergence and spread of antimicrobial-resistant infections, particularly those caused by *P. aeruginosa*.

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