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Antimicrobial Susceptibility Profile of Bacteria Isolated from Raw Sewage at a Wastewater Treatment Plant in Rio de Janeiro

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background and Aim: Given the concentration of microorganisms and emerging micropollutants, wastewater is a favorable environment for the exchange of resistance genes and the selection of antimicrobial-resistant strains, one of the most significant global threats of the 21st century, since inadequate environmental sanitation causes around 88% of deaths worldwide. The present study aimed to identify bacteria isolated from a sewage treatment plant located in Rio de Janeiro and determine their susceptibility profile to antimicrobials.

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Methods: A 10 µL aliquot of raw sewage was inoculated onto chromogenic agar and presumptive identification of microorganisms were based on their biochemical profiles. Isolates that could not be characterized using traditional biochemical tests were analyzed using polymerase chain reaction (PCR). Following the Brazilian Committee on Antimicrobial Susceptibility Testing, the resistance profile was determined by measuring the diameter of the inhibition zone and the isolates were classified as susceptible (S), resistant (R), multidrug resistant (MDR), extensively resistant (XDR) or pan-resistant (PDR).

Results: In this study, 38 strains were isolated, of which 24% were identified as Gram positive (n = 9) and 76% as Gram negative (n = 29). Among the isolated strains, Enterobacter sp. (7, 18%) was the most frequent genera and Escherichia coli (6, 15%) and Klebsiella pneumoniae (5, 13%) were the most frequent species. No pan-resistant strains were identified, however, isolates of Klebsiella pneumoniae (60%, n = 3/5), Enterobacter sp. (57%, n = 4/7) and Escherichia coli (50%, n = 3/6) presented the profile MDR and only one Enterobacter isolate was considered XDR (14%). Isolates of Morganella morgani, Proteus mirabilis, and Acinetobacter baumannii were fully sensitive to the antimicrobials tested. Regarding the antimicrobial susceptibility of Gram-positive strains, the Bacillus sp., Enterococcus sp. and Staphylococcus aureus isolates showed different resistance profiles, in which only *Bacillus* sp. and Microbacterium paraoxydans were considered high risk to health. Conclusion: The occurrence of potentially pathogenic and antimicrobial-resistant microorganisms in sewage treatment plants is a reality that has been explored over the years. Resistance to beta-lactam drugs is increasingly present in clinical practice, and these were the antimicrobials with the highest number of resistant isolates in the present study, including Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Enterobacter sp., which are listed as WHO priority. Our results revealed that sewage treatment plants act as reservoirs of bacterial resistance, facilitating the spread of resistance genes between different bacterial species. Contact of these microorganisms with the community may cause public health problems and environmental impacts, increasing the need to implement and improve environmental surveillance of resistant pathogens, establishing stricter control over the use of antimicrobials, to try to mitigate the advance of bacterial resistance.

Keywords: Antimicrobial resistance; enterobacterales; residual waters; sewage.

1. INTRODUCTION

The integrity of human, animal and environmental health is directly associated with basic sanitation conditions and water quality (Monteiro et al. 2016). Diseases related to inadequate environmental sanitation are responsible for approximately 88% of global deaths, most of which are transmitted by different aquatic matrices (Monteiro et al. 2024). Wastewater, also called sewage, resulting from anthropogenic activity, poses a risk of transmission of pathogenic microorganisms and, mainly, those resistant to antimicrobials (Monteiro et al. 2016; Joseph et al. 2019). In essence, Antimicrobial Resistance Bacteria (ARB), as well as Antimicrobial Resistance Genes (ARG), are considered a global public health problem that tends to increase, especially after the COVID-19 pandemic (Amarasiri et al. 2020; Silva et al. 2024; Wang et al. 2023).

Approximately 68 million units of antibiotics were sold in Brazil between 2020 and 2023, which may lead to an increase in cases of infections caused by multidrug-resistant or pan-resistant bacteria (Wang et al. 2023). The use of these drugs implies the elimination of residues in aquatic matrices, concentrating such substances in different compartments, including sewage system. Considering that effluent treatments currently used are not capable of eliminating these environmental contaminants, the sewage system becomes capable of amplifying the spread of ARBs and ARGs (Joseph et al. 2019; Manaia et al. 2018). Based on this, the present study aimed to identify bacteria isolated from a sewage treatment plant located in Rio de Janeiro and determine their susceptibility to antimicrobials.

2. METHODS

The methodology proposed in this pilot study consisted of collecting 500 mL of raw sewage, called influent, in sterile graduated bottles. Following all biosafety protocols, the collection was performed by the sewage treatment plant operator, allowing photographic recording of the collection process. The bottle was then properly labeled according to origin, time, type of collection and type of sample. To avoid bacterial competition, transportation took place in an isothermal box, as recommended in the Standard Methods for Examination of Water and Wastewater.

All experiments were carried out at the Microbiology Laboratory (LabMicro), Department of Biological Sciences, Sérgio Arouca National School of Public Health (ENSP), located at the Center for Research, Innovation and Surveillance in Covid-19 and Health Emergencies of the Oswaldo Cruz Foundation (FIOCRUZ).

2.1 Bacterial Identification

To obtain isolated bacterial colonies, the initial sample was diluted in sterile saline (0.9% NaCl) with different concentrations, ranging from 10^{-3} to 10^{-7} CFU. 10μL of the sample was seeded on chromogenic agar, allowing presumptive differentiation of the microorganisms. After seeding, the plates were incubated at 35±1°C for 24 hours for bacterial growth. The colonies of interest were replated and identified by Gram staining to confirm the morphological characteristics of the bacteria. Biochemical screening tests, such as Triple Sugar Iron Medium (TSI), Sulfide Indole Motility Medium (SIM), Simmons Citrate and Costa and Vernin Medium (CV) were used for presumptive identification.

In order to identify isolates that could not be characterized using traditional biochemical tests, polymerase chain reaction (PCR) analysis was performed, in which specific oligonucleotide primers for the 16S rRNA gene were amplified using the 16S rRNA full gene PCR Kit (Life Technologies) and the sequences obtained were compared based on similarity with the GenBank database. The DNA extraction protocol used was performed as described by Campos *et al*. (2012) at the National Reference Laboratory for Tuberculosis, Professor Hélio Fraga Reference Center, Sérgio Arouca National School of Public Health, Fiocruz.

2.2 Antimicrobial Sensitivity Test (AST)

To perform the sensitivity test, agar diffusion method was chosen, in which a bacterial suspension in 0.9% saline, corresponding to the 0.5 MacFarland scale, is seeded on Mueller-Hinton agar with the aid of a sterile swab. The antimicrobial discs were placed on agar and the plates were incubated in a bacteriological incubator at 37°C for 24 hours. Following the recommendations of the Brazilian Committee on Antimicrobial Susceptibility Testing (BRCAST), the isolates were tested for the following antimicrobial agents: amikacin (30μg), amoxicillin-clavulanate (10-20μg), ampicillin (10μg), cefepime (30μg), cefoxitin (30μg), ceftriaxone (30μg), ciprofloxacin (5μg), ertapenem (10μg), gentamicin (10μg), imipenem (10μg), meropenem (10μg), penicillin (10μg), and piperacycline-tazobactam (30-6μg). The sensitivity or resistance profile was determined by measuring the diameter of the inhibition zone and the isolates were classified as susceptible (S) when they did not present resistance, resistant (R) when they presented resistance to only one class of antimicrobials tested, multidrug resistant (MDR) when they were resistant to up to 3 classes, extensively resistant (XDR) when they were resistant to more than 3 classes, or pan-resistant (PDR) when they did not present susceptibility to any antimicrobial tested (BRCAST, 2024).

2.3 MAR Index

To determine the health risk, the Multiple Antibiotic Resistance Index (MAR Index) was calculated (Zagui, 2022, Afunwa et al. 2020), using the following formula:

> *MAR Index = n° ineffective antibiotics / n° antibiotics tested*

Following the interpretation proposed by Zagui (2022), intermediate resistance was considered sensitive and results equal to or greater than 0.2 were considered high risk (Zagui, 2022).

3. RESULTS AND DISCUSSION

Given the concentration of microorganisms and emerging micropollutants, wastewater is a favorable environment for the exchange of resistance genes and the selection of antimicrobial-resistant strains, one of the most significant global threats of the 21st century (Fröhler et al. 2023, Patra et al. 2024). In the present study, 38 bacterial strains were isolated, of which 24% were identified as Gram positive (n = 09), being 02 cocci and 07 bacilli, and 76% as Gram negative bacilli (n = 29). Only 5 isolates were considered non-fermenters. Among the isolated strains, the most frequent genera were *Enterobacter* sp. (n = 7, 18%), *Escherichia* sp. (n = 6, 15%), *Klebsiella* sp. (n = 5, 12.5%) and *Streptomyces* sp. (n = 4, 10%). The most frequent species were *Escherichia coli* (n = 6, 15%) and *Klebsiella pneumoniae* (n = 5, 13%), as shown in Fig. 1.

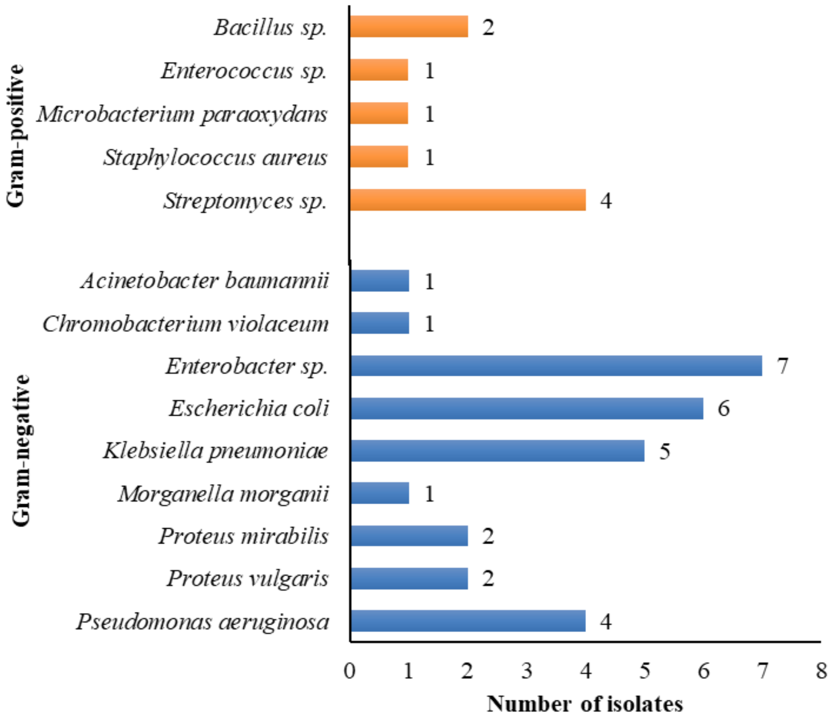

Fig. 1. Total number of isolates identified in raw sewage from a wastewater treatment plant in Rio de Janeiro

Source: Created by the authors (2024)

The most frequent isolated species, *Escherichia coli* and *Klebsiella pneumoniae,* can be explained by their presence in the intestinal microbiota of humans[14] while other bacterial isolates, such as *Proteus mirabilis*, *Proteus vulgaris* and *Pseudomonas aeruginosa*, are frequently found in wastewater due to their ability to adapt and tolerate the environment (Araújo et al. 2021,Zhang et al. 2020).

First described in 1872, *Chromobacterium violacium* is a tropical pathogen that can be commonly found in soil and water bodies in tropical and subtropical regions (Hota et al. 2022) and usually transmitted through exposure of skin wounds and traumatic injuries to contaminated soil and water (Ansari et al. 2015; Baker et al. 2008; Chowdhury et al. 2021; Menezes et al. 2015). Its zoonotic potential makes harmful to humans and animals, given its ability to cause abscesses in the skin and organs, such as the liver, lungs, kidneys, spleen, and lymph nodes, as well as septicemia with necrotizing lesions in multiple organs, resulting in the death of the patient (Hota et al. 2022,Soares et al. 2019). However, the isolate was not subjected to susceptibility testing because it was not viable for growth.

Regarding the antimicrobial susceptibility of Gram-positive strains, the *Bacillus* sp., *Enterococcus* sp. and *Staphylococcus aureus* isolates tested showed different resistance profiles, in which only *Bacillus* sp. and *Microbacterium paraoxydans* were considered high risk (Table 1). Resistant strains of *Bacillus* sp. have also been reported in other studies (Patra et al. 2024,Kalra et al. 2024), representing a relevant environmental risk, given the possibility that horizontal gene transfer favors the spread of antibiotic resistance not only among different *Bacillus* species, but mainly among other clinical pathogens, such as *Enterococcus* sp. and *Staphylococcus aureus* (Zhai et al. 2023). Although they present intermediate resistance to ampicillin and ciprofloxacin, respectively, the isolates of *Enterococcus* sp. and *S. aureus* require attention, since this classification implies a reduction in the clinical efficacy of the antimicrobials tested, in which the minimum inhibitory concentration (MIC) of the antimicrobial agent approaches blood and tissue levels (CLSI, 2005).

Table 1. Antimicrobial susceptibility testing Gram-positive isolates in raw sewage from a sewage treatment plant in Rio de Janeiro

ID*	Strain	Ami	Amp	Cfo	Cfp	Cip	Gen	Imp	Mer	Pen	Mar Index
EB1A	*Bacillus* sp.	NA	NA	NA	NA	R	NA	R	R	S	0,75
EB2B	*Bacillus* sp.	NA	NA	NA	NA	R	NA	R	R	R	1,0
EB2C	*Enterococcus* sp.	NA	I	NA	NA	S	NA	I	NA	NA	0
EB7D	*S. aureus*	S	NA	S	NA	I	S	NA	NA	R	0,2
EB10A	*M. paraoxydans*	NA	NA	R	R	R	I	R	R	NA	0,83

*Legend: AMI – Amikacin; AMP – Ampicillin; CFO – Cefoxitin; CFP – Cefepime; CIP – Ciprofloxacin; GEN – Gentamicin; IMP – Imipenem; MER – Meropenem; PEN – Benzylpenicillin; NA – Not applicable; R – Resistant; I – Intermediate; S – Sensitive. The authors (2024). * Note: The strain identification code refers to the laboratory's internal protocol*

Table 2. Antimicrobial susceptibility profile of Gram-negative bacilli isolated from a sewage treatment plant in Rio de Janeiro, Brazil

ID*	Strain	Amc	Amp	Ppt	Cfo	Ctx	Cfp	Cip	Gen	Ami	Imp	Mer	Ert	Mar Index
EB2A	*Escherichia coli*	*S*	*S*	*S*	*S*	*R*	*R*	*S*	*S*	*S*	*S*	*I*	*R*	*0,25*
EB2DI	*Acinetobacter baumannii*	*NA*	*S*	*S*	*NA*	*I*	*S*	*I*	*S*	*S*	*S*	*S*	*NA*	*0,00*
EB3AI	*Enterobacter sp.*	*R*	*R*	*S*	*R*	*S*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*0,33*
EB3B	*Proteus mirabilis*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*0,00*
EB3CI	*Escherichia coli*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*0,00*
EB3CII	*Pseudomonas aeruginosa*	*NA*	*NA*	*I*	*NA*	*NA*	*I*	*R*	*NA*	*S*	*I*	*S*	*NA*	*0,20*
EB3D	*Morganella morganii*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*0,00*
EB3E	*Klebsiella pneumoniae*	*S*	*R*	*S*	*R*	*S*	*S*	*R*	*S*	*S*	*I*	*S*	*S*	*0,25*
EB3F	*Pseudomonas aeruginosa*	*NA*	*NA*	*I*	*NA*	*NA*	*I*	*R*	*NA*	*I*	*I*	*I*	*NA*	*0,17*
EB3G	*Proteus mirabilis*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*0,00*
EB4B	*Klebsiella pneumoniae*	*S*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*0,08*
EB4C	*Klebsiella pneumoniae*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*0,00*
EB5A	*Proteus vulgaris*	*S*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*0,08*
EB5B	*Enterobacter sp.*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*R*	*R*	*0,17*
EB6A	*Escherichia coli*	*S*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*I*	*S*	*S*	*0,08*
EB6B	*Enterobacter sp.*	*R*	*R*	*S*	*R*	*S*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*0,33*
EB6C	*Klebsiella pneumoniae*	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*0,50*
EB7A	*Proteus vulgaris*	*S*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*0,08*
EB7E	*Escherichia coli*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*0,00*
EB7F	*Enterobacter sp.*	*S*	*R*	*R*	*S*	*S*	*S*	*R*	*R*	*S*	*R*	*S*	*S*	*0,42*

ID*	Strain	Amc	Amp	Ppt	Cfo	Ctx	Cfp	Cip	Gen	Ami	Imp	Mer	Ert	Mar Index
EB7G	*Escherichia coli*	S	R	S	S	S	I	R	R	S	S	S	S	0,25
EB8A	*Enterobacter sp.*	S	R	S	S	S	S	S	I	S	S	S	S	0,08
EB8B	*Pseudomonas aeruginosa*	NA	NA	I	NA	NA	I	I	NA	I	I	S	NA	0,00
EB9B	*Enterobacter sp.*	R	S	S	R	S	S	S	S	S	S	S	S	0,17
EB11B	*Escherichia coli*	R	R	R	S	S	S	S	R	S	S	S	S	0,33
EB11C	*Pseudomonas aeruginosa*	NA	NA	I	NA	NA	I	R	NA	I	I	I	NA	0,17
EB11D	*Enterobacter sp.*	S	R	S	R	S	S	S	S	S	S	S	S	0,17
EB11E	*Klebsiella pneumoniae*	R	R	S	R	I	S	S	S	S	R	I	I	0,33

Legend: AMC – Amoxicillin-Clavulanate; AMP – Ampicillin; PPT – Piperacycline-Tazobactam; CFO – Cefoxitin; CTX – Ceftriaxone; CFP - Cefepime; CIP – Ciprofloxacin; GEN – Gentamicin; AMI - Amikacin; IMP – Imipenem; MER – Meropenem; ERT – Ertapenem; NA – Not applicable; R – Resistant; I – Intermediate; S – Sensitive. The authors (2024).

** Note: The strain identification code refers to the laboratory's internal protocol*

In this perspective, the *Microbacterium paraoxydans* strain stands out with the greatest risk to health, whose sensitivity to gentamicin may be linked to exposure to the drug, demanding greater attention due to the difficulty of treatment in case of infection. *Microbacterium* sp. are Gram-positive coryneform bacilli widely distributed in the environment, such as soil and sewage, and are rare pathogens in humans. Although the epidemiological and clinical characteristics of *Microbacterium* species are scarce, cases of endophthalmitis, peritoneal dialysis-related infection, catheter-related bloodstream infections and bacteremia have been reported, mainly in immunocompromised patients (Amano et al. 2019; Bernard et al. 2015; Ko et al. 2007; Pinchman et al. 2023).

In 2024, the World Health Organization (WHO) listed the Enterobacterales order resistant to carbapenems as critical priority for monitoring due to their ability to transfer resistance genes and the severity of the infections they cause (WHO, 2024). Regarding the Gram-negative isolates subjected to antimicrobial susceptibility test, 71% were considered resistant, 32% resistant to one class of antimicrobials (n = 9), 36% resistant to at least three classes of the drugs tested (n = 10), and only 01 isolate (4%) considered extensively resistant, not showing susceptibility to more than three classes tested (Table 2). The MAR Index revealed that only 8 isolates were susceptible to all antimicrobials tested, suggesting a low health risk and may come from an environment with zero health risk, given their sensitivity to all antimicrobials, in which the absence of contact with drugs and resistance genes can be considered. However, the EB8B strain warrants attention, as its resistance profile indicates possible drug exposure, characterizing it as an isolate with intermediate resistance and potential risk to human health (Zagui, 2022; CLSI, 2005). In contrast, 36% (n = 10) of the isolates are considered to pose a high health risk.

The EB6C strain stands out due to its resistance to various beta-lactams, being classified as an extended-spectrum beta-lactamase (ESBL) *Klebsiella pneumoniae*, which confers resistance to a wide range of antibiotics, hindering the treatment of infections and increasing the risk of dissemination (Li et al. 2023; Salawudeen et al. 2023). Being capable of causing a series of infections in hospitalized patients, with the advancement of antibiotic therapy, resistant strains of *K. pneumoniae* are increasingly prevalent, favoring the dissemination of different resistance genes, especially in the hospital environment, being considered one of the greatest challenges for global public health (Li et al. 2023; Scarpate and Cossatis, 2009). According to Scarpate and Cossatis (2009), the prevalence of ESBL-positive *K. pneumoniae* in Latin America is higher than the world average (20-30%) (Scarpate and Cossatis, 2009), which may be directly related to the widespread and indiscriminate use of third-generation cephalosporins, such as ceftriaxone, and carbapenems, in which meropenem is considered a last resort treatment, disseminating this multidrug-resistant microorganism to the community, making clinical management of infections difficult (Salawudeen et al. 2023, Scarpate and Cossatis, 2009; Khanfar et al. 2009; Demirdag and Hosoglu, 2010; Silva and Junior, 2022, Silva, 2023).Among the antimicrobials tested, beta-lactams showed the highest resistance rates, including penicillins, such as ampicillin (58%, n = 14/24), cefoxitin (30%, n = 7/23), amoxicillin associated with potassium clavulanate (26%, n = 6/23) (Fig. 2).

Among the carbapenems tested, 9% of the strains tested were resistant to ertapenem (n = 2/23), 7% were resistant to imipenem (n = 2/28) and only 4% were resistant to meropenem (n = 1/28), which may be linked to the fact that carbapenems are the drugs of choice for the treatment of ESBL-positive strains, in which meropenem is considered a last resort treatment (Silva and Junior, 2022, Silva, 2023). Of the aminoglycosides tested, only cases of resistance to gentamicin were identified (13%, n = 3/24). Resistance to aminoglycosides may originate from cross-resistance, given that amikacin is a substrate for only a few aminoglycosidases, bacterial enzymes capable of modifying the structure of aminoglycosides, making strains resistant to other aminoglycosides, such as gentamicin, likely to be susceptible to amikacin (Bennett et al. 2019, Gazineo et al. 2023). This statement corroborates the results found in the present study, in which, only cases of resistance to gentamicin were identified, a drug primarily recommended in cases of severe infections, but which, due to its low cost, began to be used indiscriminately, favoring the selection of resistant strains (Gazineo et al. 2023).

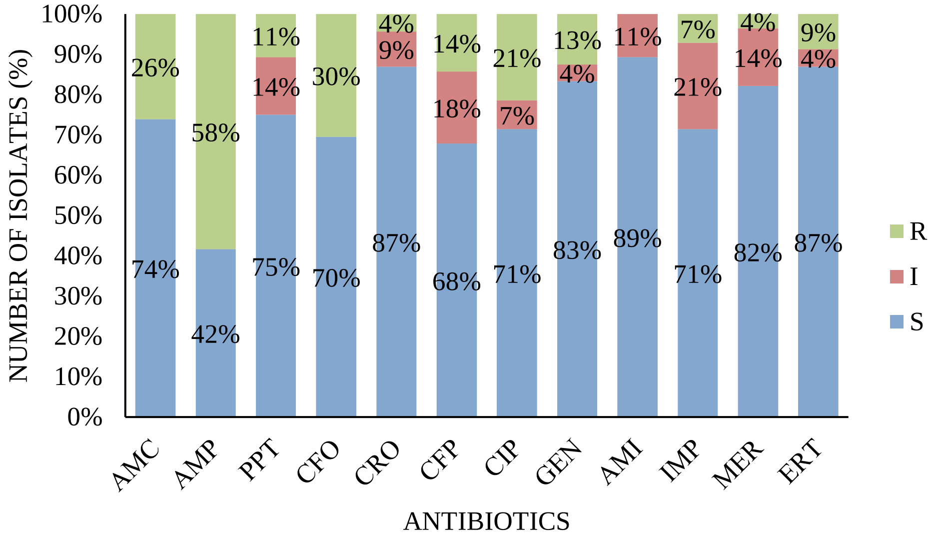

Fig. 2. Strains resistant to the tested antibiotics isolated from a sewage treatment plant in Rio de Janeiro

Legend: AMC – Amoxicillin-Clavulanate; AMP – Ampicillin; PPT – Piperacycline-Tazobactam; CFO – Cefoxitin; CTX – Ceftriaxone; CFP - Cefepime; CIP – Ciprofloxacin; GEN – Gentamicin; AMI - Amikacin; IMP – Imipenem; MER – Meropenem; ERT – Ertapenem; R – Resistant; I – Intermediate; S – Sensitive. Source: Created by the authors (2024)

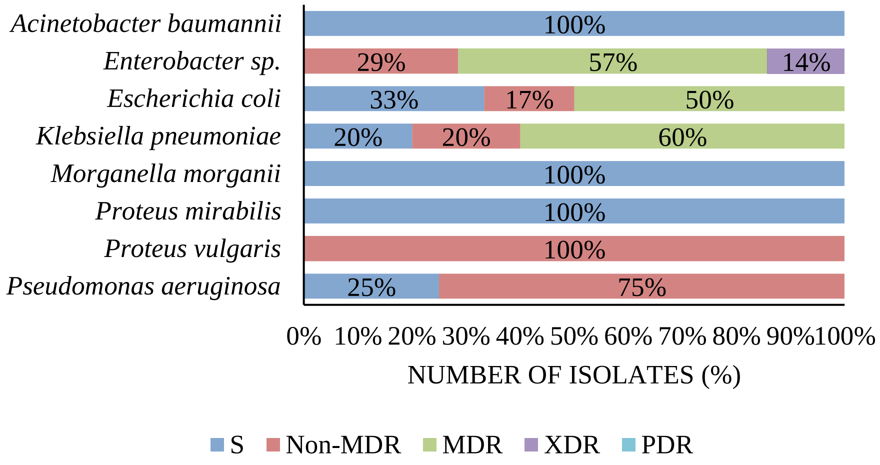

Fig. 3. Resistance profile of Gram-negative rods isolated from sewage treatment plant in Rio de Janeiro

Legend: S – Sensitive; MDR – Multidrug resistant; XDR – Extensively resistant; PDR – Pan-resistant. Source: Created by the authors (2024)

Furthermore, no pan-resistant strains were identified. However, isolates of *Klebsiella* sp. (60%, n = 3/5), *Enterobacter* sp. (57%, n = 4/7), and *Escherichia* sp. (50%, n = 3/6) presented the MDR profile, and only one *Enterobacter* isolate was considered XDR (14%). Isolates of *Morganella morgani*, *Proteus mirabilis*, and *Acinetobacter baumannii* were fully sensitive to the antimicrobials tested (Fig. 3).

Even though pan-resistant strains were not identified in this research, it is important to highlight that *A. baumannii* has intrinsic resistance to some antimicrobials and a greater tendency to acquire resistance genes from the environment (Barbosa et al. 2023). Further analyses are necessary to obtain more robust data. However, the results obtained corroborate the study by Abrantes (2022), in which the author states that (Mills and Lee, 2019) bacteria of the order Enterobacterales have a high profile of non-sensitivity to antimicrobials, including strains whose resistance occurs through exposure (intermediate), mainly to beta-lactams, including cephalosporins and carbapenems, and quinolones (Abrantes, 2022). Moreover, abiotic factors (e.g. temperature, pH and electric conductivity) can directly impact on the microbial composition of effluents, influencing the development of specific physiological and biochemical groups of bacteria. Most of the clinically and environmentally relevant ARB, are strictly chemoorganoheterotrophic, mesophilic, neutrophilic, and facultative or aerobic. This means that their ability to survive and proliferate is conditioned by the balance of all these variables. (Manaia et al. 2018)

Therefore, our results reveal that sewage treatment plants can act as large reservoirs of antimicrobial-resistant bacteria, playing a direct role in the spread of resistance genes among different bacterial species, mainly ESBL, MRSA and carbapenem resistant strains. (Katale et al. 2022) Anticipating the treatment of waste from hospitals, implementing effective methodologies for the removal and inactivation of ARBs and/or ARGs before dumping into the sewage system, may help to reduce risks to human, animal and environmental health and mitigate the advance of bacterial resistance. (Drigo et al. 2021, Waseem et al. 2017) Based on these findings, it is crucial to highlight the role of effluents in the dissemination of antimicrobial-resistant pathogens and the importance of creating epidemiological surveillance networks in these matrices.

4. CONCLUSION

Although these are preliminary results, it allows us to identify the role of effluents in bacterial resistance, with emphasis on *Bacillus* sp. and, mainly, Gram-negatives, including species such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter* sp., which are listed as WHO priority. Of the isolates identified, only 8 may have originated from an environment with no risk to health, in which there was no direct contact with drugs, given their sensitivity to the antimicrobials tested. However, resistance to beta-lactam drugs, such as ampicillin, amoxicillin and some cephalosporins, is increasingly present in clinical practice, and these were the antimicrobials with the highest number of resistant isolates in the present study.

The occurrence of potentially pathogenic and antimicrobial-resistant microorganisms in sewage treatment plant is a reality that has been explored over the years. Bacteria carrying antimicrobial resistance genes can be easily disseminated through waterways. Contact of these microorganisms with community may cause public health problems and environmental impacts, increasing the need to implement and improve environmental surveillance of resistant pathogens, establishing stricter control over the use of antimicrobials, in order to try to mitigate the advance of bacterial resistance.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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