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Original Article

Hospital sewage in Brazil: a reservoir of multidrug-resistant carbapenemase-producing *Enterobacteriaceae*

Esgoto hospitalar no Brasil: um reservatório de *Enterobacteriaceae* produtoras de carbapenemase resistente a múltiplas drogas

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Abstract

The One Health concept recognizes that human health is clearly linked to the health of animals and the environment. Infections caused by bacteria resistant to carbapenem antibiotics have become a major challenge in hospitals due to limited therapeutic options and consequent increase in mortality. In this study, we investigated the presence of carbapenem-resistant *Enterobacteriaceae* in 84 effluent samples (42 from hospital and 42 from non-hospital) from Campo Grande, midwest Brazil. First, sewage samples were inoculated in a selective culture medium. Bacteria with reduced susceptibility to meropenem and ertapenem were then identified and their antimicrobial susceptibility was determined using the Vitek-2 system. The bla_{KPC} genes were detected using PCR and further confirmed by sequencing. Carbapenem-resistant *Enterobacteriaceae* (CRE) were identified in both hospital (n=32) and non-hospital effluent (n=16), with the most common being *Klebsiella pneumoniae* and of the *Enterobacter cloacae* complex species. This is the first study to indicate the presence of the bla_{KPC-2} gene in carbapenem-resistant *Enterobacteriaceae*, classified as a critical priority by the WHO, in hospital sewage in this region. The dissemination of carbapenem antibiotic-resistant genes may be associated with clinical pathogens. Under favorable conditions and microbial loads, resistant bacteria and antimicrobial-resistance genes found in hospital sewage can disseminate into the environment, causing health problems. Therefore, sewage treatment regulations should be implemented to minimize the transfer of antimicrobial resistance from hospitals.

Keywords: sewers, *Enterobacteriaceae*, antimicrobial resistance, hospital wastewater.

Resumo

O conceito One Health reconhece que a saúde humana está claramente ligada à saúde dos animais e do ambiente. As infecções causadas por bactérias resistentes aos antibióticos carbapenêmicos tornaram-se um grande desafio nos hospitais devido às opções terapêuticas limitadas e consequente aumento da mortalidade. Neste estudo, investigamos a presença de Enterobacteriaceae resistentes a carbapenêmicos em 84 amostras de efluentes (42 hospitalares e 42 não hospitalares) de Campo Grande, Centro-Oeste do Brasil. Primeiramente, amostras de esgoto foram inoculadas em meio de cultura seletivo. As bactérias com susceptibilidade reduzida ao meropenem e ao ertapenem foram então identificadas e a sua susceptibilidade antimicrobiana foi determinada utilizando o sistema Vitek-2. Os genes blaKPC foram detectados por PCR e posteriormente confirmados por sequenciamento. Enterobacteriaceae resistentes a carbapenêmicos (CRE) foram identificadas em efluentes hospitalares (n=32) e não hospitalares (n=16), sendo as mais comuns Klebsiella pneumoniae e espécies do complexo Enterobacter cloacae. Este é o primeiro estudo a indicar a presença do gene blaKPC-2 em Enterobacteriaceae resistentes a carbapenêmicos, classificadas como prioridade crítica pela OMS, em esgoto hospitalar desta região. A disseminação de genes resistentes a antibióticos carbapenêmicos pode estar associada a patógenos clínicos. Sob condições favoráveis e cargas microbianas, bactérias resistentes e genes de resistência antimicrobiana encontrados no esgoto hospitalar podem se disseminar no meio ambiente, causando problemas de saúde. Portanto, devem ser implementadas regulamentações sobre tratamento de esgotos para minimizar a transferência de resistência antimicrobiana dos hospitais.

Palavras-chave: esgotos, Enterobacteriaceae, resistência bacteriana, águas residuais hospitalares.

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1. Introduction

Healthcare institutions generate large volumes of liquid effluents from specific activities related to healthcare. These effluents comprise a range of contaminants, such as disinfectants, drugs, bacteria, viruses and parasites; therefore, the direct discharge of these effluents into the environment adversely affects aquatic environments and human health (Fatimazahra et al., 2023). The continuous exposure of bacteria to antibiotics used in hospitals has led to the development of gene resistance, which is a public and global threat with serious health consequences (Fatimazahra et al., 2023). Owing to limited treatment options, multidrug-resistant (MDR) bacterial infections contribute to increased hospital stays and mortality rates (Antimicrobial Resistance Collaborators, 2022; WHO, 2017). By 2050, such infections could cause up to 10 million deaths globally per year and have significant economic impacts (UNEP, 2022); therefore, immediate action is required.

Carbapenemase-producing *Enterobacteriaceae* pose a significant health threat because some strains are resistant to almost all available antibiotics. *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacteriaceae* have been isolated worldwide (Lee et al., 2016; Logan and Weinstein, 2017). It is the main Ambler's class A carbapenemase found in South America (Bonelli et al., 2014) including in Brazilian medical centers, but it has been more frequently reported in hospitals in the southern and southeastern regions (Dias et al., 2021; Ferreira et al., 2019; Santos et al., 2022; Sousa et al., 2020; Tavares et al., 2015).

Many studies have highlighted the role of wastewater as a crucial environmental reservoir for antimicrobial-resistant bacteria (ARB) and genes (ARG) (Al Salah et al., 2020; Batista et al., 2023; Chagas et al., 2011; Fouz et al., 2020; Hassoun-Kheir et al., 2020). Hospital sewage is a particularly important source of ARB, especially extended-spectrum beta-lactamase and KPC-producing Gram-negative rods (Conte et al., 2017; Hassoun-Kheir et al., 2020; Picão et al., 2013; Zagui et al., 2022). Furthermore, ARGs are not degradable; therefore, they can spread among microbial communities in the environment through horizontal gene transfer, which is the main mechanism of resistance in most Gram-negative bacteria (Fouz et al., 2020).

Carbapenemase production is one of the main mechanisms conferring resistance to carbapenem antibiotics in *Enterobacteriaceae* isolated from clinical samples in the state of Mato Grosso do Sul, midwest Brazil (Biberg et al., 2015; Campos et al., 2017; Rodrigues et al., 2022). Notably, these bacteria exhibit a strong dissemination ability (Jelić et al., 2019; Picão et al., 2013). Therefore, for the first time, we report the presence of the bla_{KPC} gene in carbapenem-resistant *Enterobacteriaceae* isolated from hospital sewage in the midwest region of Brazil.

2. Material and methods

2.1. Sampling sites

Considering the lack of specific legislation, wastewater from hospitals is disposed of without prior treatment in the municipal sewage system. In this study, samples were collected from the effluent of a tertiary teaching hospital (265 beds) and from non-hospital effluent in Campo Grande, Mato Grosso do Sul State, midwest Brazil. Samples were collected once a month for six months. Forty-two samples of hospital effluent were collected, including water from the coronary care unit, medical clinic ward, pediatric ward, intensive care unit, emergency medical care, surgical clinic, material sterilization center, gynecology and obstetrics ward, and hospital effluent output. Similarly, 42 samples were also collected from non-hospital effluent, including effluent entering the hospital from the dentistry department and effluent from the clinical analysis laboratory, streams, the city center, neighborhoods, regional industry, and the university rectory, as well as a point before the sewage outlet from the hospital. Figure 1 shows a schematic of the sample collection locations. From each selected point, a 200-mL sewage sample was collected in a sterile glass bottle. All samples were transported at 4 °C and processed within 6 h.

2.2. Microbiological analysis

First, 10 µL of sewage samples were sown on MacConkey agar plates (Ionlab, Brazil) and incubated at 37 °C for 24 h (Zagui et al., 2020). Subsequently, the colonies were counted using a colony counter and classified according to the appearance and color of the colonies. Isolated colonies were streaked onto Chromogenic KPC Agar (Probac - Brazil), intended for the cultivation of clinically relevant carbapenem-resistant bacteria and incubated at 37 °C for 24 h.

The presumed-identification of carbapenem-resistant Gram-negative bacteria was interpreted according to the manufacturer's instructions; that is, growth of metallic blue colonies indicated *Klebsiella, Enterobacter*, and *Citrobacter* spp., dark pink to red color indicated *Escherichia coli* species, and a cream or translucent color was presumed to indicate *Pseudomonas* spp (Probac™, Brazil). One colony of each profile and only one colony with identical profiles were selected for isolation on Mueller–Hinton agar (Oxoid™ - United Kingdom) and subjected to disk diffusion analysis for meropenem, and ertapenem (10 µg, Sensifar™, Brazil) susceptibility according to the Brazilian Committee Antimicrobial Susceptibility Testing guidelines (BrCAST, 2021). Isolates exhibiting non-susceptibility to at least one carbapenem were stored in tryptone soy broth (Kasvi™, Brazil) with 15% glycerol at -20 °C.

All bacteria with reduced sensibility to meropenem and ertapenem were identified and their antimicrobial susceptibility was determined using the Vitek-2 system (bioMérieux, Marcy L'Etoile, France) and the results interpreted according to BrCAST guidelines (BrCAST, 2021). Isolates were tested for susceptibility to meropenem (MER), ertapenem (ERT), imipenem (IPM), amikacin (AMI), amoxicillin/clavulanate, aztreonam (AZT), ceftazidime (CAZ), cefepime (CPM), ceftriaxone (CRO), cefoxitin (CFO), ciprofloxacin (CIP), piperacillin/tazobactam (TZP), gentamicin (GEN), avibactam/ceftazidime (CZA), and ceftolozane/tazobactam (C/T). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality-control strains. The antibiotics were chosen on the basis of their routine use at the hospital.

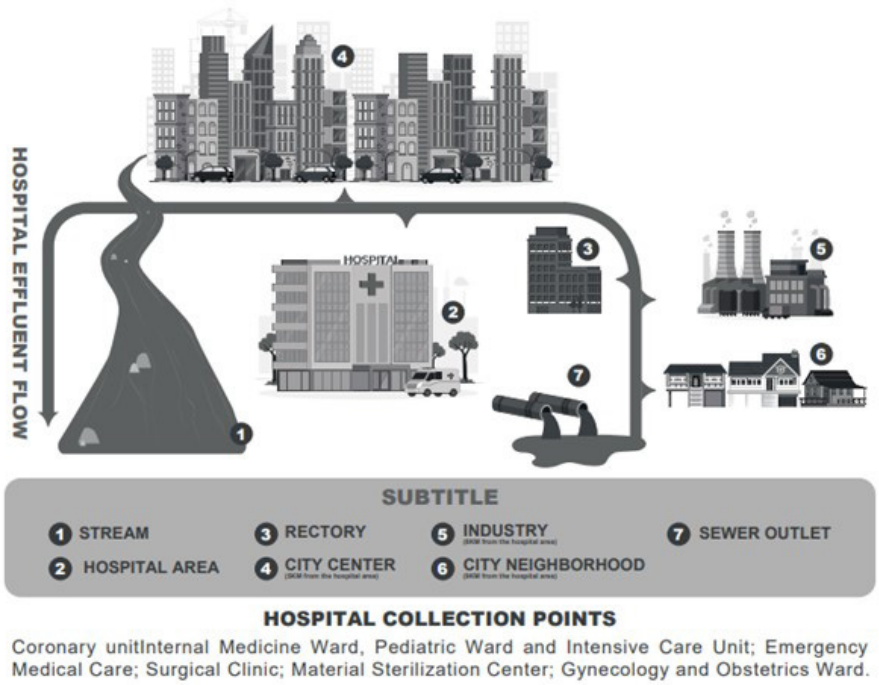

Figure 1. Schematic illustrating the sample collection locations in Campo Grande, Mato Grosso do Sul State, midwest Brazil.

For the phenotypic detection of carbapenemases, the modified carbapenem inactivation method and EDTA-CIM were employed according to CLSI guidelines (CLSI, 2018). The classification of MDR strains was performed according to Magiorakos et al. (2012), considering MDR strains as being "non-susceptible to ≥1 agent in ≥3 antimicrobial categories."

All isolates that expressed phenotypic resistance to meropenem and/or ertapenem were screened using PCR for the presence of bla_{KPC} genes.

2.3. Genotypic analysis

The genomic DNA was isolated using a boiling method from a bacterial colony grown overnight. DNA extraction and PCR were performed according to Monteiro et al. (2012) and Biberg et al. (2015). The following primers were used to amplify the bla_{KPC} gene: forward, 5' TCGCTAAACTCGAACAGG 3' and reverse, 5' TTACTGCCCGTTGACGCCCAATCC 3'. All PCR runs were performed using a positive control (ATCC BAA-1705, KPC-2) and RNase-free water as the negative control.

Following amplification, the size of the PCR products was verified using 1% agarose gels and the products were then purified using ExoSAP-IT® (Affymetrix, Inc., Santa Clara, CA) according to the manufacturer's instructions. The amplified genomic DNA was quantified via optical density measurement using a spectrophotometer (NanoDrop One ThermoFisher Scientific, Wilmington, DE, USA). Approximately 60 ng of DNA was prepared for sequencing using the BigDye Terminator v3.1 (Applied Biosystems, FosterCity, USA) kit. Sequencing was performed on a 3130XL Genetic Analyzer (Applied Biosystems, Foster City, USA). The resulting DNA sequences were analyzed using Geneious R7.1.3 (Biomatters Ltd.) to verify and edit forward and reverse reads, and to manually check the alignments.

The consensus sequences were compared with a genetic database available on National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST; GenBank accession numbers PP482160-PP482174).

2.4. Data analysis

To collate all available information regarding multidrug-resistant and carbapenemase-producing *Enterobacteriaceae* isolated from sewage in Brazilian hospitals, we searched for articles in two databases—U.S. National Library of Medicine (PubMed) and Web of Science Core Collection—to obtain all articles published from 2010 to 2023, with the search carried out in January 2024. The key terms "Enterobacteriaceae", "carbapenemase", "KPC", "beta-lactamases genes", "sewage", "hospital" and "Brazil" were used to obtain available articles. Duplicates were removed and initial screening was performed by evaluating titles, abstracts, and keywords, with an explicit focus on *Enterobacteriaceae* and the beta-lactamase genes.

2.5. Statistical analysis

Descriptive analysis and characterization of the samples were performed using the mean, standard deviation, and absolute and relative frequencies. To evaluate the significant differences between the various locations, the Kruskal–Wallis test was employed. $P<0.05$ was considered to indicate statistical significance.

3. Results

Between September 2021 and February 2022, 84 effluent samples were collected from the city of Campo Grande:

42 (50%) from hospital effluent and 42 (50%) from non-hospital effluent. From the 84 effluent samples, 7.299 x 10^3 colony forming units (CFU)/mL were isolated, with an average of 25 (standard deviation = 58.5) CFU/mL of Gram-negative bacteria (GNB) collected per sample. Table 1 shows the number of CFU/mL of isolated GNB in hospital and non-hospital effluent. When all units were compared, a statistically significant difference was observed between hospital and non-hospital effluent ($p < 0.001$).

Overall, 145 Gram-negative colonies with probable resistance to carbapenems were observed on Chromogenic KPC Agar. Among these, 78 were isolated from hospital effluent and 67 from non-hospital sources. Among these, 118 (n=81%) colonies had color suggestive of the *Enterobacteriaceae* family, whereas 27 colonies (n=19%) had color suggestive of *P. aeruginosa*.

According to the disk diffusion susceptibility test, 33.1% (48/145) of bacteria exhibited resistance to carbapenem antibiotics (ertapenem and/or meropenem). These carbapenem-resistant *Enterobacteriaceae* (CRE) were identified in both hospital (n=32) and non-hospital effluent (n=16).

According to the phenotypic test for carbapenemase production using the modified carbapenem inactivation method, 16 (33%) isolates were identified as bacteria producing class A or D Ambler group β-lactamases. All 16 belonged to the *Enterobacteriaceae* family (five (31%) *Klebsiella pneumoniae* ssp. *pneumoniae*, four (25%) *Enterobacter cloacae* complex, two (12%) *Klebsiella pneumoniae* ssp. *ozaenae*, two (12%) *Klebsiella oxytoca*, two (12%) *Citrobacter farmeri*, and one (6%) *Kluyvera intermedia*). All isolates harbored the bla_{KPC-2} gene. These bacteria were isolated only from hospital effluent, including eight (50%) from hospital sewage discharge, three (19%) from the emergency medical unit, three (19%) from the surgical clinic, and two (13%) from the coronary unit, medical clinic ward, pediatric ward, and intensive care unit. The antimicrobial susceptibility profiles of the bacteria are presented in Table 2. All 16 KPC-producing *Enterobacteriaceae* were considered MDR. Among these, two isolates (*K. pneumoniae* and *K. oxytoca*) were resistant to all antibiotics tested.

4. Discussion

Similar to reports from around the world, MDR bacteria have been identified in soil, food, aquatic environments, cattle, birds, and farms in Brazil (Bartley et al., 2019; Conte et al., 2017; Oliveira et al., 2017; Oliveira et al., 2023; Gomes et al., 2022; Nakamura-Silva et al., 2022; Ramalho et al., 2022; Zagui et al., 2022; Zagui et al., 2023). These pathogens can spread through different niches with easy contact with humans, animals, and food (Souza et al., 2023).

This study on CRE was motivated by the high frequency of isolation of CRE from clinical samples. The *Enterobacteriaceae* family is the most relevant bacteria from a clinical perspective as it includes pathogens responsible for many infections and are associated with

Table 1. Number of Gram-negative bacteria (CFU/mL) isolated from effluent collected at different locations in Campo Grande city from 2021 to 2022.

Effluent collection location	CFU/mL of effluent (n=7,299 x 10^3)			P-value*
	N (x 10^3)	Median	1º e 3º quartile	
Hospital Environment				
Coronary Care Unit, Medical clinic ward, Pediatric ward and Intensive Care Unit	951	15.0	(4.75 – 25.26)	
Emergency Medical Care	205	7.5	(4.00 – 19.76)	
Surgical Clinic	732	14.0	(3.00 – 30.00)	
Gynecology and obstetrics	355	13.0	(8.00 – 28.76)	
Hospital effluent output	1330	10.5	(5.76 – 18.00)	
Material Sterilization Center	60	6.0	(2.26 – 7.0)	
Non-Hospital Environment				<0.001
Dentistry	537	19.0	(4.50 – 40.00)	
Clinical Analysis Laboratory	489	12.0	(5.00 – 25.00)	
Stream	137	2.5	(1.00 – 6.50)	
City center	315	4.0	(9.00 – 15.00)	
Neighborhood	1445	16.0	(7.75 – 45.01)	
Industry in the region	682	18.0	(10.0 – 26.0)	
University rectory	51	3.0	(1.76 – 6.01)	

Note: *Kruskal-Wallis test

Table 2. Antimicrobial-resistance profile of 16 *Klebsiella pneumoniae* carbapenemase-producing *Enterobacteriaceae* in sewage samples collected from different sampling sites of a tertiary hospital in Campo Grande.

Species	Sampling point*	Antibiotic resistance profile	Resistance phenotype	*bla* genes
Enterobacter cloacae complex	1	MER, IPM, ERT, AMI, AMC, AZT, CAZ, CPM, CRO, CIP, TZP, GEN, C/T	MDR	KPC
	1	MER, ERT, AMC, AZT, CAZ, CPM, CRO, CIP, TZP, GEN, C/T	MDR	KPC
	2	MER, IPM, ERT, AMC, AZT, CAZ, CPM, CRO, CIP, TZP, GEN, C/T	MDR	KPC
	3	MER, ERT, AMC, AZT, CRO, CIP, TZP, GEN	MDR	KPC
Klebsiella pneumoniae ssp pneumoniae	1	MER, ERT, AMC, AZT, CAZ, CPM, CRO, CIP, TZP, GEN, CZA, C/T	MDR	KPC
	3	MER, IPM, ERT, AMC, AZT, CAZ, CPM, CRO, CIP, TZP, C/T	MDR	KPC
	3	MER, ERT, AMI, AMC, AZT, CAZ, CRO, TZP, C/T	MDR	KPC
	3	MER, ERT, AMC, AZT, CAZ, CPM, CRO, CIP, TZP, CZA, C/T	MDR	KPC
	3	MER, ERT, AMI, AMC, CAZ, CRO,TZP, GEN, C/T	MDR	KPC
Klebsiella pneumoniae ssp ozaenae	3	MER, IPM, ERT, AMI, AMC, AZT, CPM, CRO, CIP, TZP, GEN	MDR	KPC
	2	MER, IPM, ERT, AMC, AZT, CAZ, CRO, TZP, C/T	MDR	KPC
Klebsiella oxytoca	3	MER, ERT, AMI, AMC, AZT, CAZ, CPM, CRO, CIP, TZP, GEN, CZA, C/T	MDR	KPC
	4	MER, IPM, ERT, AMC, CRO, TZP, GEN	MDR	KPC
Citrobacter farmeri	4	MER, ERT, AMC, AZT, CRO, CIP, TZP, GEN, C/T	MDR	KPC
	3	MER, IPM, ERT, AMI, AMC, AZT, CAZ, CPM, CRO, CIP, TZP, GEN, C/T	MDR	KPC
Kluyvera intermedia	2	MER, IPM, ERT, AMI, AMC, AZT, CAZ, CPM, CRO, CIP, TZP, GEN, C/T	MDR	KPC

Antibiotics: meropenem (MER), ertapenem (ERT), imipenem (IPM), amikacin (AMI), amoxicilin/clavulanic acid (AMC), aztreonam (AZT), ceftazidime (CAZ), cefepime (CPM), ceftriaxone (CRO), cefoxitin (CFO), ciprofloxacin (CIP), piperacilin/tazobactam (TZP), gentamicin (GEN), avibactam/ceftazidime (CZA) and ceftolozane tazobactam (C/T). *1 – Emergency Medical Care; 2 – Surgical Clinic; 3 – Sewer outlet; 4 – Coronary Care Unit/Medical clinic ward/Pediatric ward and Intensive Care Unit.

high mortality rates worldwide (Antimicrobial Resistance Collaborators, 2022; WHO, 2017). It is also a common group of bacteria found in hospital and non-hospital effluent (Gomes et al., 2022; Picão et al., 2013; Sudeep et al., 2024).

Several studies have investigated the role of hospital wastewater on the spread of antimicrobial resistance in aquatic environments (Amador et al., 2015; Batista et al., 2023; Conte et al., 2022; Chagas et al., 2011; Conte et al., 2017; Fouz et al., 2020; Fróes et al., 2016; Hassoun-Kheir et al., 2020; Jelic et al., 2019; Karungamye et al., 2023; Picão et al., 2013; Zagui et al., 2023).

In this study, the largest number of GNB CFUs was identified in samples collected upon discharge from the hospital (Table 1). However, one external location (a neighborhood far from the hospital) had an even higher number of CFUs/mL (n=1,445). This result may be related to anthropogenic activities in this neighborhood (Karkman et al., 2019), which is the one of the most populous in the city.

As observed in many studies, carbapenem-resistant bacteria are more abundant in hospital wastewater than in non-hospital effluent (Amador et al., 2015; Cahill et al., 2019; Hassoun-Kheir et al., 2020; Sudeep et al., 2024; Zagui et al., 2020). Considering that potent carbapenem antibiotics are used exclusively in hospitals, we expected a greater density of CRE in hospital sewage than in non-hospital sewage. This appears to be a direct consequence of inadequate sewage infrastructure that allows untreated sewage to be discharged into the environment.

In Brazil, most public hospitals still function in old buildings that do not have an adequate and efficient structure for the treatment of liquid effluents containing MDR bacteria (Dias et al., 2021). Moreover, more than 50% of the municipalities return untreated sewage to nature (Zagui et al., 2020).

Excessive and improper use of antibiotics in hospital environments contribute to the accumulation of antibiotic residues and ARB in wastewater systems (Al Salah et al., 2020; Amador et al., 2015; Batista et al., 2023; Fatimazahra et al., 2023; Fouz et al., 2020; Karungamye et al., 2023; Morris et al., 2016). Therefore, the presence of CRE in non-hospital

effluent indicates the dissemination of these bacteria to aquatic environments (Oliveira et al., 2023; Suzuki et al., 2020). The occurrence of CRE in non-hospital effluent is concerning as it is a risk factor for acquiring diseases that are difficult to treat (Santos et al., 2022; WHO, 2017).

In our study, among the MDR *Enterobacteriaceae* isolates, 12.5% strains were resistant to all antibiotics tested. The spread of these bacteria, therefore, has serious implications for public health. Identifying the dissemination of MDR bacteria in hospital effluent is important for implementing control measures. It is also important to highlight that the primary goal of urban wastewater treatment is the elimination of organic and inorganic contaminants, and not specifically the removal of antibiotic residues or ARB. In addition, ARB and ARG may be found in wastewater even after treatment (Batista et al., 2023; Karungamye et al., 2023; Oliveira et al., 2023; Picão et al., 2013; Ramalho et al., 2022).

Klebsiella pneumoniae and *Enterobacter cloacae* complex species were the most common MDR *Enterobacteriaceae* isolated from the hospital effluent in this study, which is consistent with the findings of other studies (Batista et al., 2023; Chagas et al., 2011; Picão et al., 2013; Suzuki et al., 2020; Thamlikitkul et al., 2019; Zagui et al., 2022). *K. pneumoniae* is an opportunistic MDR pathogen and a major cause of common hospital-acquired illnesses in many countries (Ferreira et al., 2019; Navon-Venezia et al., 2017; Santos et al., 2022). Corroborating with results of studies conducted in São Paulo (Zagui et al., 2022) and in Curitiba, Brazil (Conte et al., 2017), the MDR phenotype was attributed only to GNB isolated from hospital sewage.

Unlike observations from the southeastern region of Brazil (Batista et al., 2023; Zagui et al., 2022), we did not identify *E. coli* with the MDR phenotype in hospital sewage. This suggests that bacterial species in wastewater may vary according to the geographical region.

Unfortunately, we were unable to directly compare bacteria isolated from hospital effluent with those obtained from clinical samples. However, in an earlier study, a *Klebsiella pneumoniae* strain previously isolated from a patient's urinary catheter was found to be genetically identical to an isolate from wastewater at the entry point of a sewage treatment plant (Röderová et al., 2016), which suggests that the bacteria identified in hospital sewage in this study may have originated from patients with infections before disseminating through the environment.

Carbapenemases can be detected by phenotypic and genotypic tests. In this study, not all bacteria that grew on the chromogenic agar (which should be selective for bacteria resistant to carbapenem) were resistant to meropenem or ertapenem in the disk diffusion test. In contrast, the phenotypic test (modified carbapenem inactivation method) used to detect carbapenemase enzymes showed excellent performance compared to bla_{KPC} gene detection using PCR. Although the number of tested bacteria was small, this result is important because most routine hospital laboratories in developing countries, such as Brazil, do not have molecular techniques for detecting ARG.

Despite two decades of experience with KPC-producing bacteria, efforts to contain their global spread have failed. The β-lactamase genes have been widely distributed geographically and are endemic in several countries, including Brazil (Ferreira et al., 2019; Lee et al., 2016; Logan and Weinstein, 2017; Sampaio and Gales, 2016; Santos et al., 2022).

The presence of KPC-producing *Enterobacteriaceae* in hospital effluent has been described in several countries (Cahill et al., 2019; Hassoun-Kheir et al., 2020; Karungamye et al., 2023; Okafor and Nwodo, 2023), and the variations in AMR gene abundance have been found to strongly correlate with socioeconomic, health, and environmental factors (Fouz et al., 2020). As can be seen in Figure 2, genes that encode resistance to β-lactamases in *Enterobacteriaceae* isolated from hospital sewage have been described so far in the southeastern region of Brazil (Batista et al., 2023; Chagas et al., 2011; Froes et al., 2016;

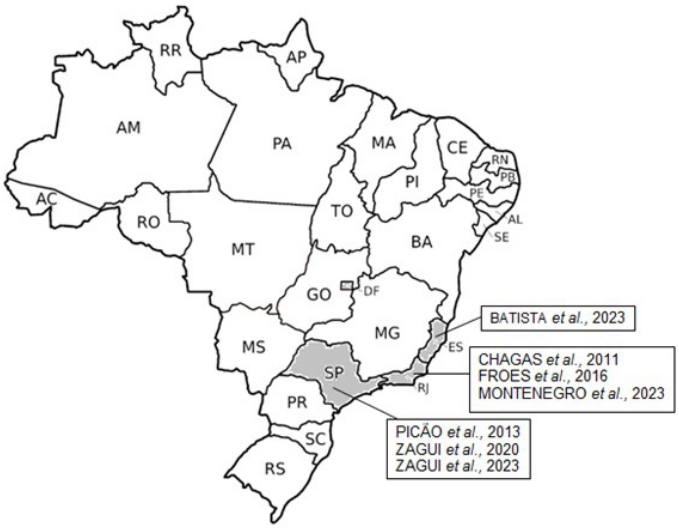

Figure 2. Distribution of studies identifying resistance β-lactamases genes in *Enterobacteriaceae* isolated from hospital sewage in Brazil, 2010–2023.

Montenegro et al., 2023; Picão et al., 2013; Zagui et al., 2020; Zagui et al., 2023).

Although *Enterobacteriaceae* species commonly occur in effluent, little is known about the existence of KPC-producing *Enterobacteriaceae* and their dissemination from hospital and non-hospital effluent in the midwest region of Brazil (Oliveira et al., 2023).

This is the first study documenting the presence of bla_{KPC} genes in GNB from hospital sewage in the midwest region of Brazil. In Goiânia, another city in the midwest region of Brazil, Resende et al. (2009) conducted a study on Gram-negative bacteria (GNB) resistant to antimicrobials in the effluent of 10 hospitals and at a sewage treatment plant. They did not find any extended-spectrum beta-lactamase or KPC-producing *Enterobacteriaceae*. On the other hand, bla_{KPC} genes were reported in MDR *Enterobacteriaceae* of non-hospital effluent (a stream) in the same city (Gomes et al., 2022).

Studies carried out in the capital of Brazil, Brasília, showed that clinically relevant GNB bearing $bla_{KPC\text{-}or\text{-}NDM}$ genes resist in Municipal sewage treatments and spread into environmental aquatic matrices (Pereira et al., 2022), and the occurrence of $bla_{KPC\text{-}or\text{-}NDM}$ carbapenem-resistant *Klebsiella pneumoniae* strains was positively associated with the number of hospitalized patients in the areas serviced by Municipal sewage treatment plants. Furthermore, high-risk clones, CC11-$bla_{KPC\text{-}2}$ and CC147-$bla_{NDM\text{-}1,}$ were detected in treated sewage (Oliveira et al., 2023).

Here, we showed that the main resistance gene ($bla_{KPC\text{-}2}$) involved in carbapenem resistance in clinics is present in hospital effluent. However, a limitation of this study is that only the bla_{KPC} gene was investigated. Further studies should, therefore, search for other carbapenem-resistance genes, such as bla_{IMP}, $bla_{NDM\text{-}1}$, $bla_{VIM,}$ and $bla_{OXA\text{-}48}$ genes (Cahill et al., 2019; Montenegro et al., 2023). In KPC-producing *Enterobacteriaceae* isolated from the sewage of hospitals, the presence of bla_{TEM}, $bla_{OXA\text{-}1like}$, $bla_{OXA\text{-}370}$, $bla_{NDM,}$ and bla_{KPC} genes has already been described in southeastern Brazil (Batista et al., 2023; Montenegro et al., 2023; Zagui et al., 2022).

Carbapenemase genes are higher in hospital wastewaters than in municipal sewage (Cahill et al., 2019; Hassoun-Kheir et al., 2020; Okafor and Nwodo, 2023). Studies have also reported their presence in river waters (Al Salah et al., 2020; Gomes et al., 2022; Jelić et al., 2019) and sewage treatment systems (Cahill et al., 2019; Pereira et al., 2022).

Nevertheless, our findings highlight the significance of hospital sewage as a potential reservoir for pathogenic bacteria, particularly those connected with the $bla_{KPC\text{-}2}$ gene, and their dissemination in aquatic environments close to hospitals. The results are concerning because carbapenem-resistant species, whether KPC-positive or -negative, fall within the critical priority group listed by the WHO (2017). These bacteria can cause various clinical manifestations, particularly in hospitalized patients and those requiring mechanical ventilation and catheters, thereby limiting therapeutic options. Moreover, by revealing the predominance of KPC-producing *Enterobacteriaceae* in this region of Brazil, this study has important implications for linking with other epidemiological studies, confirming the presence of this type of resistance, and highlighting the risk to public health. In an environmental context, the presence of bacteria carrying resistance genes highlights the risk of these genes transferring to other bacteria, causing imbalances in ecosystems. This research underscores the need to address the dissemination of potentially pathogenic and antibiotic-resistant microorganisms within the environment.

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