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Major Article

Evaluation of *in-vitro* susceptibility of ß-lactam-resistant Gram-negative bacilli to ceftazidime-avibactam and ceftolozane-tazobactam from clinical samples of a general hospital in southern Brazil

***Thaisa Noceti Carvalho*[1],[2], *Vanessa Cristine Kobs*[1], *Daniela Hille*[1], *Roseneide Campos Deglmann*[1], *Luiz Henrique Melo*[1],[2] *and Paulo Henrique Condeixa de França*[1]**

[1]. Universidade da Região de Joinville, Joinville, SC, Brasil.
[2]. Dona Helena Hospital, Joinville, SC, Brasil.

ABSTRACT

Background: The spread of carbapenemase- and extended-spectrum β-lactamase (ESBL)-producing gram-negative bacilli (GNB) represent a global public health threat that limits therapeutic options for hospitalized patients. This study aimed to evaluate the *in-vitro* susceptibility of β-lactam-resistant GNB to ceftazidime-avibactam (C/A) and ceftolozane-tazobactam (C/T), and investigate the molecular determinants of resistance.

Methods: Overall, 101 clinical isolates of Enterobacterales and *Pseudomonas aeruginosa* collected from a general hospital in Brazil were analyzed. Susceptibility to the antimicrobial agents was evaluated using an automated method, and the minimum inhibitory concentrations (MIC50/90) of C/A and C/T were determined using Etest®. The β-lactamase-encoding genes were investigated using polymerase chain reaction.

Results: High susceptibility to C/A and C/T was observed among ESBL-producing Enterobacterales (100% and 97.3% for CLSI and 83.8% for BRCAST, respectively) and carbapenem-resistant *P. aeruginosa* (92.3% and 87.2%, respectively). Carbapenemase-producing *Klebsiella pneumoniae* exhibited high resistance to C/T (80%- CLSI or 100%- BRCAST) but high susceptibility to C/A (93.4%). All carbapenem-resistant *K. pneumoniae* isolates were susceptible to C/A, whereas only one isolate was susceptible to C/T. Both antimicrobials were inactive against metallo-β-lactamase-producing *K. pneumoniae* isolates. Resistance genes were concomitantly identified in 44 (44.9%) isolates, with bla_{CTX-M} and bla_{SHV} being the most common.

Conclusions: C/A and C/T were active against microorganisms with β-lactam-resistant phenotypes, except when resistance was mediated by metallo-β-lactamases. Most C/A- and C/T-resistant isolates concomitantly carried two or more β-lactamase-encoding genes (62.5% and 77.4%, respectively).

Keywords: Antimicrobial resistance. Gram-negative bacilli. Ceftazidime-avibactam. Ceftolozane-tazobactam. *In vitro* activity. Genetic marker.

INTRODUCTION

Significant clinical and economic impacts are often reported because of bacterial resistance, since long hospital stays and the empirical use of different antimicrobial agents increase healthcare costs, as well as morbidity, and mortality rates[1]. The rapid spread of carbapenemase- and extended-spectrum β-lactamase (ESBL)-producing gram-negative bacilli (GNB) represents an important threat to global public health[2,3] and has limited the use of broad-spectrum cephalosporins and carbapenems in hospitalized patients[1,4].

In 2017, the World Health Organization (WHO) published a list of potentially critical multidrug-resistant microorganisms with global priority for the research and development of new

Corresponding author: Thaisa Noceti Carvalho. **e-mail:** thaisanoceti@gmail.com

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Conflict of interest: The authors declare that there is no conflict of interest.

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antimicrobials, including carbapenem-resistant *Pseudomonas aeruginosa* and Enterobacterales resistant to carbapenems and third-generation cephalosporins[5]. A few antimicrobial agents have been developed in recent years to combat infections caused by multidrug-resistant GNB[6,7].

Ceftazidime-avibactam and ceftolozane-tazobactam were approved by the Food and Drug Administration (FDA) and the Brazilian Health Regulatory Agency (ANVISA) for the treatment of complicated intra-abdominal and urinary infections[8,9]. Ceftazidime-avibactam exerts *in-vitro* activity against clinical ESBL-producing isolates, including Ambler classes A (serine carbapenemases [KPC]), C (cephalosporinase-AmpC), and some class D enzymes (oxacillinases), but not metallo-β-lactamases (MβL)[9,10]. With the addition of avibactam, a β-lactamase inhibitor, ceftazidime tends to expand its activity against resistant strains[6]. Ceftolozane-tazobactam, which is currently approved for the treatment of hospital-acquired and mechanical ventilation-associated bacterial pneumonia[11], is a combination of a fifth-generation cephalosporin and a known β-lactamase inhibitor. These agents together exert broad-spectrum activity against gram-negative bacteria, especially multidrug-resistant *P. aeruginosa*[12-15].

Although recently approved for clinical use and despite its proven efficacy against GNB, resistance to ceftazidime-avibactam and ceftolozane-tazobactam has been reported in several countries[16]. In this context, the present study evaluating the *in-vitro* activity of these antimicrobial agents, as well as genotypic resistance markers, is important for optimizing their use and will also contribute to the understanding of the current epidemiological scenario.

METHODS

Study characterization and selection of clinical isolates

This descriptive study focused on the phenotypic and molecular investigation of *P. aeruginosa* and Enterobacterales resistant to at least one carbapenem antibiotic or ESBL-producing antibiotic. The isolates were obtained sequentially from microbiological cultures of clinical samples collected from a general hospital in southern Brazil from January 2018. The clinical samples were subjected to routine procedures in the microbiology laboratory of the hospital for the identification of each microorganism, using the automated Microscan Walkaway Plus system (Beckman Coulter, USA) as well as Gram staining.

Phenotypic determination of antimicrobial susceptibility

The antimicrobial susceptibility profile was evaluated using the Kirby-Bauer disk diffusion method and the automated Microscan Walkaway Plus system (Beckman Coulter, USA) to determine the minimum inhibitory concentration (MIC) of each antimicrobial agent. Additionally, the MICs of ceftazidime-avibactam and ceftolozane-tazobactam were defined by a quantitative method using standardized Etest® strips that contained an exponential concentration gradient. The concentration range used for both antimicrobials was 0.016/4–256/4 mg/L and the results were interpreted using the parameters of the Clinical and Laboratory Standards Institute (CLSI) and of the Brazilian Committee on Antimicrobial Susceptibility Testing (BRCAST).

The isolates were classified as ESBL producers based on the observation of a reduction in the inhibition halos for broad-spectrum β-lactams in the antimicrobial susceptibility test and double-disk synergy test. Isolates were classified as resistant to carbapenems (CR) when resistance to meropenem, ertapenem, or imipenem was identified. The phenotypic detection of carbapenemases was performed using the enzymatic blocking method described in ANVISA Technical Note No. 01/2013[17], which provides prevention and control measures for infections caused by multidrug-resistant Enterobacterales.

Extraction of bacterial DNA and investigation of target genes

Bacterial DNA was extracted from Müller-Hinton agar cultures using heat shock, as previously described[18]. To confirm the suitability of the extracted DNA for subsequent genotype analysis, the 16S rRNA gene was identified by the polymerase chain reaction (PCR)[19].

The presence of the target genes was also investigated using PCR. All reactions were carried out in a final volume of 50 µL, using 50–500 ng of extracted DNA. The PCR-amplified products were subjected to electrophoresis on 1% agarose gel and compared with a standard.

The bla_{SHV} and bla_{CTX-M} genes were investigated in isolates with positive phenotypic tests for the presence of ESBL, using specific primers. The thermocycling conditions consisted of an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of denaturation, annealing, and extension (1 min at 72 °C) for each gene, and a final extension at 72 °C for 10 min.

To investigate the carbapenemase-encoding $bla_{OXA-48-like}$, bla_{NDM-1}, bla_{KPC}, bla_{SPM-1}, bla_{VIM}, and bla_{IMP} genes, isolates showing phenotypic resistance to carbapenems were subjected to PCR consisting of an initial denaturation at 94 °C for 3 min, followed by specific thermocycling conditions specific for each target gene. Reference strains were used to confirm the effectiveness of the target gene detection methods.

Statistical analysis

The samples were obtained using convenience sampling. Data were analyzed using descriptive statistics, with calculation of absolute and relative frequencies. Categorical variables are expressed as absolute numbers and percentages.

RESULTS

Overall, 101 bacterial isolates were included in the study: 39 (38.6%) carbapenem-resistant *P. aeruginosa*, 37 (36.6%) ESBL-producing Enterobacterales, 15 (14.8%) *Klebsiella pneumoniae* with a positive phenotypic test for KPC, four (4.0%) *K. pneumoniae* with a positive phenotypic test for MβL, three (3.0%) carbapenem-resistant isolates of the CESP group (consisting of *Citrobacter freundii*, *Enterobacter* spp., *Serratia* spp., *Providencia* spp., *Morganella morganii,* and *Hafnia alvei*), and three (3.0%) carbapenem-resistant *K. pneumoniae*.

The isolates were collected from urine samples (31.7%; n=32), rectal swabs (16.8%; n=17), wound discharge (15.8%; n=16), bronchoalveolar lavage (11.9%; n=12), and other less common sites (23.8%; n=24). Regarding the distribution of isolates among hospital units, 53.5% (n=54) were from in-patient units, 20.8% (n=21) from the intensive care unit (ICU), 19.8% (n=20) from the emergency department, and 5.9% (n=6) were isolated at the surgical center and from out-patient units. Thirty-seven (36.6%) of the 101 isolates were obtained from surveillance cultures.

In-vitro activity of ceftolozane-tazobactam

The *in- vitro* activity of ceftolozane-tazobactam against each group of microorganisms selected in this study is shown in **Table 1**. The ESBL-producing Enterobacterales and CR *P. aeruginosa* isolates showed high susceptibility. A high resistance rate was observed in the KPC-producing *K. pneumoniae* isolates. Two CR *K. pneumoniae* isolates were resistant. All CR isolates of the CESP group and MβL-producing *K. pneumoniae* were resistant to ceftolozane-tazobactam.

In-vitro activity of ceftazidime-avibactam

Table 2 shows the *in-vitro* activity of ceftazidime-avibactam against each group of microorganisms selected in this study. All ESBL-producing Enterobacterales isolates were susceptible to ceftazidime-avibactam, showing the lowest MIC values compared with the other groups of microorganisms tested. A high susceptibility rate was observed for CR *P. aeruginosa*.

The resistance rate of KPC-producing *K. pneumoniae* was low. All CR *K. pneumoniae* isolates were susceptible, whereas the MβL-producing *K. pneumoniae* isolates were resistant. Two bacterial isolates from the CESP group (*E. cloacae* and *S. marcescens*) were susceptible, and one was resistant to ceftazidime-avibactam.

Phenotypic antimicrobial susceptibility

Figure 1 summarizes the comparison of ceftazidime-avibactam and ceftolozane-tazobactam susceptibility profiles with the other antimicrobials tested in the different groups of microorganisms studied according to the CLSI breakpoints.

Genotypic resistance markers

The 16S rRNA gene was amplified in most of the isolates studied (97%, 98/101). The percentage of isolates positive for resistance genes was high (78.6%). **Table 3** shows the distribution of the bacterial isolates according to the investigated genotypic resistance markers. All the tested isolates were negative for bla_{SPM-1}, $bla_{OXA-48-like}$, and bla_{IMP}.

Coexistence of resistance genes was observed in 44 (44.9%) isolates. The most prevalent combinations were bla_{KPC}+bla_{CTX-M}+bla_{SHV} in KPC-producing *K. pneumoniae*, and bla_{CTX-M}+bla_{SHV} in ESBL-producing *Escherichia coli*. Most isolates with phenotypic resistance to ceftazidime-avibactam and ceftolozane-tazobactam concomitantly carried two or more β-lactamase-encoding genes (**Table 4**). The bla_{CTX-M}, bla_{SHV}, and bla_{KPC} were most frequently detected in isolates resistant to ceftolozane-tazobactam, whereas bla_{SHV}, bla_{CTX-M}, and bla_{NDM-1} were most frequently detected in isolates resistant to ceftazidime-avibactam.

DISCUSSION

The increasing incidence of bacterial strains isolated from clinical samples that produce carbapenemases, enzymes capable of inactivating carbapenems, and most β-lactams[20] represents the greatest challenge of antibiotic therapy in recent years[2,6]. Enterobacterales and *P. aeruginosa* are the main causative agents of severe infections associated with antibiotic resistance resulting from chromosomal mutations and the transfer of plasmid-mediated resistance[21]. Such clinical isolates commonly produce carbapenemases and exhibit multidrug resistance and pan-resistance phenotypes[22].

TABLE 1: *In vitro* activity of ceftolozane-tazobactam.

Group of microorganisms (N)	MIC frequency (%)											MIC interpretation (%)						MIC (mg/L)		
												CLSI			BRCAST					
	<1	1	2	3	4	6	8	12	24	32	48	>256	S	I	R	S	I	R	MIC 50	MIC 90	Range
Pseudomonas aeruginosa - CR (39)	15.4	46.2	56.4	66.7	87.2	92.3	94.9			97.4		100	87.2	7.7	5.1	87.2		12.8	2	6	0.38 - >256
Enterobacterales - ESBL (37)	67.6	83.8	97.3									100	97.3		2.7	83.8		16.2	<1	2	0.125 - >256
Klebsiella pneumoniae - KPC (15)			13.3		20.0		26.7	33.3	53.3	60.0	86.7	100	13.3	6.6	80.0			100	24	>256	2,0 - >256
Klebsiella pneumoniae - MβL (4)												100			100			100	>256	>256	>256 - >256
Klebsiella pneumoniae – CR (3)	33.3								66.7		100		33.3		66.6	33.3		66.6	24	48	0.50 - 48
CESP group - CR (3)							33.3					100			100			100	>256	>256	8 - >256

MIC: minimum inhibitory concentration; **S:** susceptible; **I:** intermediate; **R:** resistant; **MIC50 and MIC90 (mg/L):** concentrations that inhibit 50% and 90% of the bacterial isolates, respectively. **CLSI:** Clinical and Laboratory Standards Institute; **BRCAST:** Brazilian Committee on Antimicrobial Susceptibility Testing. **CR:** resistance to at least one carbapenem; **ESBL:** extended-spectrum β-lactamase; **KPC:** *Klebsiella pneumoniae* carbapenemase; **MβL:** metallo-β-lactamase.

TABLE 2: *In vitro* activity of ceftazidime-avibactam.

Group of microorganisms (N)	MIC frequency (%)									MIC interpretation (%)			MIC (mg/L)		
										CLSI and BRCAST					
	<1	1	2	3	4	6	8	12	24	>256	S	I	R	MIC 50	MIC 90	Range
Pseudomonas aeruginosa - CR (39)	2.6	33.3	43.6	69.2	76.9	89.7	92.3	94.9	97.4	100	92.3		7.7	3	8	0.50 - >256
Enterobacterales - ESBL (37)	89.2	97.3		100							100			<1	1	0.125 - 3
Klebsiella pneumoniae - KPC (15)	26.7	86.7	93.3							100	93.3		6.6	1	2	0.38 - >256
Klebsiella pneumoniae - MβL (4)										100			100	>256	>256	>256 - >256
Klebsiella pneumoniae - CR (3)	33.3		66.7		100						100			2	4	0.75 - 4
CESP group - CR (3)		33.3			66.7			100			66.7		33.3	4	12	1 - 12

MIC: minimum inhibitory concentration; **S:** susceptible; **I:** intermediate; **R:** resistant; **MIC50 and MIC90 (mg/L):** concentrations that inhibit 50% and 90% of the bacterial isolates, respectively. **CLSI:** Clinical and Laboratory Standards Institute; **BRCAST:** Brazilian Committee on Antimicrobial Susceptibility Testing. **CR:** resistance to at least one carbapenem; **ESBL:** extended-spectrum β-lactamase; **KPC:** *Klebsiella pneumoniae* carbapenemase; **MβL:** metallo-β-lactamase.

Studies have been conducted in different countries to evaluate *in-vitro* susceptibility to ceftazidime-avibactam and ceftolozane-tazobactam, and bacterial resistance to these antimicrobial agents has been reported in hospitalized patients with or without previous treatment[16]. Furthermore, the combination of resistance mechanisms can significantly increase the MIC of ceftazidime-avibactam and ceftolozane-tazobactam[16,23].

The ability of ceftazidime-avibactam to inhibit KPC-type β-lactamases has attracted global interest. In China, Cui et al. evaluated 347 KPC-producing *K. pneumoniae* isolates collected from patients without previous treatment with only 12 (3.5%) isolates showing reduced susceptibility to ceftazidime-avibactam[24]. In Brazil, Rossi et al. found that among 30 selected *K. pneumoniae* isolates that were not susceptible to meropenem and positive for bla_{KPC}, only one (3.3%) was resistant to ceftazidime combined with avibactam[9]. Similarly, in our study, the presence of bla_{KPC} did not influence ceftazidime-avibactam susceptibility, and the resistance rate observed (6.6%; 1/15) was consistent with the global surveillance results of carbapenem-resistant and bla_{KPC}-carrying *K. pneumoniae*[10].

Jonge et al. characterized the *in vitro* activity of ceftazidime-avibactam against 961 meropenem-non-susceptible Enterobacterales isolates from Europe, Asia, Latin America, and the Middle East using a global antimicrobial resistance surveillance program. The authors evaluated 145 MβL-producing isolates and detected a ceftazidime-avibactam resistance rate of 96.6%[10]. Similarly, all MβL-producing *K. pneumoniae* isolates in this study were resistant to ceftazidime-avibactam. Thus, ceftazidime-avibactam is a potent agent against carbapenem-resistant Enterobacterales, except for isolates in which resistance is mediated by MβL.

According to international reports, the resistance rates of *P. aeruginosa* to ceftazidime-avibactam are higher than those reported for Enterobacterales, ranging from 2.9 to 18%, whereas these rates can reach 50% in isolates resistant to carbapenems[16]. Although this study investigated carbapenem-resistant *P. aeruginosa* isolates, the rate of ceftazidime-avibactam resistance was low (7.7%; 3/39). This might be related to the absence of carbapenemase-encoding genes in most of the isolates investigated, suggesting that the detected phenotypic resistance to carbapenems is associated with other pseudomonal resistance mechanisms not analyzed here/in this study.

Studies have reported that bacteremia caused by ESBL-producing Enterobacterales is associated with higher rates of treatment failure and patient mortality when compared to bacteremia caused by non ESBL-producing strains[1,25]. Various authors have emphasized that ESBL-producing Enterobacterales strains are not associated with resistance to ceftazidime-avibactam[6,7,10,15,25,26], which was also demonstrated in our study.

López-Calleja et al. analyzed the multidrug-resistant and extensively drug-resistant non-MβL-producing *P. aeruginosa* isolates collected in Spain and reported 92.2% susceptibility to ceftolozane-tazobactam, which was the second most active antimicrobial agent after colistin[27]; this was also observed in our study (87.2%). In particular, we did not identify bla_{NDM-1} or bla_{IMP} genes in carbapenem-resistant *P. aeruginosa* isolates, and the bla_{VIM} gene detected in only one isolate was not associated with the ceftolozane-tazobactam resistance phenotype. In contrast, Teo et al. highlighted the importance of geographic variation in antimicrobial activity. The authors reported much lower

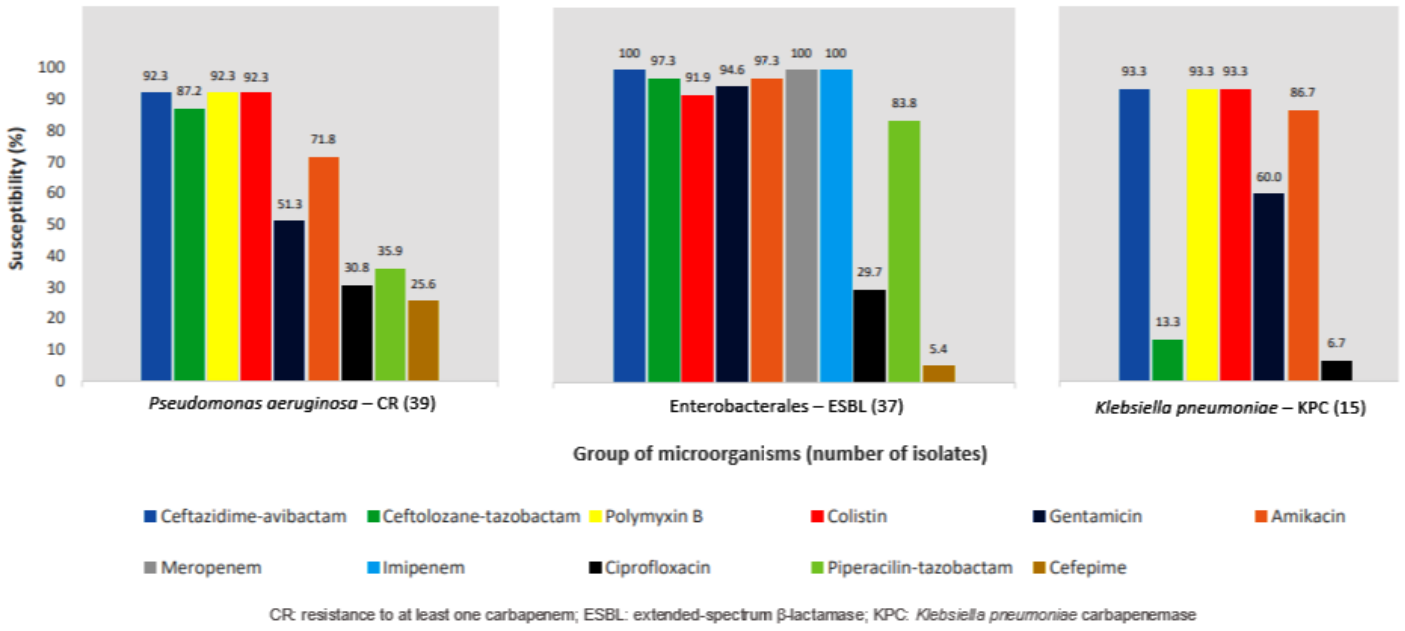

FIGURE 1: Susceptibility to ceftazidime-avibactam and ceftolozane-tazobactam compared to the other antimicrobials tested in the study.

TABLE 3: Distribution of bacterial isolates according to genotypic resistance markers.

Phenotypic resistance	Species (N)	β-Lactamase genes	Isolates (n)	Isolates (%)
ESBL	*Escherichia coli* (27)	bla_{CTX-M}	26	96.3
		bla_{SHV}	17	63
	Klebsiella pneumoniae (7)	bla_{CTX-M}	7	100
		bla_{SHV}	6	85.7
	Proteus mirabilis (2)	bla_{CTX-M}	2	100
	Klebsiella ozaenae (1)	bla_{SHV}	1	100
		bla_{CTX-M}	1	100
KPC	*Klebsiella pneumoniae* (15)	bla_{KPC}	15	100
		bla_{SHV}	14	93.3
		bla_{CTX-M}	12	80
		bla_{NDM-1}	1	6.7
		bla_{VIM}	1	6.7
CR	*Pseudomonas aeruginosa* (36)	bla_{CTX-M}	14	38.9
		bla_{SHV}	5	13.9
		bla_{KPC}	2	5.6
		bla_{VIM}	1	2.8
	Klebsiella pneumoniae (3)	bla_{CTX-M}	3	100
		bla_{SHV}	3	100
	Klebsiella pneumoniae (3)	bla_{NDM-1}	1	33.3
	Enterobacter cloacae (2)	bla_{CTX-M}	1	50
	Serratia marcescens (1)	bla_{KPC}	1	100
MβL	*Klebsiella pneumoniae* (4)	bla_{SHV}	4	100
		bla_{NDM-1}	3	75

ESBL: extended-spectrum β-lactamase; **KPC:** *Klebsiella pneumoniae* carbapenemase; **CR:** resistance to at least one carbapenem; **MβL:** metallo-β-lactamase.

TABLE 4: Presence of β-lactamase-encoding genes and phenotypic susceptibility to ceftazidime-avibactam (C/A) and ceftolozane-tazobactam (C/T) in the isolates studied.

Phenotypic resistance	Species	Isolates (n)	β-Lactamase genes	Phenotype	
				C/A	C/T
ESBL	*Escherichia coli*	15	bla_{CTX-M}, bla_{SHV}	S	S
		10	bla_{CTX-M}	S	S
		1	bla_{CTX-M}, bla_{SHV}	S	R
		1	bla_{SHV}	S	S
	Klebsiella pneumoniae	5	bla_{CTX-M}	S	S
		1	bla_{CTX-M}, bla_{SHV}	S	R
		1	bla_{CTX-M}	S	R
	Proteus mirabilis	2	bla_{CTX-M}	S	R
	Klebsiella ozaenae	1	bla_{CTX-M}, bla_{SHV}	S	R
KPC	*Klebsiella pneumoniae*	9	bla_{KPC}, bla_{CTX-M}, bla_{SHV}	S	R
		2	bla_{KPC}, bla_{SHV}	S	R
		1	bla_{KPC}, bla_{CTX-M}, bla_{SHV}, bla_{NDM-1}	S	R
		1	bla_{KPC}, bla_{CTX-M}, bla_{SHV}, bla_{VIM}	S	R
		1	bla_{KPC}, bla_{CTX-M}	S	R
		1	bla_{KPC}, bla_{SHV}	R	R
CR	*Pseudomonas aeruginosa*	7	bla_{CTX-M}	S	S
		2	bla_{CTX-M}, bla_{SHV}	S	S
		1	bla_{KPC}, bla_{CTX-M}	S	S
		1	bla_{KPC}, bla_{CTX-M}, bla_{SHV}	S	S
		1	bla_{SHV}	S	S
		1	bla_{VIM}	S	S
		1	bla_{CTX-M}	R	S
		1	bla_{CTX-M}	S	R
		1	bla_{CTX-M}, bla_{SHV}	R	R
	Klebsiella pneumoniae	2	bla_{CTX-M}, bla_{SHV}	S	R
		1	bla_{CTX-M}, bla_{SHV}, bla_{NDM-1}	S	S
	Enterobacter cloacae	1	bla_{CTX-M}	R	R
	Serratia marcescens	1	bla_{KPC}	S	R
MβL	*Klebsiella pneumoniae*	3	bla_{SHV}, bla_{NDM-1}	R	R
		1	bla_{SHV}	R	R

ESBL: extended-spectrum β-lactamase; **KPC:** *Klebsiella pneumoniae* carbapenemase; **CR:** resistance to at least one carbapenem; **MβL:** metallo-β-lactamase. **C/A:** ceftazidime-avibactam; **C/T:** ceftolozane-tazobactam. **R:** resistant; **S:** susceptible.

susceptibility rates of *P. aeruginosa* to ceftolozane-tazobactam (37.9%), which was associated with the presence of MβL, compatible with local molecular epidemiology[28].

Tuon et al. evaluated 673 GNB isolates collected from different Brazilian centers and found rates of *in-vitro* susceptibility to ceftolozane-tazobactam ranging from 40.4% to 94.9%. The susceptibility rate of *K. pneumoniae* to ceftolozane-tazobactam was low (40.4%) because of the high incidence of KPC-type carbapenemases in Brazil, an enzyme that catalyzes the hydrolysis of ceftolozane[29]. In our study, the susceptibility rate of KPC-producing *K. pneumoniae* to ceftolozane-tazobactam was even lower (13.3%). This finding might be explained by the identification of bla_{KPC} gene in all isolates tested and the concomitant presence of ESBL-encoding genes. Additionally, two isolates carrying more than one carbapenemase- and ESBL-encoding gene (bla_{KPC}+bla_{CTX-M}+bla_{SHV}+bla_{NDM-1} and bla_{KPC}+bla_{CTX-M}+bla_{SHV}+bla_{VIM}) were identified in association with ceftolozane-tazobactam resistance phenotypes. Regarding MβL-producing *K. pneumoniae*, all isolates were resistant to ceftolozane-tazobactam and most of them carried bla_{NDM-1}, suggesting that this agent should be used with caution in empirical therapies.

In contrast, we found high rates of *in-vitro* ceftolozane-tazobactam susceptibility among ESBL-producing Enterobacterales. This finding in consistent with a study that evaluated 21,952 Enterobacterales isolates from 51 countries and found that ceftolozane-tazobactam inhibited 82.4% of ESBL-producing isolates[15].

This study had some limitations. The number of isolates evaluated was relatively small, and the study was conducted at a single hospital. However, the study used clinical isolates selected in recent years to better reflect the current epidemiological scenario. Therefore, we recommend multicenter studies using a phenotypic

and genotypic approach and a greater number of multidrug-resistant isolates for better understanding of the local molecular epidemiology and for the detection of resistance to ceftazidime-avibactam and ceftolozane-tazobactam in different regions of Brazil.

In conclusion, we found high susceptibility rates to ceftazidime-avibactam and ceftolozane-tazobactam among ESBL-producing Enterobacterales and carbapenem-resistant *Pseudomonas aeruginosa*. In contrast, carbapenemase-producing *Klebsiella pneumoniae* exhibited high resistance to ceftolozane-tazobactam but high susceptibility to ceftazidime-avibactam. Our results obtained *in-vitro* confirmed that ceftazidime-avibactam and ceftolozane-tazobactam were active against microorganisms with β-lactam resistance phenotypes, except when resistance was mediated by metallo-β-lactamases. Additionally, most ceftazidime-avibactam- and ceftolozane-tazobactam-resistant isolates concomitantly carried two or more β-lactamase-encoding genes.

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