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Original article

Activity of ceftolozane-tazobactam and comparators against gram-negative bacilli: Results from the study for monitoring antimicrobial resistance trends (SMART – Brazil; 2016–2017)

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ABSTRACT

Multi-drug resistant Gram-negative bacilli (GNB) have been reported as cause of serious hospital-acquired infections worldwide. The aim of this study was to investigate the *in vitro* activity of ceftolozane-tazobactam compared to other agents against GNB isolated from patients admitted to Brazilian medical centers between the years 2016 and 2017. Presence of β-lactamase encoding genes was also evaluated.

Methods: Antimicrobial susceptibility testing of GNB isolated from intra-abdominal (IAI), respiratory (RTI), and urinary tract infections (UTI) was performed according to ISO 227-1 guidelines and interpreted following CLSI and BrCAST/EUCAST guidelines. Qualifying Enterobacteriaceae isolates were screened for the presence of β-lactamase genes by PCR followed by DNA sequencing.

Results: 1748 GNB collected from UTI (45.2%), IAI (25.7%) and RTI (29.1%) were evaluated. Ceftolozane-tazobactam remained highly active (94.7%) against *E. coli* isolates. Among *K. pneumoniae*, susceptibility rates were 85.9% and 85.4% for amikacin and colistin, whereas ceftolozane-tazobactam (44.1% susceptible) and carbapenems (55.2-62.2% susceptible) showed poor activity due to bla_{KPC-2}. Against *E. cloacae* amikacin, imipenem, and meropenem retained good activity (>90%). Ceftolozane-tazobactam was the most potent β-lactam agent tested against *P. aeruginosa* (90.9% susceptible), including ceftazidime and imipenem resistant isolates. β-lactamase encoding genes testing was carried out in 433 isolates. bla_{CTX-M} variants were predominant in *E. coli*, *P. mirabilis* and *E. cloacae*. Among the *K. pneumoniae molecularly tested*, most carried bla_{KPC} (68.5%), with all harboring $bla_{KPC-2,}$ except two isolates carrying bla_{KPC-3} or bla_{KPC-30}. ESBL encoding genes, mainly CTX-M family, were frequently detected in *K. pneumoniae*, plasmid-mediated AmpC were rare. A variety of PDC encoding genes were detected in *P. aeruginosa* isolates with five isolates harboring MBL and one KPC encoding genes.

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Conclusion: Ceftolozane-tazobactam was very active against *E. coli*, *P. mirabilis* and *P. aeruginosa* isolates and could constitute an excellent therapeutic option including for those isolates resistant to extended-spectrum cephalosporins and carbapenems but not producers of carbapenemases.

Introduction

In 2019, the World Health Organization has identified antimicrobial resistance as one of the world's top 10 global health threats[1] https://www.who.int/emergencies/ten-threats-to-global-health-in-2019. The U.S. Centers for Disease Control and Prevention (CDC) has recently reported that more than 2.8 million antibiotic-resistant infections occur in the United States each year.[2] Although antimicrobial resistance varies widely depending on the bacterial species, antimicrobial agent and geographical region, high levels of antimicrobial resistance for several bacterial species–antimicrobial combinations have been also observed among invasive isolates reported to the European Antimicrobial Resistance Surveillance Network (EARS-Net).[3] Unfortunately, Gram-negative bacilli (GNB) exhibiting resistance to multiple antimicrobial agents have been reported as important causes of serious hospital acquired infections in Brazilian hospitals.[4] Carbapenems used to be considered drugs of choice for treatment of multidrug resistant GNB[5]; however, the emergence and spread of carbapenemase encoding genes carried by mobile genetic elements has jeopardized the clinical usefulness of this important therapeutic class of antimicrobials.[5] The last report of the Brazilian Health Surveillance Agency estimated that 44.1% of *Klebsiella pneumoniae* and 42.9% of *Pseudomonas aeruginosa* isolates causing catheter-related bloodstream infections in ICU adult patients were resistant to carbapenems.[4] Although many Brazilian medical centers have been aware of their carbapenem resistance rates, the underlying mechanisms of carbapenem resistance are largely unknown.

The Study for Monitoring Antimicrobial Resistance Trends (SMART) program has generated data on the frequency of antimicrobial susceptibility of GNB associated with urinary tract (UTI), intra-abdominal (IAI) and respiratory tract (RTI) infections worldwide since 2008. The principal aim of this study was to determine the frequency of pathogens and *in vitro* activity of ceftolozane-tazobactam compared to other agents against the most frequently identified GNB isolated from patients admitted to Brazilian medical centers between the years 2016 and 2017. The presence of β-lactamase encoding genes, the main mechanism of β-lactam resistance, was also determined for selected isolates.

Methods

Bacterial isolates

Non-duplicate GNB isolates were collected consecutively from eight Brazilian medical centers between January 1, 2016, and December 31, 2017. The participating medical centers were located in the Brazilian cities of Rio de Janeiro (one center), Salvador (one center), and São Paulo (six centers). Each participating medical center collected up to 100 consecutive GNB isolated from patients with intra-abdominal (IAI) and respiratory (RTI) infections, and 50 GNB from urinary-tract infections (UTI) per year. GNB were identified at the species level at the respective participant medical center and shipped to a central microbiology laboratory (International Health Management Associates, IHMA, Schaumburg, IL, USA), where confirmation of bacterial species, antimicrobial susceptibility testing, and molecular characterization of β-lactamase encoding genes were carried out. Bacterial identification at the species level was confirmed for all isolates using MALDI-TOF spectrometry (Bruker Daltonics, Billerica, MA, USA).

Susceptibility testing

Antimicrobial susceptibility testing for amikacin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftolozane-tazobactam, ceftriaxone, ciprofloxacin, colistin, ertapenem, imipenem, meropenem, and piperacillin-tazobactam was determined by testing customized MicroScan dehydrated broth microdilution panels (Siemens Medical Solutions Diagnostics, West Sacramento, CA, USA) according to the ISO 227-1 guidelines and interpreted following both CLSI[7] and BrCAST/EUCAST[8,9] guidelines. Quality control of broth microdilution panels followed the manufacturer's and CLSI guidelines using the following ATCC strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *K. pneumoniae* ATCC 700603. Corresponding QC values tested were within the acceptable ranges as specified by CLSI. *E. coli* and *Klebsiella pneumoniae* isolates with MICs ≥2 μg/mL for ceftazidime, ceftriaxone, or aztreonam were screened as "ESBL phenotype". Enterobacteriaceae with MIC ≥ 4 μg/mL for imipenem and/or meropenem were defined as carbapenem resistant. *P. aeruginosa* isolates having MICs >8 μg/mL and >2 μg/mL, respectively, were classified as not susceptible (NS) to ceftazidime and meropenem.

Molecular characterization of β-lactamase encoding genes

All *Enterobacteriaceae* with MICs ≥4 mg/L for ceftolozane-tazobactam and/or ≥1 mg/L for ertapenem (except Proteeae *Enterobacteriaceae*) and/or ≥2 mg/L for imipenem were selected for characterization of β-lactamase content as well as *P. aeruginosa* isolates displaying ceftolozane-tazobactam MICs ≥8 mg/L and/or imipenem MICs ≥4 mg/L. For comparison reasons, 50% of the *E. coli* and *K. pneumoniae* possessing the ESBL phenotype but showing susceptibility to ertapenem, imipenem,

and ceftolozane-tazobactam were also selected for molecular characterization of β-lactamase encoding genes. Qualifying Enterobacteriaceae isolates were screened for the presence of β-lactamase genes (*bla*) encoding extended spectrum-β-lactamases (ESBLs; TEM, SHV, CTX-M, VEB, PER, GES), AmpC β-lactamases (ACC, ACT, CMY, DHA, FOX, MIR, MOX), and carbapenemases (KPC, OXA-48-like, IMP, VIM, NDM, SPM, and GIM) by multiplex PCR as described previously.[8] Limited sequencing was performed on bla_{TEM} and bla_{SHV} to identify genes encoding TEM-type and SHV-type enzymes containing amino acid substitutions common to ESBLs (SHV A146 V, G238S, G238A, E240 K; TEM E104 K, R164S, R164C, R164H, G238S). All detected genes, excluding bla_{SHV} and bla_{TEM} that did not encode ESBLs and the intrinsic, chromosomally coded bla_{AmpC} of *Citrobacter* spp. ($bla_{CMY\text{-}type}$) and *Enterobacter* spp. ($bla_{ACT\text{-}type}$ and $bla_{MIR\text{-}type}$), were amplified and sequenced in their entirety. Qualifying *P. aeruginosa* isolates were screened for the presence of *bla* encoding ESBLs (TEM, SHV, CTX-M, VEB, PER, GES), plasmid-encoded AmpC β-lactamases (ACC, ACT, CMY, DHA, FOX, MIR, MOX), and carbapenemases (KPC, OXA-24-like, IMP, VIM, NDM, SPM, and GIM) by multiplex PCR as described. All detected genes, as well as the chromosomally encoded *Pseudomonas*-derived cephalosporinase bla_{AmpC} (PDC) common to the species, were amplified and sequenced in their entirety.

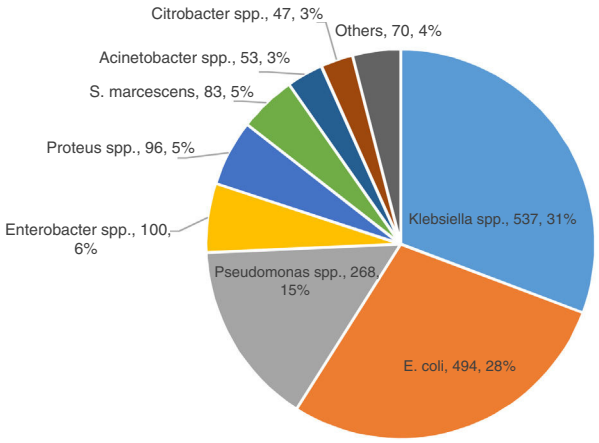

Fig. 1 – Distribution of isolates according to the bacterial species collected from participating Brazilian medical centers of the SMART Program (Brazil, 2016-2017).
a. *Acinetobacter* spp. (53): *A. baumannii* (49), *A. ursingii* (1), *A. guillouiae* (1), *A. nosocomialis* (1), and *A. pittii* (1);
b. Citrobacter spp. (47): *C. amalonaticus* (1), *C. farmeri* (3), *C. freundii* (30), and *C. koseri* (13);
c. *Enterobacter* spp. (100): *E. asburiae* (13), *E. cloacae* (81), *E. kobei* (5), and *Enterobacter* spp. (1);
d. *Klebsiella* spp. (537): *K. aerogenes* (65), *K. oxytoca* (19), *K. pneumoniae* (431), and *K. variicola* (22);
e. *Proteus* spp. (96): *P. hauseri* (2), *P. mirabilis* (91), and *P. vulgaris* (3);
f. *Pseudomonas* spp. (268): *P. aeruginosa* (265), *P. mosselii* (1), *P. otitidis* (1), and *P. putida* (1);
g. Others (70): *Achromobacter xylosoxidans* (5), *Aeromonas caviae* (1), *Aeromonas hydrophila* (1), *Burkholderia cenocepacia* (3), *Escherichia hermanii* (1), *Morganella morganii* (20), *Pluralibacter gergoviae* (2), *Providencia alcalifaciens* (1), *Providencoa rettgeri* (4), *Providencia stuartii* (6), *Raoultella ornithinolytica* (1), and *Raoutella planticola* (1), *Salmonella* spp. (2), *Stenotrophomonas maltophilia* (22).

Results

A total of 1,748 GNB were collected at the eight Brazilian medical centers between the years 2016 (N = 776) and 2017 (N = 972). Most isolates were recovered from female patients (51%) of all

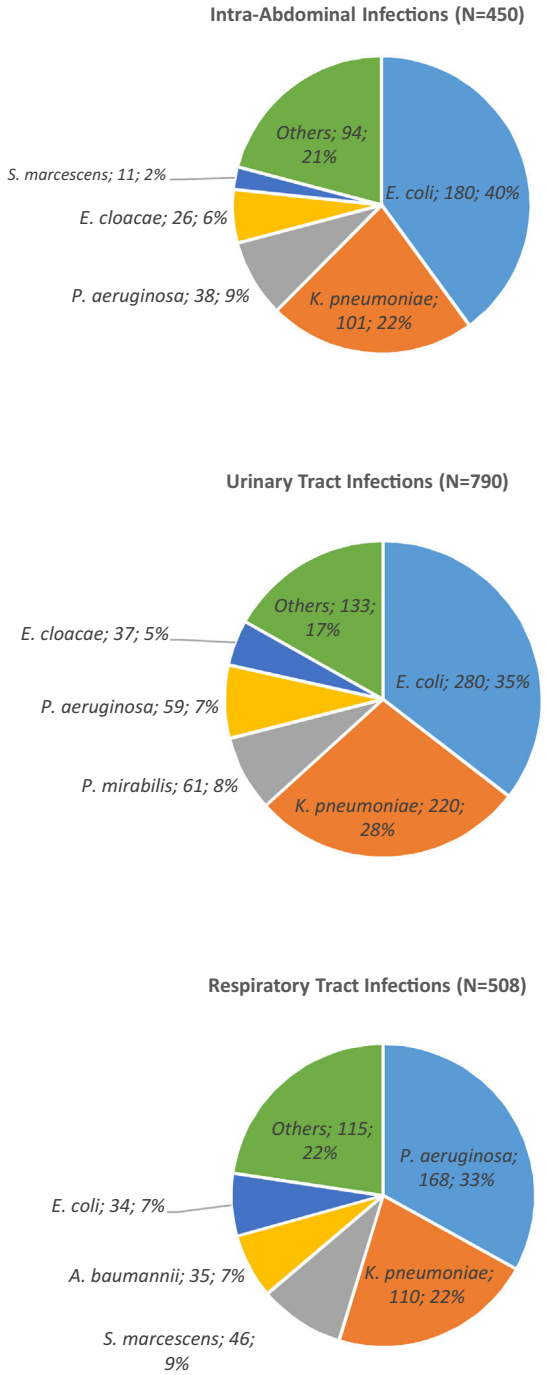

Fig. 2 – Distribution of bacterial species according to the type of infection (SMART Program- Brazil, 2016-2017).

ages. Approximately 77.9% (1362 of 1748) of GNB belonged to five bacterial species as depicted in Fig. 1. The distribution of the five most relevant bacterial species according to the body site of infection is shown in Fig. 2. Most of these pathogens were collected from urinary tract (45.2%), followed by respiratory (29.1%), and intra-abdominal infections (25.7%). In general, among all species isolated, *E. coli* (n = 494) and *K. pneumoniae* (n = 431) were the most frequently identified species from all sources of infection. While *E. cloacae*, *E. coli*, *K. pneumoniae*, and *P. mirabilis* were more frequently isolated from urine tract infections, *P. aeruginosa* (33.0%) was the most frequent species recovered from respiratory tract infections, followed by *K. pneumoniae* (23.0%), *S. marcescens* (9%), and *A. baumannii* (7%). *E. coli* (40.0%) was the most frequent species isolated from patients with intra-abdominal infections followed by *K. pneumoniae* (22.0%) as depicted in Fig. 2.

Antimicrobial susceptibility

Antimicrobial susceptibility profiles of the five most relevant pathogens causing infections in the participating Brazilian medical centers is shown in Table 1. For comparison purposes the susceptibility and resistance rates according to the CLSI and EUCAST breakpoints are shown in Table 1; however, since BrCAST/EUCAST criteria have been recommended by the Brazilian Ministry of Health,[10] the BrCAST/EUCAST susceptibility/resistance rates were considered when evaluating the antimicrobial activity of the respective antimicrobial agents, when these percentages varied.

Colistin ($MIC_{50/90}$, ≤1/≤1 μg/mL; 99.4% susceptible) and imipenem ($MIC_{50/90}$, ≤0.5/≤0.5 μg/mL; 99.4% susceptible) were the most *in vitro* active agents tested against the 494 *E. coli* isolates, followed by meropenem ($MIC_{50/90}$, ≤0.12/≤0.12 μg/mL; 99.0% susceptible), ertapenem ($MIC_{50/90}$, ≤0.06/≤0.06 μg/mL; 97.8% susceptible), and amikacin ($MIC_{50/90}$, ≤4/8 μg/mL; 97.4% susceptible). In contrast, the lowest susceptibility rates were observed for ciprofloxacin ($MIC_{50/90}$, ≤0.25/>2 μg/mL; 55.1% susceptible), followed by aztreonam ($MIC_{50/90}$, ≤1/>16 μg/mL; 71.5% susceptible), ceftriaxone ($MIC_{50/90}$, ≤1/> 32 μg/mL; 71.7% susceptible), cefepime ($MIC_{50/90}$, ≤1/>32 μg/mL, 73.3% susceptible), and ceftazidime ($MIC_{50/90}$, ≤1/>16 μg/mL, 78.1% susceptible). Ceftolozane-tazobactam ($MIC_{50/90}$, 0.25/0.5 μg/mL) remained highly active against *E. coli* isolates, 94.7% of which were susceptible to this agent, including 87.2% of 102 *E. coli* isolates exhibiting the ESBL phenotype as shown in Table 2.

Among the 431 *K. pneumoniae* evaluated in this study, the highest susceptibility rates were observed for amikacin ($MIC_{50/90}$, ≤4/32 μg/mL, 85.9% susceptible) and colistin ($MIC_{50/90}$, ≤1/>4 μg/mL, 85.4% susceptible). Ceftolozane-tazobactam ($MIC_{50/90}$, ≤4/32 μg/mL, 44.1% susceptible) as well as carbapenems (55.2-62.2% susceptible) showed poor activity against isolates of *K. pneumoniae* as displayed in Table 1. While 22.9% of the 144 *K. pneumoniae* exhibiting the ESBL phenotype remained susceptible to ceftolozane-tazobactam, 98.8% of the 168 *K. pneumoniae* non-susceptible to imipenem were resistant to this combination, as shown in Table 2. In fact, against these pathogens, only amikacin and colistin showed susceptibility rates superior to 70%.

A total of 81 *E. cloacae* were analyzed in this study. Among the extended cephalosporins, ceftolozane-tazobactam ($MIC_{50/90}$, 2/16 μg/mL, 46.9% susceptible) showed the highest susceptibility rate against this species followed by cefepime ($MIC_{50/90}$, 4/>32 μg/mL, 39.5% susceptible), ceftriaxone ($MIC_{50/90}$, >32/>32 μg/mL, 38.3% susceptible), and ceftazidime ($MIC_{50/90}$, 32/>32 μg/mL, 35.8% susceptible). In contrast, imipenem ($MIC_{50/90}$, ≤0.5/1 μg/mL, 98.8% susceptible), meropenem ($MIC_{50/90}$, ≤0.12/0.25 μg/mL, 98.8% susceptible), and amikacin ($MIC_{50/90}$, ≤4/≤4 μg/mL, 98.8% susceptible) were active against most *E. cloacae* (Table 1). Colistin ($MIC_{50/90}$, ≤1/>2 μg/mL, 95.1% susceptible) showed good *in vitro* activity against *E. cloacae*. Against the 52 *E. cloacae* isolates not susceptible to ceftazidime, only amikacin, imipenem, and meropenem retained good activity against such isolates with identical susceptibility rates (98.0%) followed by colistin (94.2% susceptible) as demonstrated in Table 2.

Ceftolozane-tazobactam ($MIC_{50/90}$, 0.5/0.5 μg/mL; 97.8% susceptible) and meropenem ($MIC_{50/90}$, ≤0.12/≤0.12 μg/mL; 98.9% susceptible) were the most active antimicrobials against the 91 *P. mirabilis* evaluated followed by piperacillin-tazobactam ($MIC_{50/90}$, ≤2/≤2 μg/mL; 96.7% susceptible), ertapenem ($MIC_{50/90}$, ≤0.06/≤0.06 μg/mL; 95.6% susceptible), and amikacin ($MIC_{50/90}$, ≤4/8 μg/mL; 95.6% susceptible). Although cefepime, ceftazidime, and ceftriaxone showed good *in vitro* activity with MIC_{50}s ≤1 μg/mL, 19.8%, 14.3%, and 22.0% of the isolates tested were resistant to these agents, respectively (Table 1). Ertapenem, imipenem, and meropenem were highly active against the 10 *P. mirabilis* isolates that exhibited the ESBL phenotype (Table 2). Ceftolozane-tazobactam (MIC_{50}, 0.5 μg/mL) and piperacillin-tazobactam (MIC_{50}, ≤2 μg/mL) also inhibited nine of these isolates (Table 2).

Ceftolozane-tazobactam was the most potent ($MIC_{50/90}$, 1/4 μg /mL) β-lactam agent tested against 265 *P. aeruginosa*, inhibiting 90.9% of isolates, while ceftazidime ($MIC_{50/90}$, 4/>32 μg/mL) imipenem ($MIC_{50/90}$, 1/16 μg /mL) and meropenem ($MIC_{50/90}$, 1/>16 μg/mL) inhibited only 66.8%, 70.2%, and 66.0% of these isolates, respectively, as shown in Table 1. Colistin ($MIC_{50/90}$, ≤1/2 μg/mL; 98.9% susceptible) and amikacin (MIC50/90, ≤4/16 μg/mL; 87.9% susceptible) were active against *P. aeruginosa*. Ceftolozane-tazobactam retained moderate activity against *P. aeruginosa* isolates that were non susceptible to ceftazidime or imipenem exhibiting susceptibility rates of 73.9% and 79.8%, respectively. In contrast, colistin was highly active against isolates displaying both phenotypes, ceftazidime non-susceptible (MIC50/90, ≤1/2 μg/mL; 96.6% susceptible) or imipenem non-susceptible ($MIC_{50/90}$, ≤1/2 μg/mL; 98.7%) as shown in Table 2.

Detection of β-lactamase encoding genes

According to the resistance profile, the molecular characterization of β-lactamase encoding genes was carried out in 433 isolates; i.e., in 36 of 81 *E. cloacae* (44.4%), 52 of 494 (10.5%) *E. coli*, 238 of 431 (55.2%) *K. pneumoniae*, 3 of 91 (3.3%) *P. mirabilis*, and 104 of 265 (39.2%) *P. aeruginosa* that fulfilled the study criteria specified in the material and methods section. The distribution of β-lactamase encoding genes according to bacterial species and medical center location is shown in Table 3. ESBL encoding genes were found in 37 of 52 selected *E. coli*. These isolates harbored a single $bla_{CTX\text{-}M}$ variant, with predominance of $bla_{CTX\text{-}M\text{-}15}$ (13 isolates; 35.1%), followed by $bla_{CTX\text{-}M\text{-}8}$ (11

Table 1 – Antimicrobial susceptibility profile of the five most frequent pathogens causing infections at participating Brazilian medical centers of the SMART Program (Brazil, 2016-2017).

Pathogen Antimicrobial Agents	Broth Microdiluition (μg/mL)		CLSI[a]		EUCAST[a]	
	MIC_{50}	MIC_{90}	S (%)	R (%)	S (%)	R (%)
Enterobacter cloacae (81)						
Amikacin	≤ 4	≤ 4	98.8	1.2	98.8	1.2
Aztreonam	> 16	> 16	39.5	60.5	38.3	60.7
Cefepime	4	> 32	46.9	53.1	39.5	60.5
Ceftazidime	32	> 32	39.5	60.5	35.8	61.2
Ceftolozane-tazobactam	2	16	59.3	40.7	46.9	53.1
Ceftriaxone	> 32	> 32	38.3	61.7	38.3	60.7
Ciprofloxacin	≤ 0.25	> 2	61.7	38.3	61.7	38.3
Colistin	≤ 1	> 1	95.1	4.9	95.1	4.9
Ertapenem	≤ 0.12	1	77.8	22.2	77.8	22.2
Imipenem	≤ 0.5	1	96.3	3.7	98.8	1.2
Meropenem	≤ 0.12	0.25	98.8	1.2	98.8	1.2
Piperacillin-tazobactam	16	> 64	50.6	49.4	43.2	56.8
E. coli (494)						
Amikacin	≤ 4	8	98.6	0.4	97.4	2.6
Aztreonam	≤ 1	> 16	80.0	20.0	71.5	28.5
Cefepime	≤ 1	> 32	76.5	23.5	73.3	26.7
Ceftazidime	≤ 1	16	84.4	15.6	78.1	21.9
Ceftolozane-tazobactam	0.25	0.5	96.8	3.2	94.7	5.3
Ceftriaxone	≤ 1	> 32	71.7	28.3	71.7	28.3
Ciprofloxacin	≤ 0.25	> 2	55.1	44.9	55.1	44.9
Colistin	≤ 1	≤ 1	99.4	0.6	99.4	0.6
Ertapenem	≤ 0.06	≤ 0.06	97.8	2.2	97.8	2.2
Imipenem	≤ 0.5	≤ 0.5	98.6	0.6	99.4	0.6
Meropenem	≤ 0.12	≤ 0.12	99.0	1.0	99.0	1.0
Piperacillin-tazobactam	≤ 2	8	93.3	6,7	90.9	9.1
K. pneumoniae (431)						
Amikacin	≤ 4	32	89.6	10.4	85.9	14.1
Aztreonam	> 16	> 16	37.4	62.6	35.0	65.0
Cefepime	32	> 32	36.7	63.3	35.7	64.3
Ceftazidime	32	> 32	39.0	61.0	34.3	65.7
Ceftolozane-tazobactam	4	> 32	49.7	50.3	44.1	55.9
Ceftriaxone	> 32	> 32	36.2	63.8	36.2	63.8
Ciprofloxacin	> 2	> 2	31.6	68.4	31.6	68.4
Colistin	≤ 1	> 4	85.4	14.6	85.4	14.6
Ertapenem	0.25	> 4	55.2	44.8	55.2	44.8
Imipenem	≤ 0.5	> 32	61.0	39.0	62.2	37.8
Meropenem	≤ 0.12	> 16	58.7	42.3	60.1	39.9
Piperacillin-tazobactam	> 64	> 64	41.5	58.5	36.7	63.3
P. aeruginosa (265)						
Amikacin	≤ 4	16	91.7	8.3	87.9	12.1
Aztreonam	8	> 16	52.5	47.5	67.6	32.4
Cefepime	8	32	68.3	31.7	68.3	31.7
Ceftazidime	4	> 32	66.8	33.2	66.8	33.2
Ceftolozane-tazobactam	1	4	90.9	9.1	90.9	9.1
Ciprofloxacin	≤ 0.25	> 2	69.8	30.2	69.8	30.2
Colistin	≤1	2	98.9	1.1	98.9	1.1
Imipenem	1	16	63.8	36.2	70.2	29.8
Meropenem	1	> 16	66.0	34.0	66.0	34.0
Piperacillin Tazobactam	16	> 64	59.6	40.4	59.6	40.4
P. mirabilis (91)						
Amikacin	≤ 4	8	97.8	2.2	95.6	4.4
Aztreonam	≤ 1	2	94.5	5.5	83.5	16.5
Cefepime	≤ 1	32	82.4	17.6	80.2	19.8

Table 1 (*Continued*)

Pathogen Antimicrobial Agents	Broth Microdiluition (μg/mL)		CLSI[a]		EUCAST[a]	
	MIC_{50}	MIC_{90}	S (%)	R (%)	S (%)	R (%)
Ceftazidime	≤ 1	2	95.6	4.4	85.7	14.3
Ceftolozane-tazobactam	0.5	0.5	98.9	1.1	97.8	2.2
Ceftriaxone	≤ 1	> 32	78.0	22.0	78.0	22.0
Ciprofloxacin	≤ 0.25	> 2	72.5	27.5	72.5	27.5
Ertapenem	≤ 0.06	≤ 0.06	95.6	4.4	95.6	4.4
Imipenem	1	2	55.0	45.0	92.3	7.7
Meropenem	≤ 0.12	≤ 0.12	98.9	1.1	98.9	1.1
Piperacillin-tazobactam	≤ 2	≤2	97.8	2.2	96.7	3.3

[a] CLSI, Clinical Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MIC, minimal inhibitory concentration; R, Resistant; S, susceptible.

isolates; 29.5%), $bla_{CTX\text{-}M\text{-}2}$ (8 isolates; 21.6%), $bla_{CTX\text{-}M\text{-}14}$ (3 isolates; 8.1%), and $bla_{CTX\text{-}M\text{-}9}$ (2 isolates; 5.4%) as displayed in Table 3. While variants like $bla_{CTX\text{-}M\text{-}14\ and}$ $bla_{CTX\text{-}M\text{-}9}$ seemed to be restricted to medical centers located, respectively, in São Paulo and Rio de Janeiro, $bla_{CTX\text{-}M\text{-}2,}$ $bla_{CTX\text{-}M\text{-}8,}$ and $bla_{CTX\text{-}M\text{-}15}$ were widely distributed. Only four *E. coli* isolates collected from two medical centers located in São Paulo and another located in Salvador (2 isolates) were shown to carry $bla_{KPC\text{-}2}$. One of these isolates also carried $bla_{CTX\text{-}M\text{-}8}$. Among the three *P. mirabilis* exhibiting the ESBL phenotype, $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}8}$, and $bla_{CTX\text{-}M\text{-}15}$ were identified in three distinct medical centers located in São Paulo and Rio de Janeiro. A single *E. cloacae* isolate harboring $bla_{KPC\text{-}2}$ was collected in Salvador. In addition to $bla_{KPC\text{-}2,}$ this isolate also harbored $bla_{CTX\text{-}M\text{-}15.}$ No other carbapenemase encoding genes were identified among *E cloacae* isolates evaluated. In contrast, 11 of 36 (30.6%) *E. cloacae* isolates were shown to carry $bla_{CTX\text{-}M\text{-}15.}$

Among 238 *K. pneumoniae* isolates that were eligible for molecular characterization, 165 (69.3%) were shown to carry bla_{KPC} with almost all harboring $bla_{KPC\text{-}2.}$ Single isolates carrying $bla_{KPC\text{-}3}$ or $bla_{KPC\text{-}30}$ were collected from a single medical center located in São Paulo. OXA-48, IMP or VIM encoding genes were not detected among the studied isolates. In contrast, two isolates harboring $bla_{NDM\text{-}1}$ were identified in the medical centers located in Salvador and Rio de Janeiro. While ESBL encoding genes, mainly belonging to the CTX-M family, were very frequently detected in *K. pneumoniae* isolates, plasmid-mediated AmpCs like $bla_{CMY\text{-}2}$ and $bla_{CMY\text{-}141}$ were very rare. The most frequently detected ESBL encoding genes were $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}2,}$ and $bla_{CTX\text{-}M\text{-}14}$ as shown in Table 3. In many occasions, bla_{KPC2} was associated with $bla_{CTX\text{-}M\text{-}15}$ (38 isolates), $bla_{CTX\text{-}M\text{-}14}$ (33 isolates), and $bla_{CTX\text{-}M\text{-}2}$ (32 isolates).

Among the 10 *P. aeruginosa* isolates exhibiting MICs ≥ 32 μg/mL for ceftolozane-tazobactam, five isolates harboring MBL encoding genes were detected with $bla_{SPM\text{-}1}$ (two isolates), $bla_{IMP\text{-}1,}$ $bla_{IMP\text{-}74,}$ and $bla_{VIM\text{-}2}$ identified, respectively, in single isolates. One *P. aeruginosa* isolate harboring $bla_{KPC\text{-}2}$ was found and shown to be resistant to ceftolozane-tazobactam, imipenem, and meropenem with MICs of 16, 32 and 16 μg/mL, respectively. A high number of *Pseudomonas* Derived Cephalosporinase (PDC), also denominated AmpC, encoding genes was detected in our collection with PDC-35, -5, -16, -3, being the most frequently observed as shown in Table 3. In contrast, ESBL encoding genes were rarely found in *P. aeruginosa*, with four isolates from a single center located in São Paulo carrying $bla_{CTX\text{-}M\text{-}2}$ and another isolated from a distinct medical center carrying $bla_{GES\text{-}1}$.

The distribution of beta-lactamases according to the participating Brazilian medical center is shown in Fig. 3. The size of each rectangle is proportional to the frequency of each beta-lactamase encoding gene found in the respective medical center. Although *K. pneumoniae* isolates harboring $_{KPC\text{-}2}$ were found in all medical centers, its frequency varied among the institutions. The same observation can be extrapolated for various PDC types in *P. aeruginosa* demonstrating the importance of local epidemiology.

Discussion

Surveillance studies are important not only for supporting new drug development but also for determining the antimicrobial susceptibility profile for guiding the selection of the most appropriate antimicrobial therapy, when more comprehensive data are not available (11)]. The enhanced understanding of the resistance mechanisms is a key factor in the search for new antimicrobial agents, allowing for the development of drugs that are effective despite such mechanisms. Ceftolozane-tazobactam is one of the most recently approved cephalosporin-beta-lactamase inhibitor combination for treating infections caused by Gram-negative bacilli, including *P. aeruginosa,* in Brazil.[12] Investigating the susceptibility profile of Brazilian Gram-negative bacilli and the mechanisms of resistance involving these organisms are crucial to support an effective clinical decision.

In our study, ceftolozane-tazobactam showed high antibacterial activity against *E. coli* with >90% susceptibility regardless of the resistance mechanism. The same favorable resistance profile was observed in *E. coli* isolated from China,[13] Canada[14] and US.[15] Although this class of drug is not recommended as first line treatment for ESBL infections, the favorable profile renders an alternative for treating ESBL-producing *E. coli*. Ceftolozane-tazobactam was also very active against *P. mirabilis,* including the ESBL-producing isolates.[15,16] Of note, high clinical cure rates with ceftolozane-tazobactam treatment of IAI and UTI caused by ESBL has been observed in a pooled analysis of ceftolozane-tazobactam clinical trials.[17]

Table 2 – Susceptibility rates to distinct antimicrobial agents of the five most frequent pathogens according to the phenotype of resistance.

E. cloacae non susceptible to ceftazidime (52)	Broth Microdiluition μg/mL		CLSI[a]		EUCAST[a]	
	MIC_{50}	MIC_{90}	S (%)	R (%)	S (%)	R (%)
Amikacin	≤ 4	≤ 4	97.9	2.1	98.0	2.0
Aztreonam	> 16	> 16	0	100	3.8	96.2
Cefepime	16	> 32	12.2	87.8	5.7	94;3
Ceftazidime	> 32	> 32	0	100.0	0	100.0
Ceftolozane-tazobactam	8	32	32.6	67.4	19.2	80.8
Ceftriaxone	> 32	> 32	0	100	5.7	94.3
Ciprofloxacin	2	> 2	42.8	57.2	44.2	55.8
Colistin	≤ 1	≤ 1	94.2	5.8	94.2	5.8
Ertapenem	0.5	2	63.2	37.8	65.3	34,7
Imipenem	≤ 0.5	1	95.9	4.1	98.0	2.0
Meropenem	≤ 0.12	0.25	97.9	2.1	98.0	2.0
Piperacillin-tazobactam	64	> 64	20.4	79.6	11.5	88.5
ESBL-producing *E. coli* (102)						
Amikacin	≤ 4	8	97.1	2.9	94.1	2.9
Aztreonam	> 16	> 16	22.6	77.4	2.9	97.1
Cefepime	32	> 32	5.8	94.2	0	100.0
Ceftazidime	8	32	37.3	62.7	21.5	78.5
Ceftolozane-tazobactam	0.5	2	92.2	7.8	87.2	12.8
Ceftriaxone	> 32	> 32	0	100.0	0	100.0
Ciprofloxacin	> 2	> 2	19.6	80.4	19.6	80.4
Colistin	≤ 1	≤ 1	99.0	1.00	99.0	1.00
Ertapenem	≤ 0.06	0.12	97.1	2.9	97.0	3.0
Imipenem	≤ 0.5	≤ 0.5	99.0	1.0	99.0	1.0
Meropenem	≤ 0.12	≤ 0.12	99.0	1.0	99.0	1.0
Piperacillin-tazobactam	4	32	86.3	13.7	80.3	19.7
ESBL-producing *K. pneumoniae* (144)						
Amikacin	≤ 4	16	92.4	7.6	88,9	11.1
Aztreonam	> 16	> 16	2.1	97.9	0.7	99.3
Cefepime	> 32	> 32	0.7	99.3	0.7	99.3
Ceftazidime	> 32	> 32	4.2	95.8	0.7	99.3
Ceftolozane-tazobactam	16	> 32	36.1	63.9	22.9	77.1
Ceftriaxone	> 32	> 32	0.7	99.3	0.7	99.3
Ciprofloxacin	> 2	> 2	2.1	97.9	2.1	97.9
Colistin	≤ 1	> 4	79.9	20.1	79.9	20.1
Ertapenem	0.5	> 4	53.5	46.5	53.5	46.5
Imipenem	≤ 0.5	> 32	63.2	36.8	66.0	44.0
Meropenem	≤ 0.12	> 16	60.4	39.6	63.2	36.8
Piperacillin-tazobactam	> 64	> 64	22.2	77.8	13.2	86.8
K. pneumoniae non susceptible to imipenem (168)						
Amikacin	≤ 4	> 32	79.2	20.8	69.9	30.1
Aztreonam	> 16	> 16	1.2	98.8	0.0	100.0
Cefepime	> 32	> 32	0.6	99.4	0.0	100.0
Ceftazidime	> 32	> 32	2.4	97.6	0.6	99.4
Ceftolozane-tazobactam	> 32	> 32	1.2	98.8	0.6	99.4
Ceftriaxone	> 32	> 32	0.0	100.0	0.0	100.0
Ciprofloxacin	> 2	> 2	3.0	97.0	2.5	97.5
Colistin	≤ 1	> 4	71.4	28.6	71.4	28.6
Ertapenem	> 4	> 4	1.2	98.8	0.6	99.4
Imipenem	32	> 32	0.0	100.0	0.0	100.0
Meropenem	> 16	> 16	1.8	98.2	0.6	99.4
Piperacillin-tazobactam	> 64	> 64	1.2	98.8	0.6	99.4
P. aeruginosa non susceptible to ceftazidime (88)						
Amikacin	≤ 4	> 32	85.2	14.8	77.3	22.7
Aztreonam	> 16	> 16	11.4	88.6	29.6	70.4
Cefepime	32	> 32	19.3	80.7	19.3	80.7
Ceftazidime	> 32	> 32	0.0	100.0	0.0	100.0

Table 2 (*Continued*)

E. cloacae non susceptible to ceftazidime (52)	Broth Microdiluition μg/mL		CLSI[a]		EUCAST[a]	
	MIC_{50}	MIC_{90}	S (%)	R (%)	S (%)	R (%)
Ceftolozane-tazobactam	4	32	73.9	26.1	73.9	26.1
Ciprofloxacin	1	> 2	47.7	52.3	47.7	52.3
Colistin	≤ 1	2	96.6	3.4	96.6	3.4
Imipenem	2	32	50.0	50.0	53.4	46.6
Meropenem	4	> 16	44.3	55.7	44.3	55.7
Piperacillin-tazobactam	> 64	> 64	8.0	92.0	8.0	92.0
P. aeruginosa non susceptible to imipenem (96)						
Amikacin	≤ 4	> 32	86.5	13.5	77.2	22.8
Aztreonam	16	> 16	30.2	69.8	46.8	53.2
Cefepime	8	> 32	50.0	50.0	43.0	57.0
Ceftazidime	8	> 32	54.2	45.8	48.1	51.9
Ceftolozane-tazobactam	2	16	82.3	17.7	79.8	20,2
Ciprofloxacin	0.5	> 2	53.1	46.9	48.1	51.9
Colistin	≤ 1	2	99.0	1.0	99.0	1.0
Imipenem	8	32	0.0	82.3	0.0	100,0
Meropenem	16	> 16	13.5	86.5	3.8	96.2
Piperacillin-tazobactam	32	> 64	41.7	58.3	32.9	67.1
P. mirabilis ESBL (10)						
Amikacin	≤ 4	8	100.0	0.0	100.0	0.0
Aztreonam	2	16	70.0	30.0	30.0	30.0
Cefepime	32	> 32	0.0	100.0	0,0	100.0
Ceftazidime	2	8	80.0	20.0	40.0	60,0
Ceftolozane-tazobactam	0.5	1	100.0	0.0	90.0	10.0
Ceftriaxone	> 32	> 32	0.0	100.0	0.0	100,0
Ciprofloxacin	> 2	> 2	0.0	100.0	0.0	100.0
Ertapenem	≤ 0.06	≤ 0.06	100.0	0.0	100.0	0,0
Imipenem	1	2	60.0	40.0	100.0	0.0
Meropenem	≤ 0.12	≤ 0.12	100.0	0.0	100.0	0.0
Piperacillin-tazobactam	≤ 2	8	100.0	0.0	90.0	0.0

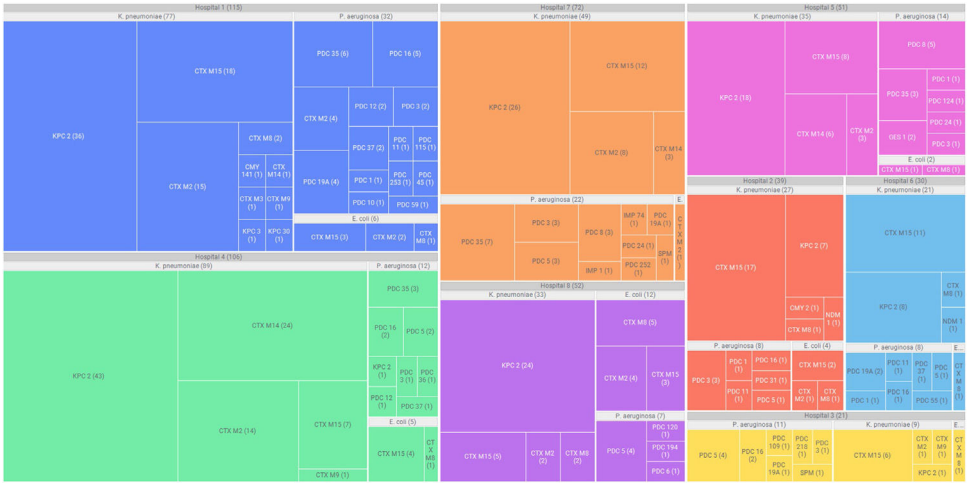

Fig. 3 – Distribution frequency of beta-lactamase encoding genes according to participating Brazilian medical centers. The size and value of each rectangle is proportional to the frequency of each beta-lactamase encoding gene found in that respective medical center.

It is important to notice that ciprofloxacin resistance rates were very high (>50%) among *E. coli* isolates, emphasizing that this fluoroquinolone should not be prescribed empirically in our setting. Overall, *E. cloacae* had a low susceptibility rate to ceftolozane-tazobactam, and especially for those isolates resistant to ceftazidime. This result was in concert with the observation of Robin et al.[18] The hyperexpression of chromosomal AmpC could justify this result. In addition,

Table 3 – Distribution of beta-lactamase encoding genes according to bacterial species and medical center location.

Bacterial Species/ β-lactamase encoding genes (Number)	Number of Isolates detected by geographic location (Number)	Other β-lactamase encoding genes
E. coli (52)		
ESBL (37)		
$bla_{CTX-M-2}$ (8)	SP (7), RJ (1)	
$bla_{CTX-M-8}$ (11)	SP (9), RJ (1), Salvador (1)	
$bla_{CTX-M-9}$ (2)	RJ (2)	
$bla_{CTX-M-14}$ (3)	SP (3)	
$bla_{CTX-M-15}$ (13)	SP (11), RJ (2)	
Carbapenemase (4)		
bla_{KPC-2}(4)	SP (2), Salvador (2)	
E. cloacae (12)		
ESBL (12)		
$bla_{CTX-M-15}$ (11)	SP (11), RJ (1), Salvador (1)	
Bla_{SHV-12}(1)	SP (1)	
Carbapenemase (1)		
bla_{KPC-2}(1)	Salvador (1)	
P. mirabilis (3)		
ESBL (3)		
$bla_{CTX-M-2}$ (1)	RJ (1)	
$bla_{CTX-M-8}$ (1)	SP (1)	
$bla_{CTX-M-15}$ (1)	SP (1)	
K. pneumoniae (238)		
ESBL (173)		
$bla_{CTX-M-2}$ (43)	SP (43)	bla_{KPC-2} (32)
$bla_{CTX-M-3}$ (1)	SP (1)	
$bla_{CTX-M-8}$ (6)	SP (4), RJ (1), Salvador (1)	bla_{KPC-2} (2)
$bla_{CTX-M-9}$ (3)	SP (3)	
$bla_{CTX-M-14}$ (34)	SP (34)	bla_{KPC-2} (33)
$bla_{CTX-M-15}$ (84)	SP (56), RJ (17), Salvador (11)	bla_{KPC-2} (38); bla_{NDM-1}(2)
$bla_{CTX-M-35}$ (2)	SP (2)	
$bla_{CTX-M-141}$ (1)	Salvador (1)	bla_{KPC-2} (1)
AmpC (2)		
bla_{CMY-2} (1)	SP (1)	
$bla_{CMY-141}$(1)	SP (1)	
Carbapenemase (165)		
bla_{KPC-2} (163)	SP (148), RJ (7), Salvador (8)	
bla_{KPC-3} (1)	SP (1)	
bla_{KPC-30} (1)	SP (1)	
bla_{NDM-1} (2)	RJ (1), Salvador (1)	
P. aeruginosa (104)		
PDC (104)		
PDC-1 (4)	SP (2), RJ (1), Salvador (1)	
PDC-3 (11)	SP (8), RJ (3)	
PDC-5 (15)	SP (13), Salvador (1), RJ (1)	bla_{IMP-1} in SP (1); bla_{SPM-1} in SP (2)
PDC-6 (1)	SP (1)	
PDC-8 (8)	SP (8)	
PDC-10 (1)	SP (1)	
PDC-11 (3)	SP (1), RJ (1), Salvador (1)	
PDC-12 (3)	SP (3)	
PDC-16 (11)	SP (9), Salvador (1), RJ (1)	
PDC-19A (8)	SP (6), Salvador (2)	bla_{VIM-2} in SP (1)
PDC-24 (2)	SP (2)	
PDC-31 (1)	RJ (1)	
PDC-35 (19)	SP (19)	bla_{IMP-74} in SP (1); $bla_{CTX-M-2}$ in SP (3); bla_{GES-1} in SP (2);
PDC-36 (1)	SP (1)	
PDC-37 (4)	SP (3), Salvador (1)	bla_{KPC-2} in SP (1)
PDC-45 (1)	SP (1)	

Table 3 (*Continued*)

Bacterial Species/ β-lactamase encoding genes (Number)	Number of Isolates detected by geographic location (Number)	Other β-lactamase encoding genes
PDC-55 (1)	Salvador (1)	
PDC-59 (1)	SP (1)	$bla_{CTX\text{-}M\text{-}2}$ in SP (1)
PDC-109 (1)	SP (1)	
PDC-115 (2)	SP (2)	
PDC-120 (1)	SP (1)	
PDC-124 (1)	SP (1)	
PDC-194 (1)	SP (1)	
PDC-218 (1)	SP (1)	
PDC-252 (1)	SP (1)	
PDC-253 (1)	SP (1)	

SP, São Paulo; RJ, Rio de Janeiro.

association with other mechanisms of beta-lactam resistance could be present in these isolates, such as alteration in outer membrane protein(s), because the susceptibility rate to ertapenem was inferior to those observed for imipenem and meropenem.[19] Previous studies have shown that AmpC hyperproducing *E. cloacae* isolates were usually resistant to ceftolozane-tazobactam.[20]

Among the five most frequent organisms studied, *K. pneumoniae* was one of the most difficult to treat pathogen, because these microorganisms were usually resistant to first line treatment drugs like imipenem. The resistance rates to ceftolozane-tazobactam and colistin were also high in our study. This pattern of susceptibility seems to be largely affected by the presence of $bla_{KPC\text{-}2,}$ which in many cases was associated with $bla_{CTX\text{-}type}$, ESBL encoding genes belonging to the CTX-M-family. Our resistance rates were higher than those reported previously by Pfaller et al., who had evaluated the activity of cetolozane-tazobactam in bacterial isolates collected in Latin America.[21] This difference could be attributed to distinct inclusion criteria like medical centers and the study time period, for example. In fact, it has been widely reported that KPC-2 is endemic in many Brazilian hospitals.[4] In our study, $bla_{KPC\text{-}2}$ was the most frequent carbapenemase encoding gene found in the participating medical centers with frequencies varying among the institutions. While $bla_{KPC\text{-}3}$ has been reported frequently in some Latin American countries, it has been found rarely in Brazil.[22] In fact, to date, $bla_{KPC\text{-}3}$ has only been reported in *A. baumannii* isolated from a Brazilian medical center located in São Luís, Maranhão.[23] To the best of our knowledge, this is the first study to detect new variants of $bla_{KPC,}$ such as $bla_{KPC\text{-}3}$ and $bla_{KPC\text{-}30}$, in *K. pneumoniae* in Brazilian medical centers. $bla_{KPC\text{-}3}$ and $bla_{KPC\text{-}30}$ were found to be harbored by *K. pneumoniae* isolated from urine and intra-abdominal abscess of patients hospitalized at the same Brazilian medical center located in the city of São Paulo. NDM-1-producing *K. pneumoniae* isolates were unfrequently detected in our study. Only two isolates displaying this genotype were identified: one in urine and another in intra-abdominal abscess of patients hospitalized in Rio de Janeiro and Salvador, respectively.

Ceftolozane-tazobactam has been shown previously to be the most effective agent for *P. aeruginosa* isolates regardless of resistance to other antimicrobial agents.[24] Ceftolozane-tazobactam also demonstrated good activity against isolates non-susceptible to ceftazidime and/or imipenem. The activity of ceftolozane-tazobactam was not affected by hyperproduction of AmpC or other mechanism of resistance as expected.[25] In contrast, its activity was affected by production of carbapenemases including metallo-β-lactamases (MBL). In this study, $bla_{KPC\text{-}2}$ was detected in a single *P. aeruginosa* isolate collected in São Paulo, as observed previously in some medical centers.[26–30] In addition, a few MBL-producing *P. aeruginosa* isolates were detected in our study. SPM-1-producing *P. aeruginosa* ST277, an MBL producer clone, was widely disseminated in Brazilian hospitals in the 2000s.[31] Our results seem to corroborate the results previously reported by Cacci et al., who noticed a decline in the frequency of the SPM-1-producing *P. aeruginosa* ST277 clone at the intensive care unit of a hospital located in Rio de Janeiro city, where SPM-1 was previously endemic.[32] In our study, only two SPM-1-producing *P. aeruginosa* isolates were identified in two distinct medical centers located in the city of São Paulo. In one of these medical centers, two isolates of *P. aeruginosa* were shown to harbor genes encoding the IMP variants, $bla_{IMP\text{-}1}$ and $bla_{IMP\text{-}74}$. To the best of our knowledge, $bla_{IMP\text{-}74}$ had not been identified previously in any Brazilian medical center. It was isolated from a urine sample of 44-y-o female patient who had been hospitalized to treat of a urinary tract infection.

Conclusion

In this study, ceftolozane-tazobactam was shown to be very active against *E. coli*, *P. mirabilis* and *P. aeruginosa* isolates and could constitute an excellent therapeutic option including for those isolates resistant to extended-spectrum cephalosporins and carbapenems but not producers of carbapenemases. However, the activity of ceftolozane-tazobactam against *K. pneumoniae* has been jeopardized by the spread of ESBL and KPC-2-producing *K. pneumoniae* in Brazilian medical centers. In addition, the co-production of beta-lactamases by such species also compromise the activity of ceftolozane-tazobactam. In this context, surveillance studies like SMART are essential for helping to delineate the changes in the epidemiology of Gram-negative infections over time, not only in Brazil, but also worldwide.

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