ORIGINAL ARTICLE

Evaluation of *in vitro* activity of ceftolozane-tazobactam in combination with other classes of antibacterial agents against *Enterobacterales* and *Pseudomonas aeruginosa*—the EM200 study

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Abstract

Ceftolozane-tazobactam is a cephalosporin/β-lactamase inhibitor combination developed for use against some β-lactam- and multidrug-resistant Gram-negative organisms. This study aimed to evaluate the *in vitro* activity of ceftolozane–tazobactam against clinical bacterial isolates at the University Hospital of Marrakech. This is a descriptive and analytical prospective study. A total of 143 *Enterobacterales* and 48 *Pseudomonas aeruginosa* isolates were collected from January 2018 to December 2018 from patients with respiratory, urinary and intra-abdominal infections. The identification was made by Phoenix automated system (BioMérieux). MIC50/90 were tested by broth microdilution for ceftolozane-tazobactam, and other drugs using dried panels. Antimicrobial susceptibility results were interpreted according to CLSI guidelines. Ceftolozane-tazobactam inhibited 98% of *Escherichia coli* ($MIC_{50/90}$; 0.25/0.5 μg/mL). The susceptibility rate of *Klebsiella pneumoniae* to ceftolozane-tazobactam was 68.8% ($MIC_{50/90}$, 0.5/>32 μg/mL); other *Enterobacterales* have shown susceptibility rates of 80.4% ($MIC_{50/90}$; 0.5/8 μg/mL). In carbapenemase-producing *K. pneumoniae*, the bla_{OXA-48} mutation was found in two isolates. Susceptibility of *P. aeruginosa* to ceftolozane-tazobactam was 91.7% ($MIC_{50/90}$, 0.5/>32 μg/mL). In non-carbapenemase-producing *P. aeruginosa, AmpC* mutations were found in all isolates. Ceftolozane-tazobactam was satisfactorily active against a wide range of tested isolates and offers clinicians a potential therapeutic option even against resistant strains in patients with intra-abdominal infections, urinary tract infections and nosocomial pneumonia.

Keywords: Antimicrobial susceptibility, ceftolozane-tazobactam, *Enterobacterales*, minimum inhibitory concentrations, *Pseudomonas aeruginosa*

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Introduction

Enterobacterales are responsible for severe respiratory infection, urinary tract infection and intra-abdominal infection due to antibiotic resistance [1]. They are represented mainly by *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Proteus mirabilis* [1]. *Pseudomonas aeruginosa* is a pathogen that has expanded in hospitals, and causes fatal infections [2]. Infectious diseases pose a serious threat to global health [1,2].

Antibiotics have saved millions of lives every year, but today many bacteria are multidrug-resistant. The excessive and irrational use of antibiotics leads to the adaptation of bacteria to develop resistance to antibiotics [3]. The European Center for the Control of Infectious Diseases evaluates 33 000 cases of deaths per year due to bacterial resistance [4]; the problem has become critical in hospitals, because of the therapeutic problems observed mainly in Gram-negative bacteria such as *Enterobacterales* and *P. aeruginosa* [3,5].

WHO has issued a global action plan to develop new antibiotics [6], like ceftolozane-tazobactam (Zerbaxa™), which is a combination of ceftolozane, a third-generation cephalosporin,

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and tazobactam, an inhibitor of several β-lactamases. It is a new antibiotic, with activity against multi-resistant Gram-negative bacteria. Zerbaxa™ was approved by the US Food and Drug Administration and by the European Medicines Agency for complicated intra-abdominal infections, serious kidney infections (acute pyelonephritis), complicated infections of the urinary tract and hospital-acquired pneumonia, including ventilator-associated pneumonia [7,8]. *In vitro* studies have shown previously that the potential value of ceftolozane-tazobactam lies in its activity against *Enterobacterales* producing extended-spectrum β-lactamases (ESBL) [9], as part of a carbapenem saving strategy [10], and against *P. aeruginosa* combining several mechanisms of antibiotic resistance (including efflux pumps and overexpression of *AmpC*) [11,12].

The aim of this study was to evaluate the *in vitro* activity of ceftolozane-tazobactam against *Enterobacterales* and *P. aeruginosa* collected from different infection sites in the Marrakech University Hospital.

Materials and methods

Type of study method

This is a descriptive and analytical prospective study, extended over 1 year from January 2018 to December 2018, in two university hospital centres in Morocco: Marrakech and Rabat.

Bacterial isolates

Gram-negative aerobic bacteria, comprising *E. coli*, *Klebsiella* spp., *Enterobacter* spp. and *Proteus* spp., and Gram-negative anaerobic bacteria, comprising only *P. aeruginosa*, were isolated from different infection sites including respiratory infections, urinary infections and intra-abdominal infections. They were not collected sequentially (isolated strains were stored at −80°C or −20°C). Bacteria were identified using the Phoenix® automated Microbiology Identification System (Becton Dickinson, Franklin Lakes, NJ, USA).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed at International Health Management Associates (IHMA) report 2018 following the 2017 Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. Minimum inhibitory concentrations (MIC) were interpreted according to CLSI MIC values for ceftolozane-tazobactam, ceftazidime, meropenem, cefepime, piperacillin-tazobactam, amikacin, ciprofloxacin, ertapenem, imipenem, ceftriaxone, levofloxacin and colistin, and were determined using broth microdilution method panels manufactured by TREK Diagnostic Systems (East Grinstead, UK) according to CLSI guidelines for antimicrobials. A suspension using colonies and normal saline, equivalent to 0.5 McFarland standard concentrations, was incubated at 35°C for 16–20 hours.

For isolates of *Enterobacterales*, ceftolozane-tazobactam MIC were considered susceptible at ≤2 μg/mL. For isolates of *P. aeruginosa*, ceftolozane-tazobactam MIC was considered susceptible at ≤4 μg/mL.

Whole-genome sequencing of non-susceptible *P. aeruginosa* and *K. pneumoniae* to ceftolozane-tazobactam

Strains with ceftolozane-tazobactam MIC values ≥ 8 μg/mL were examined.

Genomic DNA was extracted using Qiagen DNeasy Ultra-Clean kits (Qiagen, Valencia, CA, USA) and quantified using the Nanodrop™ ND-1000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA).

For whole-genome sequencing, DNA sequences were obtained on the Illumina HiSeq sequencing instrument (Illumina, San Diego, CA, USA) with 2 × 150 bp pair-end reads with a target coverage depth of approximately 150×. All analyses were carried out using Qiagen's CLCBio Genomics Workbench version 11.

For β-lactamase resistance, genes were identified by Illumina whole-genome sequencing. β-lactamase gene inclusion was 72% and 80% for minimum nucleotide sequence identity and minimum sequence length, respectively.

For porin gene identification, *ompk35* and *ompK36* in *K. pneumoniae* and *oprD* in *P. aeruginosa* were searched by TBLASTN; for *ftsI* (encoding PBP3) gene analysis, searching was on a species-specific basis in *de novo* assemblies of each genome.

The appropriate multilocus sequence typing scheme and allelic profile of each of the guided assemblies was determined computationally (using the 'find best match using k-mer spectra' tool in CLC genomics).

Statistical analysis

Statistical analysis for comparison of susceptibilities of different isolates to ceftolozane-tazobactam was performed using SPSS 18 software (SPSS Inc., Chicago, IL, USA).

Results

The isolates comprised 142 *Enterobacterales* (49 *E. coli*, 48 *K. pneumoniae,* 16 *Enterobacter* spp. and 16 *Proteus* spp., 8 *Klebsiella oxytoca*, 3 *Enterobacter aerogenes*, 1 *Klebsiella variicola* and 1 *Proteus vulgaris*), and 48 *P. aeruginosa*. The clinical isolates were collected from intensive care units (57.4%, *n* = 109) and from non-intensive care units (46.6%, *n* = 81). The 190 Gram-

TABLE 1. MIC values distribution for all antimicrobials tested against *Enterobacterales* and *Pseudomonas aeruginosa* isolates

		MICs (μg/mL)										
Organisms	**Antimicrobial agent**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**
Escherichia coli (n = 49)	Ceftolozane-tazobactam		7	26	15	0	0	1	0	0	0	
	Piperacillin-tazobactam						30	5	6	4	1	3
	Cefoxitin						4	16	23	6		
	Cefotaxime					36	1	0	0	3	9	
	Ceftriaxone					36	0	0	0	2	11	
	Ceftazidime					38	1	2	2	2	4	
	Cefepime					37	2	0	4	0	6	
	Meropenem	49	0	0	0	0	0	0	0			
	Imipenem				48	1	0	0	0	0	0	
	Ertapenem	48	0	1								
	Aztreonam					37	1	2	1	8		
	Amikacin							32	13	4		
	Ciprofloxacin			32	2	15						
	Levofloxacin					33	0	16				
	Colistin		49	0	0							
Klebsiella pneumoniae (*n* = 48)	Ceftolozane-tazobactam		1	7	16	6	3	1	7	0	7	
	Piperacillin-tazobactam						6	8	6	5	9	14
	Cefoxitine						5	26	8	9		
	Cefotaxime					13	2	1	0	0	32	
	Ceftriaxone					15	0	0	1	0	32	
	Ceftazidime					13	1	1	3	8	22	
	Cefepime					16	0	0	3	11	18	
	Ertapenem	27	3	6	1	1	1	9				
	Imipenem				31	7	4	3	1	0	2	
	Meropenem	36	1	1	0	3	4	1	2			
	Aztreonam					13	2	1	1	31		
	Amikacin							45	2	1		
	Ciprofloxacin			20	3	25						
	Levofloxacin					30	2	16				
	Colistin					42	0	6				
Enterobacter cloacae (*n* = 16)	Ceftolozane-tazobactam			4	3	0	0	3	3	1	2	
	Piperacillin-tazobactam						6	1	0	0	1	8
	Cefoxitine							1	0	15		
	Cefotaxime					6	1	0	0	0	9	
	Ceftriaxone					7	0	0	0	0	9	
	Ceftazidime					7	0	0	0	0	9	
	Cefepime					8	5	1	0	0	2	
	Ertapenem	7	0	0	1	4	2	2				
	Imipenem				14	0	0	2	0	0	0	
	Meropenem		13	1	0	0	0	1	1	0		
	Aztreonam					7	0	0	0	9		
	Amikacin							16	0	0	0	
	Ciprofloxacin			6	0	10						
	Levofloxacin					9	1	6				
	Colistin					16	0	0				
Proteus mirabilis (*n* = 16)	Ceftolozane-tazobactam			6	9	1	0	0	0	0	0	
	Piperacillin-tazobactam					16	0	0	0	0	0	
	Cefoxitine						9	6	0	1		
	Cefotaxime					16	0	0	0	0	0	
	Ceftriaxone					16	0	0	0	0	0	
	Ceftazidime					16	0	0	0	0	0	
	Cefepime					16	0	0	0	0	0	
	Ertapenem	16	0	0	0	0	0	0	0			
	Imipenem				3	10	3	0	0	0	0	
	Meropenem	16	0	0	0	0	0	0	0			
	Aztreonam					16	0	0	0	0	0	
	Amikacin							14	2	0	0	
	Ciprofloxacin				1	0	0					
	Levofloxacin					1	0	0				
Pseudomonas aeruginosa (*n* = 48)	Ceftolozane-tazobactam				24	11	1	6	0	0	6	
	Piperacillin-tazobactam							12	19	4	1	12
	Cefepime					3	19	8	4	4	10	
	Ceftazidime					0	18	15	2	5	8	
	Meropenem		10	10	0	7	1	2	5	3		
	Imipenem			1	4	26	7	1	1	4	5	
	Aztreonam						1	26	8	13		
	Amikacin							39	4	1	4	
	Ciprofloxacin			35	4	0	9					
	Levofloxacin					35	5	8				
	Colistin					47	1	0				
Other *Enterobacterales* (*n* = 14)	Ceftolozane-tazobactam			11	3							
	Piperacillin-tazobactam						9	5				
	Cefoxitin						3	7	1	3		
	Cefotaxime					14						
	Ceftazidime					14						
	Ceftriaxone					14						
	Cefepime					14						
	Ertapenem	14										
	Imipenem				11	3						
	Meropenem		14									
	Aztreonam					14						
	Amikacin							14				
	Ciprofloxacin			14								
	Levofloxacin					14						
	Colistin					13		1				

negative isolates included 113 isolates from urinary tract infections, 56 from intra-abdominal infections and 21 from lower respiratory tract infections. Table 1 shows the antibiotics assessed, and the range and MIC values of each drug. Table 2 shows the susceptibility profile of different organisms to several antibiotics and their MIC50/90.

Ceftolozane-tazobactam activity against *Enterobacterales*

The activity of ceftolozane-tazobactam against *E. coli* was high ($MIC_{50/90}$; 0.25/0.5 μg/mL). Ceftolozane-tazobactam susceptibility rate against *E. coli* was 98%. Susceptibility rates to other antibiotics were 100% to carbapenem; 100% to amikacin and 100% to colistin (Table 3).

Susceptibility rate of *K. pneumoniae* to ceftolozane-tazobactam was 68.8%, with medium activity ($MIC_{50/90}$, 0.5/>32 μg/mL), with higher susceptibility to amikacin (100%), colistin (87.5%), imipenem (79.2%), meropenem (79.2%) and ertapenem (77.1%) (Table 2).

Ceftolozane-tazobactam showed 80.4% inhibition ($MIC_{50/90}$; 0.5/8 μg/mL) against *Enterobacterales* (*Enterobacter cloacae*, *Proteus mirabilis*, *K. oxytoca*, *K. variicola*, *Enterobacter aerogenes*, *Proteus vulgaris*) compared with susceptibilities to colistin of 63%, to meropenem of 89.1% and to amikacin of 100%.

TABLE 2. $MIC_{50/90}$ and percentage susceptible of antimicrobials tested against 190 isolates

Organism	Antimicrobial agent	%S CLSI[a]	MIC_{50}[b]	MIC_{90}[b])	Range
Pseudomonas aeruginosa (*n* = 48)	Ceftolozane-tazobactam	87.5	0.5	>32	0.5 to >32
	Amikacin	91.7	≤4	16	≤4 to >32
	Aztreonam	72.9	4	>16	2 to >16
	Cefepime	70.8	4	32	≤1 to >32
	Ceftazidime	72.9	4	>32	2 to >32
	Ciprofloxacin	81.3	≤0.25	>2	≤0.25 to >2
	Colistin	100	≤1	≤1	≤1 to 2
	Imipenem	77.1	1	32	≤0.5 to >32
	Levofloxacin	83.3	≤1	>4	≤1 to >4
	Meropenem	79.2	0.5	8	≤0.12 to >16
	Piperacillin-tazobactam	72.9	8	>64	4 to >64
Escherichia coli (*n* = 49)	Ceftolozane-tazobactam	98.0	0.25	0.5	0.12–4
	Amikacin	100	≤4	8	≤4 to 16
	Aztreonam	81.6	≤1	>16	≤1 to >16
	Cefepime	79.6	≤1	>32	≤1 to >32
	Cefotaxime	73.5	≤1	>32	≤1 to >32
	Cefoxitin	87.8	8	16	≤2 to >16
	Ceftazidime	83.7	≤1	16	≤1 to >32
	Ceftriaxone	73.5	≤1	>32	≤1 to >32
	Ciprofloxacin	67.4	≤0.25	>2	≤0.25 to >2
	Colistin	100	≤1	≤1	≤1 to ≤1
	Ertapenem	100	≤0.06	≤0.06	≤0.06 to 0.25
	Imipenem	100	≤0.5	≤0.5	≤0.5 to 1
	Levofloxacin	67.4	≤1	>4	≤1 to >4
	Meropenem	100	≤0.12	≤0.12	≤0.12 to ≤0.12
	Piperacillin-tazobactam	91.8	≤2	16	≤2 to 64
Klebsiella pneumoniae (*n* = 48)	Ceftolozane-tazobactam	68.8	0.5	>32	0.12 to >32
	Amikacin	100	≤4	≤4	≤4 to 16
	Aztreonam	33.3	>16	>16	≤1 to >16
	Cefepime	33.3	16	>32	≤1 to >32
	Cefotaxime	27.1	>32	>32	≤1 to >32
	Cefoxitin	81.3	4	>16	≤2 to >16
	Ceftazidime	31.3	16	>32	≤1 to >32
	Ceftriaxone	31.3	>32	>32	≤1 to >32
	Ciprofloxacin	47.9	2	>2	≤0.25 to >2
	Colistin	87.5	≤1	>4	≤1 to >4
	Ertapenem	77.1	≤0.06	>4	≤0.06 to >4
	Imipenem	79.2	≤0.5	4	≤0.5 to >32
	Levofloxacin	66.7	≤1	>4	≤1 to >4
	Meropenem	79.2	≤0.12	4	≤0.12 to >16
	Piperacillin Tazobactam	52.1	16	>64	≤2 to >64
Other 22*Enterobacterales* (*n* = 46)	Ceftolozane-tazobactam	80.4	0.5	8	0.25 to >32
	Amikacin	100	≤4	≤4	≤4 to 8
	Aztreonam	80.4	≤1	>16	≤1 to >16
	Cefepime	93.5	≤1	2	≤1 to 32
	Cefotaxime	76.1	≤1	>32	≤1 to >32
	Cefoxitin	58.7	4	>16	≤2 to >16
	Ceftazidime	80.4	≤1	>32	≤1 to >32
	Ceftriaxone	78.3	≤1	>32	≤1 to >32
	Ciprofloxacin	71.7	≤0.25	>2	≤0.25 to >2
	Colistin	63.0	≤1	>4	≤1 to >4
	Ertapenem	82.6	≤0.06	1	≤0.06 to >4
	Imipenem	89.1	≤0.5	2	≤0.5 to 4
	Levofloxacin	80.4	≤1	4	≤1 to >4
	Meropenem	95.7	≤0.12	≤0.12	≤0.12 to 8
	Piperacillin Tazobactam	80.4	≤2	64	≤2 to >64

[a]%S, represents the percent susceptible by CLSI 2017 guidelines (EUCAST guidelines for colistin were applied for *Enterobacterales*).
[b]MIC_{50}, MIC_{90}, and range in μg/mL; no intermediate breakpoint.

From 15 ceftolozane-tazobactam non-susceptible *K. pneumoniae* isolates, molecular analysis of the resistance support was detected in 4/15 (27%). The carbapenemase gene bla_{OXA-48} was found in two isolates. ESBL production was confirmed in all isolates—$bla_{CTX-M-15}$ (four of four), bla_{SHV-1} (two of four), bla_{OXA-1} (two of four) and bla_{TEM-1B} (two of four). Mutations of *ompK35* and *ompK36* were detected in three of the isolates tested (Table 3).

Ceftolozane-tazobactam activity against *P. aeruginosa*

The susceptibility rate to ceftolozane-tazobactam against *P. aeruginosa* was 91.7%, with ($MIC_{50/90}$ values of 0.5/>32 μg/mL) (Table 3). Colistin was the most active drug with 100% of isolates susceptible ($MIC_{50/90}$, ≤1/≤1 μg/mL); susceptibility to amikacin-cefepime was 91.7%.

All six ceftolozane-tazobactam-resistant *P. aeruginosa* isolates produced *PDC* (*Ampc* mutation) and bla_{OXA-50}; four had the β-lactamases genes bla_{PER-1} and bla_{VIM-2}, and two had bla_{OXA-4}. Mutation of *oprD* was detected in three isolates; all isolates were wild-type for *ftsI* (Table 4).

Discussion

Ceftolozane-tazobactam could be an important treatment option, including against multidrug-resistant strains. This type of study is interesting to evaluate the activity of ceftolozane-tazobactam against a selection of *P. aeruginosa* and *Enterobacterales* isolates. Our study is the first in Morocco.

In the current study, ceftolozane-tazobactam was active against 82.4% of *Enterobacterales*: 98% of *E. coli*, 68.8% of *K. pneumonia* and 80.4% of other *Enterobacterales*, which is similar to other studies assessing the activity of ceftolozane-tazobactam against Gram-negative isolates. Karlowsky *et al.* [14] reported a susceptibility of 89.7% of *Enterobacterales*, Kuo *et al.* [15] found 81.9% of *K. pneumoniae* and 91.9% of *E. coli*, Sader *et al.* [16] reported 98.5% of *E. coli and* 89.6% of *K. pneumoniae*, and Shortridge *et al.* [17] reported 95.5% of *Enterobacterales*. The variation in susceptibility profiles to ceftolozane-tazobactam can be explained by intrinsic and extrinsic mechanisms.

In our study, the susceptibility of *K. pneumoniae* to ceftolozane-tazobactam was the weakest. This was due to the incidence of carbapenemase and ESBL in this microorganism, most of them having a high rate of ceftolozane hydrolysis, such as bla_{OXA-48}, $bla_{CTX-M15}$, SHV and TEM. This is in accord with the results of Tuon *et al.* [18]. Carbapenem use results in heightened rates of carbapenem-resistant infections, limiting treatment options and growing mortality. Carbapenem resistance and ESBL detected in *Enterobacterales*, usually due to plasmid β-lactamases enzymes, have also become important issues [19].

In this study, the susceptibility of ceftolozane-tazobactam was 87.5% against *P. aeruginosa,* similar to reported susceptibility rates above 80% [15–17,20,21]. Ceftolozane-tazobactam was the third most active of the β-lactam agents tested against *P. aeruginosa* after colistin and amikacin-meropenem. The same result was reported by Garcia-Fernandez *et al.* [22], who found a susceptibility rate of 91.3%, which was the third most active, after

TABLE 3. Whole-genome sequencing data for *Klebsiella pneumoniae* examined

MIC C/T (μg/mL)	OmpK 35	OmpK 36	PBP3-ftsI[a]	β-lactamase summary (72% ident, 80% coverage)[b]	MLST
8	No lesion	Lesion	WT	CTX-M-15; OXA-1; SHV-1; TEM-1B	628
>32	No lesion	No lesion	WT	CTX-M-15; OXA-48; SHV-1-like	Novel-2
>32	Lesion	No lesion	WT	CTX-M-15; OXA-1; OXA-48; SHV-11	37
8	No lesion	Lesion	WT	CTX-M-15; OXA-1; SHV-11-like; TEM-1B-like	392

Abbreviations: MLST, multilocus sequence typing; WT, wild-type.
[a]PBP3-ftsI: Peniclin Binding Porin 3- FtsI gene.
[b]Threshold for β-lactamase gene inclusion was 72% and 80% for minimum nucleotide sequence identity and minimum sequence length, respectively.

TABLE 4. Whole-genome sequencing data for *Pseudomonas aeruginosa* isolates examined

C/T MIC (μg/mL)	*oprD*	PBP3(*ftsI*)	WGS β-lactamase summary[a]	Class C (intrinsic)	MLST[b]
>32	Lesion	WT	PDC-252-like; OXA-50-like; PER-1; VIM-2; OXA-4	PDC-252-like	233
>32	No lesion	WT	PDC-40-like; VIM-2; OXA-50-like	PDC-40-like	270
>32	No lesion	WT	PDC-72-like; OXA-50-like; PER-1	PDC-72-like	277
>32	Lesion	WT	PDC-119-like; VIM-2; PER-1; OXA-4; OXA-50-like	PDC-119-like	233
>32	No lesion	WT	PDC-183-like; OXA-50-like; VIM-2	PDC-183-like	769
>32	Lesion	WT	PDC-252-like; OXA-50-like; PER-1	PDC-252-like	233

Abbreviations: MLST[b], multilocus sequence typing; WGS[a], whole-genome sequencing; WT, wild-type.

colistin (95.0%) and amikacin (93.8%). The activity of ceftolozane-tazobactam is specifically more important in the context of intensive care units, where this microorganism is an extreme worry in the management of nosocomial infections because of its resistance to different antibiotics.

Our results show the presence of intrinsic mechanisms in ceftolozane-tazobactam-resistant *P. aeruginosa* isolates by producing *AmpC* overexpression by single point mutations in the bla_{PDC} gene (*AmpC* gene), and of extrinsic mechanisms by the presence of metallo-β-lactamase (VIM), associated with mutation of porin (*OprD*). This result is consistent with four studies demonstrating the presence of a carbapenemase in ceftolozane-tazobactam-resistant *P. aeruginosa* [23–26]. Ceftolozane-tazobactam has a better safety profile compared with colistin, which has high frequencies of nephrotoxicity, neurotoxicity, and allergic and topical reactions, as do aminoglycosides [27].

The main strength of this study was the genome sequencing of ceftolozane-tazobactam-resistant *P. aeruginosa* and *K. pneumonie* and the isolates collected were tested using a broth microdilution MIC method semi-quantitatively, which remains the reference method. It is also highly accurate in particular for these antimicrobials: colistin, cefepime and more recently, ceftolozane-tazobactam [28–33].

2A study limitation was the low number of *Enterobacterales* that were ceftolozane-tazobactam-resistant with genome sequencing. Although the study provided new information about the activity of ceftolozane-tazobactam against *Enterobacterales* and *P. aeruginosa* isolates. The study does not provide data about the incidence of infections in a given region.

Conclusions

Ceftolozane-tazobactam demonstrated relevant activity against most of the *Enterobacterales* and *P. aeruginosa* isolates. Ceftolozane-tazobactam could be considered a therapeutic alternative for the treatment of complicated urinary infections, complicated intra-abdominal infections and nosocomial pneumonia.

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Conflicts of interest

The authors have stated that there are no conflicts of interest.

References

[1] Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez Zorn B, Guerra B, et al. Plasmids carrying antimicrobial resistance genes in *Enterobacteriaceae*. J Antimicrob Chemother 2018;7:1121–37.

[2] Dantas RCC, Silva RTE, Ferreira ML, Gençalves IR, Araujo BF, De Compos PA, et al. Molecular epidemiological survey of bacteremia by multidrug resistant *Pseudomonas aeruginosa*: the relevance of intrinsic resistance mechanisms. PLoS One 2017;12:e0176774.

[3] Desenclos J-C, De Valk H. Les maladies infectieuses émergentes : importance en santé publique, aspects épidémiologiques, déterminants et prévention. Med Maladies Infect 2005;35:49–61.

[4] Monnet DL. Consommation d'antibiotiques et résistance bactérienne. Ann Fr Anesth Réanim 2000;19:409–17.

[5] European Centre for Disease Prevention and Control. 33000 people die every year due to infections with antibiotic-resistant bacteria. 2018. Available from: https://www.ecdc.europa.eu/en/news-events/33000-people-die-every-year-due-infections-antibiotic-resistant-bacteria. [Accessed 6 January 2020].

[6] Diallo O, Baron S, Jimeno MT, Abat C, Dubourg G, Chaudet H, et al. Mortalité des bactériémies causées par des bactéries difficiles à traiter (« Difficult to Treat Resistance » DTR). Med Maladies Infect 2019;49(Suppl. I):S48.

[7] World Health Organization. WHO publishes list of bacteria for which new antibiotics are urgently needed. Available from: https://www.who.int/fr/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed. [Accessed 2 September 2019].

[8] Zhanel GG1, Chung P, Adam H, Zelenitsky S, Denisuik A, Schweizer F, et al. Ceftolozane/tazobactam: a novel cephalosporin/β-lactamase inhibitor combination with activity against multidrug-resistant Gram-negative bacilli. Drugs 2014;74:31–51.

[9] US Food & Drug Administration. Ceftolozane Tazobactam – Injection products. Available from: from, https://www.fda.gov/drugs/development-resources/ceftolozane-tazobactam-injection-products. [Accessed 25 September 2019].

[10] Moya B, Zamorano L, Juan C, et al. Activity of a new cephalosporin, CXA-101 (FR264205), against beta-lactam-resistant *Pseudomonas aeruginosa* mutants selected in vitro and after antipseudomonal treatment of intensive care unit patients. Antimicrob Agents Chemother 2010;54:1213–7.

[11] Saran O, Sulik-Tyszka B, Basak GW, Wróblewska MM. Activity of ceftolozane/tazobactam against Gram-negative rods of the family Enterobacteriaceae and *Pseudomonas* spp. isolated from onco-hematological patients hospitalized in a clinical hospital in Poland. Med Sci Monit 2019;25:305–11.

[12] Moya B, Beceiro A, Cabot G, et al. Pan-beta-lactam resistance development in *Pseudomonas aeruginosa* clinical strains: molecular mechanisms, penicillin-binding protein profiles, and binding affinities. Antimicrob Agents Chemother 2012;56:4771–8.

[13] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing – twenty-seventh informational supplement. CLSI document M100S. Wayne, PA: CLSI; 2017 (ISBN 1-56238-923-8).

[14] Karlowsky JA, Kazmierczak KM, Young K, Motyl MR, Sahm DF. In vitro activity of ceftolozane/tazobactam against phenotypically defined extended-spectrum β-lactamase (ESBL)-positive isolates of *Escherichia coli* and *Klebsiella pneumoniae* isolated from hospitalized patients (SMART 2016). Diagn Microbiol Infect Dis 2019;96:114925.

[15] Kuo S-C, Liu C-E, Lu P-L, Chen Y-S, Lu M-C, Ko W-C, et al. The SMART Asia-Pacific Group. Activity of ceftolozane/tazobactam against gram-negative pathogens isolated from lower respiratory tract infections in the Asia-Pacific region: SMART 2015–2016. Int J Antimicrob Agents 2020. https://doi.org/10.1016/j.ijantimicag.2020.105883.

[16] Sader HS, Farrell DJ, Flamm RK, Jones RN. Ceftolozane/tazobactam activity tested against aerobic gram-negative organisms isolated from intra-abdominal and urinary tract infections in European and United States hospitals, 2012. J Infect 2014;69:266–77.

[17] Shortridge D, Pfaller MA, Castanheira M, Flamm RK. Antimicrobial activity of ceftolozane-tazobactam tested against Enterobacteriaceae and Pseudomonas aeruginosa with various resistance patterns isolated in U.S. hospitals (2013–2016) as part of the surveillance program: program to assess ceftolozane-tazobactam susceptibility. Microb Drug Resist 2018;24:563–77.

[18] Tuon FF, Cieslinski J, Rodriguesb SS, Serrab FB, Paula MD. Evaluation of in vitro activity of ceftolozane–tazobactam against recent clinical bacterial isolates from Brazil – the EM200 study. Braz J Infect Dis 2020;24:96–103.

[19] Tängdén T, Giske CG. Global dissemination of extensively drug-resistant carbapenemase-producing *Enterobacteriaceae*: clinical perspectives on detection, treatment and infection control. J Intern Med 2015;277:501–12.

[20] Kuti JL, Pettit RS, Neu N, Cies JJ, Lapin C, Muhlebach MS, et al. Microbiological activity of ceftolozane/tazobactam, ceftazidime, meropenem, and piperacillin/tazobactam against *Pseudomonas aeruginosa* isolated from children with cystic fibrosis. Diagn Microbiol Infect Dis 2015;83:53–5.

[21] Sutherland CA, Nicolau DP. Susceptibility profile of ceftolozane/tazobactam and other parenteral antimicrobials against *Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* from US hospitals. Clin Ther 2015 jul;37:1564–71.

[22] García-Fernández S, García-Castillo M, Bou G, et al. Activity of ceftolozane–tazobactam against *Pseudomonas aeruginosa* and *Enterobacterales* isolates recovered in Intensive Care Units in Spain: the SUPERIOR multicentre study. Int J AntimicrobAgents 2019;53:682–8.

[23] Araujo BF, Ferreira ML, De Campos PA, et al. Clinical and molecular epidemiology of multidrug-resistant *P. aeruginosa* carrying aac(6_)-ib-cr, qnrs1 and blaspm genes in Brazil. PLoS One 2016;11:1–15.

[24] Dantas RCC, Silva RTE, Ferreira ML, et al. Molecular epidemiological survey of bacteremia by multidrug resistant *Pseudomonas aeruginosa*: the relevance of intrinsic resistance mechanisms. PLoS One 2017;12:1–14.

[25] Dantas RCC. Estudo epidemiológico molecular da resistênciaaos carbapenêmicos em *Pseudomonas aeruginosa* isoladas desangue: produção de β-lactamases, perda de porina OprD ehiperexpressão de bombas de efluxo. Universidade Federal de Uberlândia; 2015.

[26] Cacci LC, Chuster SG, Martins N, do Carmo PR, Girão VPC, Nouér SA, et al. Mechanisms of carbapenem resistance in endemic *Pseudomonas aeruginosa* isolates after an SPM-1 metallo-β-lactamase producing strain subsided in an intensive care unit of a teaching hospital in Brazil. Mem Inst Oswaldo Cruz 2016;111:551–8.

[27] Bassetti M, Russo A, Carnelutti A, La Rosa A, Righi E. Antimicrobial resistance and treatment: an unmet clinical safety need. Expert Opin Drug Saf 2018;17:669–80.

[28] Sid Ahmed MA, Abdel Hadi H, Hassan AAI, Abu Jarir S, Al-Maslamani MA, Eltai NO, et al. Evaluation of in vitro activity of ceftazidime/avibactam and ceftolozane/tazobactam against MDR *Pseudomonas aeruginosa* isolates from Qatar. J Antimicrob Chemother 2019. pii:dkz379. Epub ahead of print.

[29] Castanheira M, Duncan LR, Mendes RE, Sader HS, Shortridge D. Activity of ceftolozane-tazobactam against *Pseudomonas aeruginosa* and *Enterobacteriaceae* isolates collected from respiratory tract specimens of hospitalized patients in the United States during 2013 to 2015. Antimicrob Agents Chemother March 2018;62. e02125-17.

[30] Flynt LK, Veve MP, Samuel LP, et al. Comparison of Etest to broth microdilution for testing of susceptibility of *Pseudomonas aeruginosa* to ceftolozane-tazobactam. J Clin Microbiol 2017;55:334–5.

[31] Simar S, Sibley D, Ashcraft D, et al. Colistin and polymyxin B minimal inhibitory concentrations determined by Etest found unreliable for gram-negative bacilli. Ochsner J 2017;17:239–42.

[32] Smith KP, Brennan-Krohn T, Weir S, et al. Improved accuracy of cefepime susceptibility testing for extended-spectrum-beta-lactamase producing *Enterobacteriaceae* with an on-demand digital dispensing method. J Clin Microbiol 2017;55:470–8.

[33] Rogacka AR, Xavier BB, Janssens L, Lammens C, Zarkotou O, Pournaras S, et al. Evaluation of colistin stability in agar and comparison of four methods for MIC testing of colistin. Eur J Clin Microbiol Infect Dis 2018;37:345–53.