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Short Communication

In vitro activity of ceftolozane-tazobactam against *Enterobacterales* and *Pseudomonas aeruginosa* causing urinary, intra-abdominal and lower respiratory tract infections in intensive care units in Portugal: The STEP multicenter study

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ABSTRACT

The STEP surveillance study was designed to increase knowledge about distribution of multidrug-resistant (MDR) *Enterobacterales* and *Pseudomonas aeruginosa* in Portugal, focusing on the intensive care unit (ICU). Antimicrobial susceptibility of common agents was also evaluated and compared with that of one of the latest therapeutic introductions, ceftolozane-tazobactam (C/T). Clinical isolates of *Enterobacterales* (n=426) and *P. aeruginosa* (n=396) from patients admitted in Portuguese ICUs were included. Activity of C/T and comparators was investigated using standard broth microdilution. Isolates were recovered from urinary tract (UTI, 36.9%), intra-abdominal (IAI, 24.2%) and lower respiratory tract (LRTI, 38.9%) infections. In *P. aeruginosa*, overall distribution of MDR/extremely-drug resistant (XDR)/pan-drug resistant (PDR) isolates accounted for 21.2%, 23.2% and 0.8%, respectively. C/T was the most potent agent tested against *P. aeruginosa* and MDR/XDR/PDR phenotypes. In *Escherichia coli*, extended-spectrum beta-lactamases (ESBL) and carbapenemase (CP) phenotypes accounted for 16.6% and 1.7%, respectively, whereas in *Klebsiella* spp., ESBL and CP-phenotypes represented 28.5% and 17.9%, respectively. Overall, susceptibility of C/T against *Enterobacterales* was 86.9%. C/T was the least affected agent in *E. coli* (99.4% susceptibility), whereas its activity was moderate in *Klebsiella* spp. (71.5%) and *Enterobacter* spp. (70.4%), due in part to a high rate of ESBL and CP-phenotypes. In *Enterobacterales*, bla_{KPC} was the most prevalent CP gene (63.0%), followed by bla_{OXA-48} (33.3%) and bla_{VIM} (3.7%). These microbiological results reinforce C/T as a therapeutic option in ICU patients with UTI, IAI or LRTI due to *P. aeruginosa* or *Enterobacterales* isolates, but not for CP producers.

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1. Introduction

Public health authorities have highlighted an increasing worldwide prevalence of antimicrobial resistance. This increase has been demonstrated in surveillance studies, particularly in Gram-negatives included in the so-called ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp.) microorganisms [1,2]. Moreover, in Europe, the European Centre for Disease Prevention and Control (ECDC) has shown that antimicrobial resistance remains a serious threat, and is more important in the Mediterranean area than in Northern countries [3]. In Portugal, there are scarce data on antimicrobial surveillance, activity of antimicrobials and molecular epidemiology of resistance mechanisms. In invasive isolates recovered in Portugal in 2017, resistance to third-generation cephalosporins in *Escherichia coli* and *K. pneumoniae* accounted for 15.6% and 44.9%, respectively [3]. Resistance to carbapenems increased in *E. coli* from <0.1% to 0.3%, and in *K. pneumoniae* from 1.8% to 8.6% during the period 2014 to 2017 [3], and the predominant carbapenemase (CP) in Portugal was the bla_{KPC} [4,5]. In *P. aeruginosa* isolates, resistance rates to piperacillin-tazobactam, ceftazidime and carbapenems were 24.2%, 18.6% and 18.3%, respectively [3]. There is even less information on high-risk areas for resistance, such as intensive care units (ICUs).

New β-lactam-β-lactamase inhibitors combinations have recently been developed to mitigate the effects of multidrug-resistant (MDR) organisms [6–8]. Ceftolozane-tazobactam (C/T) was initially approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for complicated urinary tract infections (cUTI) and complicated intra-abdominal infections (cIAI). This combination has also been recently approved by the FDA for hospital-acquired and ventilator-associated bacterial pneumonia [9]. In the STEP (Susceptibility Testing on *Enterobacterales* and *Pseudomonas aeruginosa)* study, the in vitro activity of C/T and comparators was assessed against *Enterobacterales* and *P. aeruginosa* clinical isolates prospectively collected from ICU patients with UTI, IAI, and lower respiratory tract infections (LRTI) in Portugal.

2. Material and Methods

2.1. Study design and setting

A prospective, multicenter study was designed to assess the in vitro activity of C/T and comparator antimicrobials against clinical isolates of *Enterobacterales* and *P. aeruginosa* prospectively recovered in Portuguese ICUs (June 2017-July 2018). Eleven Portuguese hospitals participated in the study (Fig. S1). University Hospital Ramón y Cajal in Madrid (Spain) acted as coordinator laboratory (hereafter central laboratory). Species identification was performed at each participant site and confirmed at the central laboratory using MALDI-TOF (Bruker-Daltonics, Bremen, Germany). The ethics committees of all participating sites in Portugal approved the study.

2.2. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined at the central laboratory using the standard broth microdilution method (BMD) using frozen 96-well plates (Thermo Fisher Scientific, Cleveland, OH) [10]. The antimicrobials tested were: amikacin (AMK), amoxicillin-clavulanic acid (AMC), aztreonam, cefepime (FEP), cefotaxime (CTX), ceftazidime (CAZ), C/T, ciprofloxacin (CIP), colistin (CST), fosfomycin, gentamicin, imipenem (IPM), meropenem (MEM), piperacillin-tazobactam (TZP), tigecycline (TGC) and tobramycin. *E. coli* ATCC25922, *E. coli* ATCC35218, *K. pneumoniae* ATCC700603 and *P. aeruginosa* ATCC27853 were used as quality control. Interpretation of results and quality control were performed in accordance with European Committee on Antibiotic Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) guidelines [11,12]. For comparison purposes, the definition of susceptible (S) [susceptible plus susceptible, increased exposure (I), formerly intermediate for EUCAST] was applied to both EUCAST and CLSI. Moreover, resistant (R) clinical category was considered for phenotype definition. C/T breakpoints used were the following: [*Enterobacterales*, EUCAST (S, ≤2/4 mg/L; R, >2/4 mg/L) and CLSI (S, ≤2/4 mg/L; I, 4/4 mg/L; R, ≥8/4 mg/L); *P. aeruginosa*, EUCAST (S, ≤4/4 mg/L; R, >4/4 mg/L) and CLSI (S, ≤4/4 mg/L; I, 8/4 mg/L; R, ≥16/4 mg/L)].

2.3. Phenotypic classification of isolates

To evaluate the activity of C/T against *Enterobacterales*, the following phenotypes were defined according to susceptibility to β-lactam antibiotics: 1) Extended-spectrum β-lactamase (ESBL) phenotype and 2) CP-phenotype. All isolates were evaluated according to CLSI and EUCAST screening criteria for suspected ESBL (MICs ≥2 mg/L for CTX, CAZ and/or FEP) producers [12]. Phenotypic confirmation of ESBL production was performed using the double-disk synergy (DDS) test. Suspected CP isolates displaying MICs of >1 mg/L for IPM and/or >0.12 mg/L for MEM were evaluated [12,13]. Phenotypic confirmation of CP production was performed using the ROSCO KPC/MBL and OXA-48 Confirm Kit (Rosco Diagnostica A/S, Taastrup, Denmark) following manufacturer instructions.

In *P. aeruginosa* isolates, the following resistant phenotypes were also defined using EUCAST interpretative criteria: 1) TZP-CAZ-R: combined TZP and CAZ non-susceptibility; 2) TZP-CAZ-MER-R: combined TZP, CAZ and MER non-susceptibility; 3) MDR: non-susceptibility to at least one agent in three or more antimicrobial categories; 4) Extensively drug-resistant (XDR): non-susceptibility to at least one agent in all but two or fewer antimicrobial categories; and 5) Pan-drug-resistant (PDR): non-susceptibility to all antimicrobials tested (except C/T) [14].

2.4. Molecular characterization

bla_{ESBL} and $bla_{carbapenemase}$ genes in *Enterobacterales* isolates with ESBL or CP-phenotype were characterized as previously described [15]. In *P. aeruginosa* isolates resistant to a C/T, CP genes were investigated using Cepheid Xpert® Carba-R assay (Cepheid, Sunnyvale, CA, USA).

3. Results

3.1. Bacterial isolates

A total of 822 clinical isolates of *Enterobacterales* (n=426) and *P. aeruginosa* (n=396) were collected from June 2017 to July 2018 from patients admitted in Portuguese ICUs. Isolates were recovered from UTI (n=303, 36.9%), IAI (n=199, 24.2%) and LRTI (n=320, 38.9%). Only one isolate per patient was included. Distribution of isolates by species and infection type is shown in Table S1.

3.2. Antimicrobial susceptibility of Enterobacterales isolates

Among *E. coli* isolates, 16.6% had a positive DDS test expressing an ESBL-phenotype (n=29), 1.7% a CP-phenotype (n=3) and 81.7% (n=143) were non-ESBL-CP. In *Klebsiella* spp. isolates, 28.5% had a positive DDS test expressing an ESBL-phenotype (n=43), 17.9% CP-phenotype (n=27) and 53.6% (n=81) non-ESBL-CP. Distribution of ESBL and CP-phenotypes by source of infection is shown in Fig. S2.

Antimicrobial activity, MIC_{50}/MIC_{90} and MIC range of C/T and comparator agents for *Enterobacterales* broken down by species

Table 1
Antimicrobial activity of ceftolozane-tazobactam in *Enterobacterales* broken down by species and source of infection using EUCAST breakpoints.

Organisms	IAI		LRTI		UTI		TOTAL	
	n (%)	[a]S	n (%)	S	n (%)	S	n	S
Enterobacterales	119 (27.9)	88.2	94 (22.1)	78.7	213 (50.0)	89.7	426	86.9
E. coli	51 (29.1)	100.0	16 (9.1)	100.0	108 (61.7)	99.1	175	99.4
ESBL-*E. coli*	7 (24.1)	100.0	3 (10.31)	100.0	19(65.5)	100	29	100
Klebsiella spp.	38 (25.2)	73.7	46 (30.5)	60.9	67 (44.4)	77.6	151	71.5
ESBL-*Klebsiella* spp.	15 (34.9)	66.7	9 (20.9)	44.4	19 (44.2)	52.6	43	55.8
CP-*Klebsiella* spp.	6 (22.2)	16.7	12 (44.4)	16.7	9 (33.3)	44.4	27	25.9
Enterobacter spp.	15 (40.5)	73.3	13 (35.1)	84.6	9 (24.3)	44.4	37	70.3
Citrobacter spp.	3 (30.0)	100	4 (40.0)	100	3 (30.0)	100	10	100
M. morganii	2 (22.2)	100.0	1 (11.1)	100	6 (66.7)	83.3	9	88.9
Proteus spp.	5 (20.0)	100.0	4 (16.0)	100.0	16 (64.0)	100.0	25	100.0
Serratia spp.	4 (26.7)	100.0	7 (46.7)	100.0	4 (26.7)	100.0	15	100.0
Raoultella spp.	1 (33.3)	100	2 (66.6)	100	0	-	3	100
P. rettgeri	0	-	1 (100)	100	0	-	1	100

[a] Abbreviations: Susceptible (%) [susceptible (S) plus susceptible, increased exposure (I)]; ESBL, extended-spectrum β-lactamases; CP, carbapenemases; IAI, intraabdominal infection; LRTI, lower respiratory tract infection; UTI, urinary tract infection; EUCAST, European Committee on Antibiotic Susceptibility Testing.

are shown in Table S2. Data related to species with less than 10 isolates are reported in the text (e.g. *Morganella morganii* n=9). The most potent agent against *E. coli* was C/T [99.4/99.4% S EUCAST/CLSI; $MIC_{50/90}$, 0.5/1 mg/L], along with the carbapenems, MEM (98.9/98.9% S; $MIC_{50/90}$, ≤0.25/≤0.25 mg/L) and IPM (98.9/98.9%S; $MIC_{50/90}$, ≤0.25/≤0.25 mg/L). Conversely, susceptibility rates against *E. coli* of other β-lactams were less than 91% [e.g. AMC (34.3/49.2% S) or TZP (85.2/91.4% S)]. C/T in vitro activity was maintained regardless of the source of infection: IAI, 100% S; LTRI, 100% S; and UTI, 99.1% S (Table 1). In ESBL-*E. coli*, C/T showed the highest activity (100/100% S, $MIC_{50/90}$, 1/1 mg/L) along with the carbapenems, MEM and IPM (both 100% S, $MIC_{50/90}$, ≤0.25/≤0.25 mg/L). Only one (0.6%) *E. coli* isolate was resistant to C/T. This isolate expressed a CP-phenotype (MIC=16/4 mg/L) (Fig. 1).

In *Klebsiella* spp., the more active antimicrobials were AMK (92.8/97.3% S, EUCAST/CLSI; $MIC_{50/90}$ ≤8/16, mg/L) and CST (92.7%S; $MIC_{50/90}$ ≤2/≤2, mg/L). C/T overall susceptibility was 71.5/76.8% ($MIC_{50/90}$, 1/>64 mg/L). The effect of ESBL and CP-phenotypes on C/T susceptibility is reflected in Fig. 1. Activity of C/T against non-ESBL-CP-*Klebsiella* spp. isolates (n=81) was 95.1/98.8% S. In ESBL-phenotype, the carbapenems were the most active compounds [IPM (100/95.3% S) and MEM (97.7/95.4% S)] followed by CST (97.7% S). In CP-phenotype, CST (77.8% S), AMK (70.4/92.6% S) and TGC (74.1% S) presented the highest activity.

Regarding C/T activity by source of infection, in all *Klebsiella* spp. isolates, activity in IAI, LRTI and UTI was 73.7%, 60.9% and 76.6% S, respectively. C/T activity against ESBL-phenotype was in all sources <67.0% and susceptibility rate ranged from 16.7% to 44.4% against CP-phenotype (Table 1). There were 43 (28.8%) *Klebsiella* spp. isolates resistant to C/T (EUCAST breakpoints). Distribution by phenotypes was as follows: 4 non-ESBL-CP (3 *Klebsiella aerogenes*, 1 *K. pneumoniae*), 19 ESBL-phenotype and 20 CP-phenotype.

Against less common *Enterobacterales*, C/T activity was excellent in *Citrobacter* spp. (100/100% S EUCAST/CLSI; $MIC_{50/90}$, 0.5/2 mg/L), *M. morganii* (89.0/89.0% S), *Proteus mirabilis* (100/100%, $MIC_{50/90}$, 1/1 mg/L), *Providencia rettgerii* (100/100% S), *Raoultella* spp. (100/100% S) and *Serratia* spp. (100/100% S, $MIC_{50/90}$, 0.5/1 mg/L). Nevertheless, C/T showed lower activity in *Enterobacter* spp. (70.3/73.0% S, $MIC_{50/90}$, 1/16 mg/L) (Table S2).

3.3. Molecular characterization of Enterobacterales isolates

Twenty-nine *E. coli* isolates expressing ESBL-phenotype were found. Distribution of β-lactamase genes was as follows: bla_{CTX-M} (n=18), $bla_{CTX-M+TEM}$ (n=5), bla_{SHV} (n=3), and bla_{TEM} (n=1). Two isolates with an ESBL-phenotype were not confirmed by conventional PCR. Distribution of ESBL-*Klebsiella* spp. isolates (n=43) were: $bla_{CTX-M+TEM}$ (n=23), bla_{CTX-M} (n=6), $bla_{CTX-M+SHV+TEM}$ (n=6), $bla_{CTX-M+SHV}$ (n=3), bla_{SHV} (n=3), bla_{TEM} (n=1) and $bla_{SHV+TEM}$ (n=1).

Regarding CP genes, in CP-*E. coli* (n=3), one bla_{OXA-48} was found (two isolates were not confirmed by the molecular assay previously indicated). In CP-*Klebsiella* spp. (n=27), bla_{KPC} (n=17), bla_{OXA-48} (n=8), and bla_{VIM} (n=1) genes were detected. CP production was not confirmed in one isolate. A bla_{ESBL} gene was also found in 24 of 27 CP-*Klebsiella* spp. isolates. Overall, concerning the type of confirmed CP gene in both *E. coli* and *Klebsiella* spp. isolates (n=27), bla_{KPC} was the most prevalent (17/27, 63.0%), followed by bla_{OXA-48} (9/27, 33.3%) and bla_{VIM} (1/27, 3.7%).

In *Enterobacter* spp. isolates resistant to C/T (n=11), two ESBL-producers were identified (bla_{SHV} and bla_{CTX-M}). No CP genes were detected.

3.4. Antimicrobial susceptibility of Pseudomonas aeruginosa isolates

Of 396 clinical isolates, 21.2% (n=84) were classified as MDR, 23.2% (n=92) as XDR and 0.8% (n=3) as PDR, whereas only 17.0% (n=67) were susceptible to every antipseudomonal agent tested.

Antimicrobial activity, MIC_{50}/MIC_{90} and MIC range of C/T and comparator agents tested against *P. aeruginosa* and MDR/XDR phenotypes are shown in Table S3. C/T was the most potent antimicrobial tested against *P. aeruginosa* (94.7/95.5% S; $MIC_{50/90}$, 1/4 mg/L), followed by amikacin and tobramycin, applying EUCAST (both 88.9% S) or colistin and amikacin, with CLSI (96.2% S and 94.2% S, respectively). Rates of resistance within antipseudomonal β-lactams were as follows: TZP (35.9/24.5% R, EUCAST/CLSI), CAZ (42.2/30.1% R), FEP (47.2/23.7% R) and MEM (24.5/36.1% R).

C/T was also the best agent against MDR and XDR phenotypes (100% and 79.4/82.7% S, respectively) (Table S3). This activity was retained against isolates resistant to different antipseudomonal agents and combinations (e.g. TZP-CAZ-MEM-R, 70.8% S), in which C/T showed a better activity than comparators like CIP, CST or AMK (Table 2). MIC distributions of C/T against *P. aeruginosa* show how XDR/PDR phenotypes represent the resistant population (Fig. 1). Of 21 (5.3%) *P. aeruginosa* isolates resistant to C/T, 19 were XDR and 2 PDR. The bla_{VIM} gene was detected in 3 of 21 (14.3%) isolates.

The proportion of MDR (19-26%) and XDR (20-26%) isolates was similar between types of infection. However, PDR isolates were only found in UTI (Fig. S2). The analysis of susceptibility rates by source of infection also showed C/T to be the most potent agent in all of them, and the activity was as follows: 91.3% S in IAI, 98.2% S

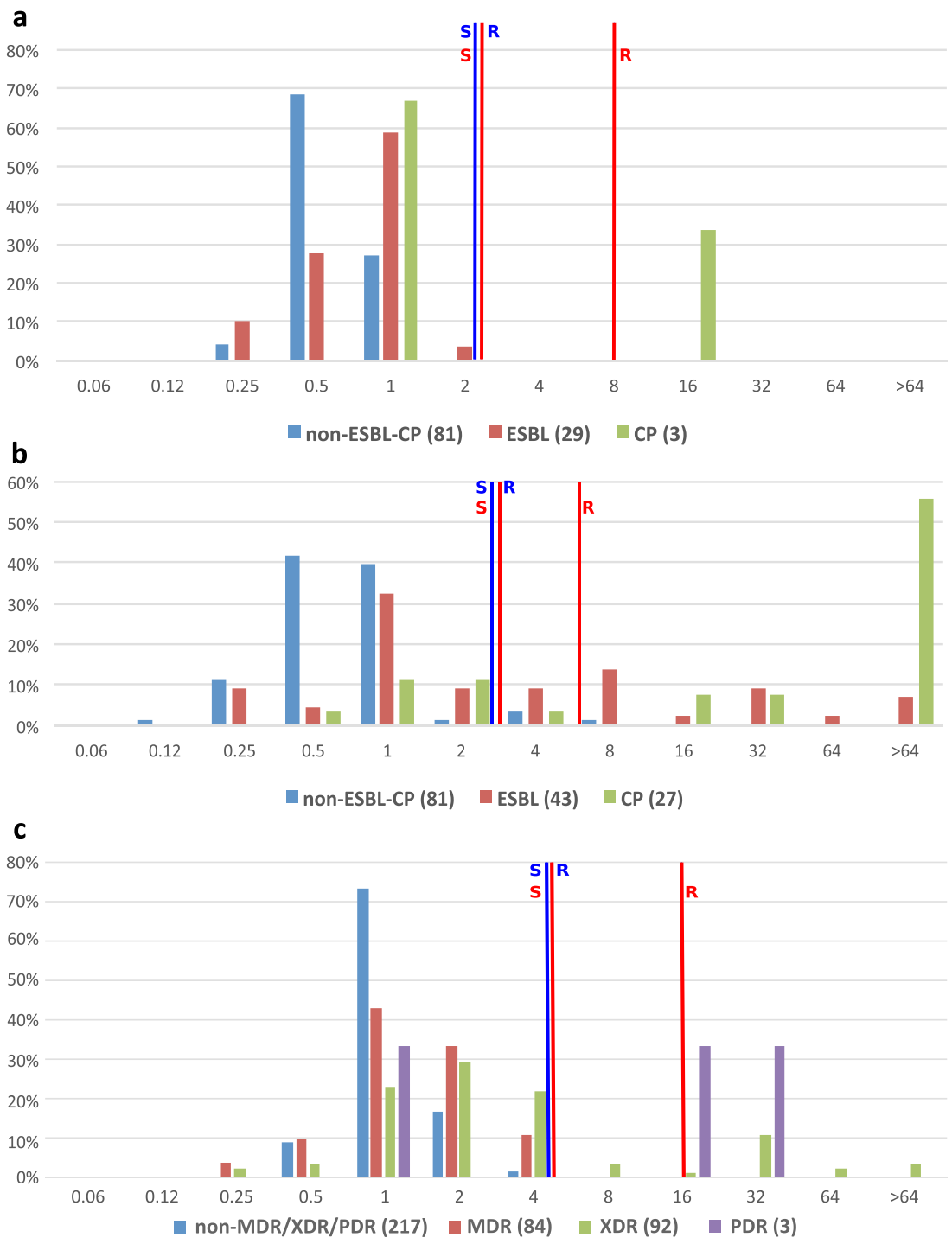

Fig. 1. MIC distribution of C/T in *E. coli* (a), *Klebsiella* spp. (b) and P. *aeruginosa* (c). EUCAST and CLSI breakpoints are displayed with blue and red lines, respectively. Abbreviations: ESBL, extended-spectrum ß-lactamases; CP, carbapenemases; MDR, multidrug-resistant; XDR, extremely-drug resistant; PDR, pan-drug resistant.

in LRTI and 88.9% S in UTI, greater than other antipseudomonal β-lactams [TZP (≤76.3%), CAZ (≤64.4%) and MEM (≤77.8%)]. Regarding other antibiotics, susceptibility rates were CST (≤81.3%), CIP (≤63.8%) and AMK (≤93.8%) (Table 2).

3.5. Antimicrobial susceptibility according to different participating hospitals

Overall activity of C/T in *Enterobacterales* by participant hospital was diverse (from 67.6% S to 96.4% S). There was no difference regarding C/T activity between *E. coli* isolates (in 10 of 11 sites, 100% S), whereas in *Klebsiella* spp. isolates, susceptibility to C/T ranged from 44.4% to 100%. This was mostly because of a diverse proportion of ESBL-*Klebsiella* spp. isolates (0-65.2%, and only one site was without ESBL) and CP-phenotypes (0-44.4%, and three sites without CP) (Table S4 and Fig. S1).

In *P. aeruginosa* isolates, C/T activity differed slightly between hospitals (87.1% S to 100% S) and was 100% S in 4 of 11 hospitals. MDR phenotype was not found in only one center, and the proportion of MDR in the others was 14.3% to 33.3%. Regarding XDR, this phenotype was not detected in one hospital, and ranged from 18.5% to 35.5% in the other centers (Table S5 and Fig. S1).

Table 2
Susceptibility[a] (%) of ceftolozane-tazobactam and comparators by resistance phenotypes and source of infection in *P. aeruginosa* according to EUCAST breakpoints.

	C/T	TZP	CAZ	MEM	CST	CIP	AMK
All *P. aeruginosa* (396)	94.7	64.1	57.8	75.5	78.3	62.9	88.9
TZP-R[b] (142)	85.2	-	10.6	46.5	75.4	39.4	78.2
CAZ-R (167)	87.4	24.0	-	50.9	74.3	46.7	80.2
MEM-R (97)	78.4	21.7	15.5	-	72.2	23.7	67.0
TZP-CAZ-R (127)	83.5	-	-	43.3	74.8	35.4	76.4
TZP-CAZ-MEM-R (72)	70.8	-	-	-	73.6	16.7	61.1
MDR (84)	100.0	51.7	41.4	79.3	70.1	57.1	94.0
XDR (92)	79.4	7.6	9.8	22.8	68.5	12.0	65.2
IAI-*P. aeruginosa* (80)	91.3	76.3	57.5	72.5	81.3	63.8	83.8
LTRI-*P. aeruginosa* (226)	98.2	59.7	55.3	75.7	79.7	63.3	93.8
UTI-*P. aeruginosa* (90)	88.9	64.4	64.4	77.8	72.2	61.1	81.1

Abbreviations: C/T, ceftolozane-tazobactam; TZP, piperacillin-tazobactam; CAZ, ceftazidime; MEM, meropenem; CST, colistin; CIP, ciprofloxacin; AMK, amikacin; MDR, multidrug-resistant; XDR, extremely-drug resistant; IAI, intraabdominal infection; LRTI, lower respiratory tract infection; UTI, urinary tract infection; EUCAST, European Committee on Antibiotic Susceptibility Testing.
[a] Susceptible [susceptible (S) plus susceptible, increased exposure (I)]
[b] Resistant

4. Discussion

To the best of our knowledge, this is the first surveillance study in Portugal focusing on antimicrobial susceptibility profiles in isolates causing infections in ICUs. This study provides information about distribution of ESBL-CP-phenotypes in *Enterobacterales* and MDR/XDR/PDR phenotypes in *P. aeruginosa* in this country.

Results in *Enterobacterales* reaffirm that prevalence of CP-producing *Enterobacterales* (CPE) is increasing in Portugal [16]. Data from EARS-Net showed levels of carbapenem resistance no greater than 0.3% or 8.6% in *E. coli* and *K. pneumoniae*, respectively [3]. In contrast, this study showed higher rates in *E. coli* (up to 1.1% or 2.6% R, applying EUCAST or CLSI, respectively) and *Klebsiella* spp. (up to 17.2% or 19.9% R, EUCAST/CLSI). Only *K. pneumoniae* isolates rates were up to 20.8% R in EUCAST and CLSI. Previous studies of CP distribution in Portugal showed a dominance of *Klebsiella pneumoniae* carbapenemase (KPC; >85%) within confirmed CPE with the absence of OXA-48 [4,5]. This enzyme was not detected in Portugal until 2013 [17]. Our survey indicated the relevant prevalence of OXA-48 (33.3%) in CPE in a setting where KPC-enzymes (63.0%) continue to dominate.

C/T was the most active antimicrobial tested along with carbapenems against *E. coli* and other less represented *Enterobacterales* species. This is consistent with other studies performed in other countries [18–20]. Indeed, C/T was 100/100% S (EUCAST/CLSI) against ESBL-*E. coli*. The activity against *Klebsiella* spp. isolates was more modest (71.5/76.8% S) and also consistent with earlier studies [18,21]. A high proportion of ESBL and CP-phenotypes was found in our survey in *Klebsiella* spp. isolates, 28.5% and 17.9%, respectively. Nevertheless, C/T activity against non-ESBL-non-CP *Klebsiella* spp. was 95.1/98.8% S. Due to a diverse proportion of ESBL and CP-phenotypes (Fig. S1), there were differences in C/T activity by source of infection (e.g. C/T susceptibility in *Klebsiella* spp. was 60.9/73.7/77.6% S in LRTI, IAI and UTI, respectively). Previous studies have reported less activity of C/T in *Enterobacter* spp., with ESBL-production or AmpC-overproduction possible reasons for this observation [22].

On the other hand, high resistant rates were found in *P. aeruginosa* isolates from ICU patients in this survey. Resistance to antipseudomonal β-lactams (>24.5% R in TZP, CAZ and MEM) was considerably above the rates described in the last EARS-Net report [3]. In fact, no antimicrobial expressed a susceptibility rate >90% S (EUCAST breakpoint) against *P. aeruginosa*, except for C/T. Despite the high rates of resistance in *P. aeruginosa* isolates in this survey, C/T was the best antimicrobial tested (94.7/95.5% S, EUCAST/CLSI), followed by AMK and TOB. This susceptibility rate was maintained against MDR (100% S) and XDR (79.4/82.7% S) isolates, and against different antipseudomonal resistant phenotypes. These results agree with recent European surveys, with C/T susceptibility rates up to 90% [19,21,22]. C/T was also superior to every comparator agent analysed by source of infection, consolidating its position as a treatment for *P. aeruginosa*-causing infections. In nearly 15% of the *P. aeruginosa* isolates, resistance to C/T was due to the presence of a metallo-beta-lactamase. In the remaining C/T-resistant isolates, other mechanisms might be involved, such as Guiana extended-spectrum (GES) enzymes or multiple mutations leading to overexpression and structural modifications of AmpC [23–25]. The differences in C/T activity by participant hospital (susceptibility rates in *Enterobacterales* from 67.6% to 96.4% or 87.1% to 100% in *P. aeruginosa*) and by source of infection reflect the importance of surveillance studies to enhance knowledge of local epidemiology and impact local guidelines.

In conclusion, C/T was the most potent agent tested against *P. aeruginosa* recovered from ICU patients; this activity was maintained regardless of a resistant phenotype. In *Enterobacterales*, C/T exhibited good overall activity against *E. coli*, although it might be affected by local epidemiology in other species, such as *Klebsiella* spp. and *Enterobacter* spp. These microbiological results reinforce C/T as a therapeutic option in ICU patients with UTI, IAI or LRTI due to *P. aeruginosa* or *Enterobacterales* isolates, but not for CP producers. Furthermore, this study provides information about molecular epidemiology of CPE in Portugal, particularly the presence of OXA-48 in a setting where KPC enzymes are the dominant CP.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2020.105887.

References

[1] Castanheira M, Deshpande LM, Mendes RE, Canton R, Sader HS, Jones RN. Variations in the occurrence of resistance phenotypes and carbapenemase genes among Enterobacteriaceae isolates in 20 Years of the SENTRY Antimicrobial Surveillance Program. Open Forum Infect Dis 2019;6:S23–33. doi:10.1093/ofid/ofy347.

[2] Shortridge D, Gales AC, Streit JM, Huband MD, Tsakris A, Jones RN. Geographic and temporal patterns of antimicrobial resistance in Pseudomonas aeruginosa over 20 years from the SENTRY Antimicrobial Surveillance Program, 1997-2016. Open Forum Infect Dis 2019;6:S63–8. doi:10.1093/ofid/ofy343.

[3] European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe 2017. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC. 2018.

[4] Manageiro V, Romão R, Moura IB, Sampaio DA, Vieira L, Ferreira E, et al. Molecular epidemiology and risk factors of carbapenemase-producing Enterobacteriaceae isolates in Portuguese Hospitals: results from European Survey on carbapenemase-producing Enterobacteriaceae (EuSCAPE). Front Microbiol 2018;9:2834. doi:10.3389/fmicb.2018.02834.

[5] Manageiro V, Ferreira E, Almeida J, Barbosa S, Simões CAntibiotic Resistance Surveillance Program in Portugal (ARSIP). Predominance of KPC-3 in a Survey for Carbapenemase-Producing Enterobacteriaceae in Portugal. Antimicrob Agents Chemother 2015;59:3588–92. doi:10.1128/AAC.05065-14.

[6] van Duin D, Bonomo RA. Ceftazidime/avibactam and ceftolozane/tazobactam: second-generation β-lactam/β-lactamase inhibitor combinations. Clin Infect Dis 2016;63:234–41. doi:10.1093/cid/ciw243.

[7] Moya B, Zamorano L, Juan C, Pérez JL, Ge Y, Oliver A. 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;54:1213–17. doi:10.1128/AAC.01104-09.

[8] Castanheira M, Rhomberg PR, Flamm RK, Jones RN. Effect of the β-lactamase inhibitor vaborbactam combined with meropenem against serine carbapenemase-producing Enterobacteriaceae. Antimicrob Agents Chemother 2016;60:5454–8. doi:10.1128/AAC.00711-16.

[9] https://www.fda.gov/news-events/press-announcements/fda-approves-new-treatment-hospital-acquired-and-ventilator-associated-bacterial-pneumonia n.d.

[10] CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 11th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2018. CLSI standard M07n.d.

[11] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0. 2020.

[12] CLSI. Performance standards for antimicrobial susceptibility testing. 28th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2018. CLSI supplement M100n.d.

[13] Livermore DM. beta-Lactamases in laboratory and clinical resistance. Clin Microbiol Rev 1995;8:557–84.

[14] Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pan-drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81. doi:10.1111/j.1469-0691.2011.03570.x.

[15] Ruiz-Garbajosa P, Hernández-García M, Beatobe L, Tato M, Méndez MI, Grandal M, et al. A single-day point-prevalence study of faecal carriers in long-term care hospitals in Madrid (Spain) depicts a complex clonal and polyclonal dissemination of carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 2016;71:348–52. doi:10.1093/jac/dkv355.

[16] Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DLEuropean Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) working group. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries, May 2015. Euro Surveill 2015;20. doi:10.2807/1560-7917.ES.2015.20.45.30062.

[17] Manageiro V, Ferreira E, Pinto M, Caniça M. First description of OXA-48 carbapenemase harbored by Escherichia coli and Enterobacter cloacae from a single patient in Portugal. Antimicrob Agents Chemother 2014;58:7613–14. doi:10.1128/AAC.02961-14.

[18] Pfaller MA, Bassetti M, Duncan LR, Castanheira M. Ceftolozane/tazobactam activity against drug-resistant Enterobacteriaceae and Pseudomonas aeruginosa causing urinary tract and intraabdominal infections in Europe: report from an antimicrobial surveillance programme (2012–15). J Antimicrob Chemother 2017;72:1386–95. doi:10.1093/jac/dkx009.

[19] 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 2017;62 e02125-17. doi:10.1128/AAC.02125-17.

[20] García-Fernández S, García-Castillo M, Bou G, Calvo J, Cercenado E, Delgado M, et al. Activity of ceftolozane/tazobactam against Pseudomonas aeruginosa and *Enterobacterales* isolates recovered from intensive care unit patients in Spain: The SUPERIOR multicentre study. Int J Antimicrob Agents 2019;53:682–8. doi:10.1016/j.ijantimicag.2019.02.004.

[21] Bouxom H, Fournier D, Bouiller K, Hocquet D, Bertrand X. Which non-carbapenem antibiotics are active against extended-spectrum β-lactamase-producing Enterobacteriaceae? Int J Antimicrob Agents 2018;52:100–3. doi:10.1016/j.ijantimicag.2018.03.014.

[22] Robin F, Auzou M, Bonnet R, Lebreuilly R, Isnard C, Cattoir V, et al. In vitro activity of ceftolozane-tazobactam against Enterobacter cloacae complex clinical isolates with different β-lactam resistance phenotypes. Antimicrob Agents Chemother 2018;62(9). doi:10.1128/AAC.00675-18.

[23] Del Barrio-Tofiño E, Zamorano L, Cortes-Lara S, López-Causapé C, Sánchez-Diener I, Cabot G, et al. Spanish nationwide survey on Pseudomonas aeruginosa antimicrobial resistance mechanisms and epidemiology. J Antimicrob Chemother 2019;74(7):1825–35. doi:10.1093/jac/dkz147.

[24] Giani T, Arena F, Pollini S, Di Pilato V, D'Andrea MM, Henrici De Angelis L, et al. Italian nationwide survey on Pseudomonas aeruginosa from invasive infections: activity of ceftolozane/tazobactam and comparators, and molecular epidemiology of carbapenemase producers. J Antimicrob Chemother 2018;73:664–71. doi:10.1093/jac/dkx453.

[25] Cabot G, Bruchmann S, Mulet X, Zamorano L, Moyà B, Juan C, et al. Pseudomonas aeruginosa ceftolozane-tazobactam resistance development requires multiple mutations leading to overexpression and structural modification of AmpC. Antimicrob Agents Chemother 2014;58:3091–9. doi:10.1128/AAC.02462-13.