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SUSCEPTIBILITY

In Vitro Activity of Ceftolozane-Tazobactam against *Enterobacter cloacae* Complex Clinical Isolates with Different β-Lactam Resistance Phenotypes

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ABSTRACT The study evaluated the *in vitro* activity of ceftolozane-tazobactam (C/T) against 94 unique clinical isolates of *Enterobacter cloacae* complex (ECC). No difference was observed according to the ECC cluster. The *in vitro* activity greatly varied depending on the β-lactamase-producing profile: 100%, 67%, and 19% of wild-type, extended-spectrum β-lactamase (ESBL)-producing, and AmpC-overproducing strains, respectively, were susceptible to C/T. The use of C/T could be of interest for the treatment of some infections caused by ESBL-producing AmpC-nonoverexpressing ECC isolates.

KEYWORDS *E. cloacae* complex, ECC, ceftolozane, tazobactam, TOL-TAZ, ESBL, AmpC

The species belonging to the *Enterobacter* genus are responsible for 5 to 10% of infections among patients hospitalized in intensive care units (ICUs) and are primarily due to the members of the *Enterobacter cloacae* complex (ECC) (1, 2). Actually, ECC is composed of 13 clusters, among which three (C-III, VI, and VIII) are the most frequently recovered from human clinical specimens (3, 4). All ECC members intrinsically harbor a chromosomal *ampC* gene coding for a cephalosporinase (2, 5–7). Among these third-generation cephalosporin (TGC)-resistant isolates, approximately one-third have acquired plasmid-mediated extended-spectrum β-lactamases (ESBLs), while the remaining two-thirds express high-level production of cephalosporinase (HL-CASE) caused by *ampC* derepression that results from chromosomal mutations (6).

Ceftolozane-tazobactam (C/T) is a novel TGC combined with a classical inhibitor of β-lactamase (ratio of 2:1), which has recently been approved for the treatment of complicated intra-abdominal and urinary tract infections (8). Although ceftolozane has been developed to be more stable than other TGCs against natural AmpC produced by *P. aeruginosa* (9), much less is known about its activity against other intrinsically AmpC-producing species, such as ECC. Indeed, previous studies have mainly described the *in vitro* activity of C/T against *Enterobacter* spp. with no distinction of species and/or phenotypes of resistance (10–12). In addition, no data are available about the *in vitro* activity of C/T according to the ECC cluster.

The purpose of the study was to (i) evaluate the *in vitro* activity of C/T against a collection of ECC clinical isolates, representing relevant clusters and exhibiting various

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phenotypes of β-lactam susceptibility profiles, and (ii) compare it to those of commonly used β-lactams.

Besides the reference strain of *E. cloacae* subsp. *cloacae* ATCC 13047 (belonging to C-XI), a total of 93 ECC clinical isolates (representing 12 clusters) collected from the University Hospital of Caen were included in the study (3). Note that the strains were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (Microflex LT; Bruker Daltonics, Bremen, Germany), and ECC members were clustered by *hsp60* sequencing as previously described (7). MICs of C/T (C was provided by Cubist Pharmaceuticals, and T was purchased from Abcam Biochemicals), piperacillin-tazobactam (TZP), cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), ertapenem (ETP), and imipenem (IMP) were determined by the broth microdilution reference method in accordance with EUCAST guidelines (http://www.eucast.org/). ECC isolates were classified into four β-lactam susceptibility phenotypes: wild-type (WT; no resistance to TGCs), ESBL (resistance to at least one TGC with a positive double-disk synergy test), HL-CASE (resistance to at least one TGC with a negative double-disk synergy test and a significant difference in TGC-mediated inhibition with or without 250 mg/liter cloxacillin), and ESBL+HL-CASE (resistance to at least one TGC with a positive double-disk synergy test and a significant difference in TGC-mediated inhibition with or without 250 mg/liter cloxacillin). To confirm the HL-CASE phenotype (especially in isolates producing ESBLs), we quantified the levels of expression of the chromosomal *ampC* gene by reverse transcription-quantitative PCR using specific primers (see Table S1 in the supplemental material). Total RNAs were extracted as previously described (7). Transcript levels were determined by the $\Delta\Delta C_T$ method using the *rpoB* gene as a housekeeping control gene (Table S1), and the fold change (FC) of expression was calculated between TGC-resistant strains and WT strains of the same cluster. HL-CASE was defined if the FC was higher than 2. ESBLs were characterized as previously described (13–15).

Twelve of the 13 clusters were represented in the study (Table S2). Among them, C-III (21%, 20/94), C-VI (20%, 19/94), and C-VIII (28%, 26/94) were predominant, as previously described (Table S2) (4). Note that none of the studied clusters expressing a WT phenotype exhibited an intrinsic resistance to the C/T in spite of the genetic variability of the *ampC* gene (7).

Among the 94 isolates, four antimicrobial susceptibility phenotypes were distinguished: WT, 34% (32/94); ESBL alone, 10% (9/94); ESBL+HL-CASE, 20% (19/94); and HL-CASE, 36% (34/94) (Table 1 and Table S2). By using the disk method with or without cloxacillin (250 mg/liter), the HL-CASE phenotype was not highlighted in 21% of isolates (4/19) presenting an ESBL+HL-CASE combined phenotype. In contrast, the expression of *ampC* allowed us to accurately discriminate between all ESBL and ESBL+HL-CASE phenotypes ($P < 0.0001$) (Fig. 1). Among the 28 isolates expressing an ESBL phenotype (ESBL alone and ESBL+HL-CASE), four genes encoding such β-lactamases were identified: $bla_{\text{CTX-M-15}}$ (17/28; 61%), $bla_{\text{SHV-12}}$ (9/28; 32%), $bla_{\text{CTX-M-9}}$ (2/28; 7%), and $bla_{\text{TEM-15}}$ (1/28; 4%). Note that one isolate coproduced $bla_{\text{CTX-M-15}}$ and $bla_{\text{SHV-12}}$ genes (Table S3). The distribution of ESBLs was similar to that recently described in French *E. cloacae* isolates (CTX-M-15, 52%; SHV-12, 38%; CTX-M-9, 10%) (16). Besides ESBL production, plasmid-mediated AmpC β-lactamase genes were also identified in two isolates ($bla_{\text{CMY-4}}$ and $bla_{\text{DHA-1}}$), and one strain harbored the acquired OXA-48-like carbapenemase OXA-204 (Table S3).

For the 32 isolates with a WT phenotype, all were categorized as susceptible for all tested β-lactams, except for one strain that was not susceptible to CAZ (MIC of 2 mg/liter), according to EUCAST breakpoints (Table 1). MICs of C/T ranged from 0.12 to 0.5 mg/liter with MIC_{50} and MIC_{90} at 0.25 and 0.5 mg/liter, respectively (Table 1). These MIC values were identical to MIC_{50} (0.25 mg/liter) and MIC_{90} (0.5 mg/liter) values published for ceftazidime-susceptible *Enterobacter* strains (12, 17).

For the nine isolates expressing an ESBL phenotype, all were resistant to TGCs (CTX, CRO, and CAZ), while TZP and FEP retained activity against 22% and 44% of strains, respectively (Table 1). Six isolates (67%) were categorized as susceptible to C/T, with MICs between 0.25 and 4 mg/liter (Table 1). MIC_{50} and MIC_{90} were at 1 and 2 mg/liter, which is similar to values

TABLE 1 MICs of different β-lactams against a collection of 94 strains (93 clinical isolates and ATCC 13047) of ECC according to resistance phenotypes

ECC clinical isolate (no.)	MIC (mg/liter)			EUCAST susceptibility breakpoint (mg/liter)	Susceptible strains (%)
	MIC_{50}	MIC_{90}	Range		
All (94)					
Ceftolozane-tazobactam	1	16	0.12–128	≤1	51
Imipenem	0.25	0.5	0.12–4	≤2	99
Ertapenem	0.25	2	0.01–32	≤0.5	70
Cefepime	0.5	16	0.03–>256	≤1	54
Ceftazidime	64	256	0.25–>256	≤1	33
Cefotaxime	64	>256	0.25–>256	≤1	34
Ceftriaxone	128	>256	0.25–>256	≤1	34
Piperacillin-tazobactam	64	256	2–256	≤8	37
Wild type (32)					
Ceftolozane-tazobactam	0.25	0.5	0.12–0.5	≤1	100
Imipenem	0.25	0.5	0.12–0.5	≤2	100
Ertapenem	0.06	0.12	0.01–0.25	≤0.5	100
Cefepime	0.03	0.06	0.03–0.06	≤1	100
Ceftazidime	0.5	1	0.25–2	≤1	97
Cefotaxime	0.5	1	0.25–1	≤1	100
Ceftriaxone	0.5	1	0.25–1	≤1	100
Piperacillin-tazobactam	2	4	2–8	≤8	100
ESBL alone (9)					
Ceftolozane-tazobactam	1	2	0.25–4	≤1	67
Imipenem	0.25	0.5	0.12–0.5	≤2	100
Ertapenem	0.125	0.5	0.03–1	≤0.5	89
Cefepime	4	256	0.06–64	≤1	44
Ceftazidime	64	128	32–128	≤1	0
Cefotaxime	256	>256	4–>256	≤1	0
Ceftriaxone	256	>256	2–>256	≤1	0
Piperacillin-tazobactam	64	128	8–128	≤8	22
ESBL+HL-CASE (19)					
Ceftolozane-tazobactam	8	128	1–128	≤1	11
Imipenem	0.5	1	0.25–4	≤2	95
Ertapenem	0.5	8	0.12–32	≤0.5	53
Cefepime	4	256	0.12–>256	≤1	11
Ceftazidime	128	256	32–>256	≤1	0
Cefotaxime	256	>256	64–>256	≤1	0
Ceftriaxone	256	>256	128–>256	≤1	0
Piperacillin-tazobactam	128	256	32–>256	≤8	0
HL-CASE (34)					
Ceftolozane-tazobactam	4	16	0.25–32	≤1	24
Imipenem	0.25	0.5	0.12–1	≤2	100
Ertapenem	1	2	0.03–4	≤0.5	47
Cefepime	2	8	0.12–16	≤1	35
Ceftazidime	128	256	2–>256	≤1	0
Cefotaxime	256	>256	16–>256	≤1	0
Ceftriaxone	256	>256	32–>256	≤1	0
Piperacillin-tazobactam	128	256	8–256	≤8	3

(2 and 4 mg/liter, respectively) reported in a previous study on 15 ESBL-producing *Enterobacter* strains (18). Also, a recent study reported 85% (40/47) of *Enterobacter* isolates were susceptible to C/T (19). This is in accordance with the facts that tazobactam inhibits most class A β-lactamases (including ESBLs) and that C/T remains active against >80% of ESBL-producing *Escherichia coli* clinical isolates (10–12, 17).

All 53 isolates showing an HL-CASE phenotype, including 19 that coproduced an ESBL, were categorized as resistant to TGCs (CTX, CRO, and CAZ), and only 19% were susceptible to C/T (Table 1). The percentages of susceptible strains were comparable between ESBL+HL-CASE and HL-CASE isolates for TZP (0 versus 3%), ETP (53 versus 47%), and IMP (95 versus 100%) but different for FEP (11 versus 35%) (Table 1). MIC_{50} and MIC_{90} of C/T were higher for ECC isolates with an ESBL+HL-CASE phenotype (8 and 128 mg/liter,

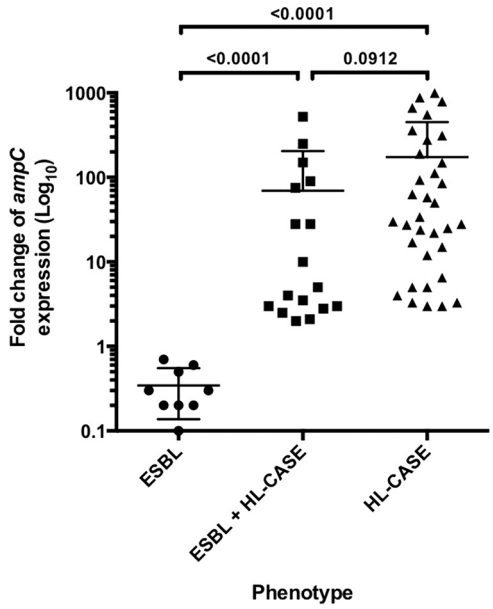

FIG 1 Fold change of expression of the *ampC* chromosomal gene according to the resistant phenotype: production of an ESBL, AmpC overproduction (HL-CASE), and ESBL+HL-CASE. The fold change (expressed as $\log_{10}$ values) was calculated between resistant strains and wild-type strains of the same cluster. HL-CASE was defined if the fold change was higher than 2.

respectively) than those for HL-CASE strains (4 and 16 mg/liter, respectively) (Table 1). Consequently, eight isolates (24%) were categorized as susceptible to C/T among HL-CASE isolates, whereas only two (11%) remained susceptible to the combination in the group of ESBL+HL-CASE strains (Table 1). Compared to ESBL producers, this poorer activity of C/T against HL-CASE ECC isolates is due to the fact that tazobactam is not effective against AmpC β-lactamases (8). In this subgroup (HL-CASE ECC), the percentage of strains inhibited by $\leq$1 mg/liter (corresponding to the EUCAST breakpoint) of C/T varied between 14 and 36% (10–12, 17), which is similar to our results. Surprisingly, for the two studies where resistance mechanisms were specified (12, 19), 50 to 75% of HL-CASE strains remained susceptible to C/T, which is much higher than proportions reported here. Interestingly, 30% (28/94) of ECC isolates were not susceptible to ETP (including one not susceptible to IMP), of which only two were susceptible to C/T (MICs of 1 mg/liter), suggesting that C/T is not a good option for the treatment of infections caused by non-carbapenemase-producing enterobacterial isolates showing reduced carbapenem susceptibility.

In summary, there is no difference in β-lactamase-producing profiles for C/T according to the ECC cluster. In contrast, the *in vitro* activity of C/T greatly varies depending on the β-lactam susceptibility profile.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00675-18.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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