Ceftolozane/Tazobactam Susceptibility Testing in Extended-Spectrum Betalactamase- and Carbapenemase-Producing Gram-Negative Bacteria of Various Clonal Lineages

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Nowadays, multidrug-resistant bacteria are considered as an increasing serious threat to public health worldwide. Global and local surveillance data are helpful in the application of the most efficient antimicrobial agent in bacterial infections. In the current study, we aimed to analyze the activity of the previously cleared agent ceftolozane/tazobactam (C/T) in African and European multidrug-resistant Gram-negative bacteria. Susceptibility testing was performed on 147 extended-spectrum β-lactamase (107 *Escherichia coli* and 40 *Klebsiella pneumoniae*) and 103 carbapenemase-producing Gram-negative bacteria using Etest according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints. Among the extended-spectrum β-lactamase producing isolates, 91 *Escherichia coli* isolates (85%) and 23 *Klebsiella pneumoniae isolates* (57.5%) were susceptible towards C/T whereas out of the 103 carbapenemase-producing isolates 102 (99.0%) were C/T-resistant. C/T should be included in susceptibility testing to fairly administer this antimicrobial agent in infections caused by multidrug-resistant bacteria. It may be considered as a therapy option for infections caused by extended-spectrum β-lactamase-producing bacteria once susceptibility to this antimicrobial combination has been confirmed.

Keywords: ceftolozane, multi-drug resistance, ESKAPE, Etest, susceptibility, ESBL, carbapenemase

Introduction

The multidrug resistance of bacteria causing life-threatening infections is a continuously increasing problem for every nation's health care system [1, 2]. In particular, the combat against pathogens of the ESKAPE group (*Enterococci*, *Staphylococci*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacteriaceae*) is an ongoing challenge in clinical practice [3]. Since phase III trials showed its therapeutic efficacy in complicated urinary tract infections (cUTI) [4] and complicated intra-abdominal infections (cIAI) [5], the novel cephalosporin ceftolozane (formally known as CXA-101 and FR264205) in a fixed combination (also known as CXA-201) with the well-known beta-lactamase inhibitor tazobactam from MSD Sharp & Dohme was approved in Europe for those indications in September 2015. Another ongoing phase 3 trial is currently exploring the treatment of ventilator-associated and nosocomial pneumonia (ASCPECT-NP) [6]. There are already few case reports demonstrating the effective treatment [7].

Studies have shown that this novel antibiotic agent exhibited enhanced in vitro activity against extended-spectrum β-lactamase (ESBL) producing isolates when combined with tazobactam, especially against clinically highly relevant *E. coli* isolates carrying the CTX-M-type genes [8, 9]. It is also one of the most active β-lactam agents against *Pseudomonas*, including drug-resistant strains [10–12] and shows a much slower development of resistance than most other agents (e.g., ceftazidime, ciprofloxacin, or meropenem) [13]. Moreover, a number of case reports could show its effectiveness as off-label use in the treatment of *Pseudomonas aeruginosa*-associated bacteremia [14] and polymicrobial osteomyelitis, including multidrug-resistant (MDR) *Stenotrophomonas maltophilia* [15].

The purpose of the current survey was to determine the in vitro activity of ceftolozane/tazobactam (C/T) in pre-characterized ESBL-producing Gram-negative bacterial species and its impact on different carbapenemase-producing bacteria.

Materials and Methods

Bacterial Strains. We analyzed 147 ESBL-producing isolates, including 107 *Escherichia coli* and 40 *Klebsiella pneumoniae* isolates (Table 1). These isolates originated from clinical specimens of hospitalized patients at Aga Khan University Hospital in Nairobi, Kenya, in 2011. The isolates were previously identified using matrix-assisted laser desorption ionization–time of flight (MALDI–TOF) mass spectrometry. The detection of the pathogens as ESBL-producers was determined by standard susceptibility testing and Etest [16].

Table 1. Susceptibility testing against ceftolozane/tazobactam was performed in the following Gram-negative bacteria with ESBL (n = 147) production

CTX-M producing isolates	
Species	*n*
Klebsiella pneumoniae	40
Escherichia coli	107
Total	147

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Table 2. Susceptibility testing against ceftolozane/tazobactam was performed in the following Gram-negative bacteria with carbapenemase (n = 103) production

Carbapenemase producing isolates	
Species	*n*
Acinetobacter baumannii	39
Klebsiella pneumoniae	33
Escherichia coli	16
Enterobacter cloacae	5
Pseudomonas aeruginosa	3
Citrobacter freundii	2
Klebsiella oxytoca	2
Serratia marcescens	2
Enterobacter asburiae	1
Total	103

Furthermore, we analyzed 103 carbapenemase-producing isolates, including 39 *Acinetobacter baumannii*, 33 *Klebsiella pneumoniae*, 16 *Escherichia coli*, 5 *Enterobacter cloacae*, 3 *Pseudomonas aeruginosa*, 2 *Citrobacter freundii*, 2 *Klebsiella oxytoca*, 2 *Serratia marcescens*, and 1 *Enterobacter asburiae* (Table 1). The previous gene analysis (Table 2) resulted in 58 Ambler class D β-lactamases (mostly OXA-23- and -48-like), 18 Ambler class A (KPC-2 and -3), and 30 metallo-β-lactamases: 17 NDM (mostly NDM-1/−6), 10 VIM (mostly VIM-1 and -2), and 2 IMP isolates (IMP-14 and -4). Isolates were collected in part from HELIOS University Clinic Wuppertal in 2015, previously identified by MALDI–TOF mass spectrometry, and genetically classified by polymerase chain reaction (PCR). Few carbapenemase-producing bacterial isolates were provided by the National Reference Center (NRZ) in Bochum, Germany. All isolates were stored at −80 °C. Control strains *K. pneumoniae* (DSM 26371, 30104, 26371), *E. coli* (DSM 22311, 1103), and *P. aeruginosa* (DSM 1117) were used for quality control purposes.

Etest. The C/T Etest strips from Liofilchem (Roseto degli Abruzzi, Italy) were used for the susceptibility testing. The 80 °C cryobank isolates were inoculated in brain heart infusion (BHI) medium and incubated at 36 °C 18 to 24 h to gain an adequate enrichment for the subculture on the solid culture medium. The isolates were sub-cultured on MacConkey agar plates and incubated at 36 °C for 18 to 24 h. A suspension of growth from these plates was then prepared in BD Phoenix™ inoculum broth and adjusted to a McFarland standard of 0.5. These were streaked on Mueller Hinton II agar plates using cotton-tipped swab. We applied the Etest strip to the plates and incubated them for additional 24 h. The minimum inhibitory concentration (MIC) was determined by reading the value at the point where the elliptical inhibition zone intersected with the MIC scale on the strip. We applied the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (susceptible if MIC ≤ 1 mg/L and resistant if MIC > 1 mg/L) [17].

Ethics

No ethical approval was necessary since we performed only in vitro assays involving anonymized strains from laboratory collections. No relation to specific individuals is traceable.

Results

ESBL-Producing Isolates. C/T showed good activity when tested against the group of ESBL-producing isolates (Table 3). Out of the 147 ESBL-producing isolates, 77.6% (n = 114) were susceptible towards C/T according to the EUCAST clinical breakpoints for *Enterobacterales* (Table 4) [17]. In particular, the ESBL-producing *E. coli* isolates showed a higher susceptibility rate (85%). Only 15% (16 isolates) of the *E. coli* isolates were resistant towards C/T and additional 10 (62.5%) *E. coli* isolates indicated a lower MIC range up to 6 mg/L for C/T. C/T demonstrated no activity towards 2 of the 16 ESBL-producing isolates (>256 mg/L). In contrast, the *K. pneumoniae* isolates (n = 17) showed a higher resistance rate towards C/T (42.5%), most of them within the lower MIC range between 1 and 6 mg/L (88.2%). Similarly, 2 of the 17 ESBL-producing *K. pneumoniae* isolates were not affected in their growth at all by C/T (MIC > 256 mg/L).

Carbapenemase-Producing Isolates. Nearly all carbapenemase-producing isolates were resistant towards C/T (Table 3). All but a single NDM isolate were highly resistant to C/T (MIC > 256 mg/L), whereas the OXA- and KPC-producing isolates showed a broad variety of MIC values between 1.5 mg/L and > 256 mg/L. Among the OXA-producing isolates, 35 were *A. baumannii*, 12 were *K. pneumoniae*, 9 were *E. coli* isolates, 1 was *C. freundii*, and another one was *S. marcescens.* Typically, the KPC-producing isolates were mostly identified as *K. pneumoniae* (n = 14), followed by 3 *E. coli,* 1 *C. freundii*, and 1 *K. oxytoca*. Similarly, all VIM-producing isolates, including all 3 *P. aeruginosa* isolates, and both IMP-producing *E. cloacae* isolates demonstrated overgrowth (MIC > 256 mg/L). Overall, 55 of the 103 (53.4%, data not shown) carbapenem-resistant isolates were not inhibited in their growth by the antibiotic combination C/T. Particularly, a representative number (n = 33) of *A. baumannii* isolates showed rather high MIC levels (84.6% MIC ≥ 32 mg/L, data not shown).

Table 3. The genotypic-characterized carbapenemase-producing Gram-negative bacteria (total n = 103)

	OXA (n = 58)									IMP (n = 2)	
	OXA-23[1]	OXA-48	OXA-72	OXA-181	OXA-164	OXA-232	OXA-58	OXA-66	OXA-162	IMP-14	IMP-4/−28
A. baumannii	26		5		2		1	1			
K. pneumoniae		11				1					
E. coli		4		4		1					
E. cloacae										1	1
C. freundii									1		
S. marcescens		1									
	NDM (n = 17)					KPC (n = 18)			VIM (n = 10)		
	NDM-1/−6[1]	NDM-3	NDM-2	NDM-5	NDM-9	KPC-2	KPC-3	VIM-1	VIM-2	VIM-4	VIM-11
A. baumannii	4		1		1						
K. pneumoniae	5					8	6	1	1		
E. coli	1	2		1		1	2				
E. cloacae	1							1		1	
P. aeruginosa									2		1
C. freundii								1			
K. oxytoca						1		1			
S. marcescens								1			
E. asburiae	1										

Table 4. The results of the susceptibility testing against ceftolozane/tazobactam in ESBL-producing (total *n* = 147) and carbapenemase-producing (total *n* = 103) Gram-negative bacteria according to the EUCAST guidelines 2018 [17]

	susceptible (EUCAST MIC ≤ 1 mg/L)	resistant (EUCAST MIC > 1 mg/L)	n
CTX-M producing isolates			
Escherichia coli	91 (85%)	16 (15%)	107 (100%)
Klebsiella pneumoniae	23 (57.5%)	17 (42.5%)	40 (100%)
n	114 (77.6%)	33 (22.6%)	147 (100%)
Carbapenemase producing isolates			
Acinetobacter baumannii[a]	0 (0%)	39 (100%)	39 (100%)
Klebsiella pneumoniae	0 (0%)	33 (100%)	33 (100%)
Escherichia coli	1 (6.3%)	15 (93.8%)	16 (100%)
Enterobacter cloacae	0 (0%)	5 (100%)	5 (100%)
Pseudomonas aeruginosa[a]	0 (0%)	3 (100%)	3 (100%)
Citrobacter freundii	0 (0%)	2 (100%)	2 (100%)
Klebsiella oxytoca	0 (0%)	2 (100%)	2 (100%)
Serratia marcescens	0 (0%)	2 (100%)	2 (100%)
Enterobacter asburiae	0 (0%)	1 (100%)	1 (100%)
n	1 (1.0%)	102 (99.0%)	103 (100%)

[a]Other breakpoints apply: >4 mg/L.

Discussion

In the last 10 to 20 years, we have witnessed a dramatic increase in the proportion of bacterial pathogens resistant to multiple antimicrobial agents. Indeed, the driving force behind the increasing rates of resistance is ultimately the abuse and misuse of antimicrobial agents, whether inadequately administered to patients and livestock or unintentionally released into the environment. This issue is very important regarding the resistance towards quinolones, carbapenems, and third-generation cephalosporins. The latter relates to the increased prevalence of extended-spectrum β-lactamases among *Enterobacterales.* Surveillance studies of antimicrobial resistance and antibiotic consumption have drawn attention to this phenomenon and should be used to drive political campaigns to contain resistance [18, 19]. Ceftolozane/tazobactam (C/T) has been approved few years ago and represents a therapy option in particular infections associated with Gram-negative bacteria, including ESBL-producing isolates. However, continuous monitoring of the efficacy of CT in such MDR bacteria should be conducted worldwide. Therefore, we investigated its activity in European and African isolates. The recommended dosage of C/T for the approved indications – complicated urinary tract infection and intra-abdominal infection – is 1000 mg ceftolozane and 500 mg tazobactam in a fixed combination administered intravenously every 8 h over 1 to 2 weeks in patients with a creatinine clearance of at least 50 mL/min. Despite C/T showing a good overall in vitro activity against extended-spectrum β-lactamase (ESBL) phenotypes (77.6%), only 57.5% of *K. pneumoniae* were susceptible in contrast to 85% of *E. coli.* This circumstance resembles the work of Farrell et al. [20]. Unlike their results, none of these isolates was tested positive for carbapenemase production. Nevertheless, some *Enterobacterales* members tend to bypass susceptibility testing methods for carbapenemase production when harboring acquired metallo-β-lactamases (MBLs) [21, 22]; therefore, we cannot exclude that some of the tested ESBL-producing isolates also produced carbapenemases. Similar to our results, Sader et al. found notably lower susceptibility rates in ESBL-phenotypes of *K. pneumoniae* in comparison to *E. coli* isolates [10].

Almost all carbapenemase-producing isolates have been tested resistant (99.0%) and in more than half of them (53.4%, data not shown) overgrowth was observed (MIC > 256 mg/L), which supports the stated lacking antimicrobial activity of C/T against carbapenemase producing isolates by Cho et al. [23]. Particularly *A. baumannii* showed high MIC levels (85.4% MIC > 32 mg/L). In our study, the OXA-23 positive isolates were exclusively *A. baumannii*, and OXA-23 was detected in more than half of those isolates, which is in line with the results of Castanheira et al. that it is the most common Ambler class D β-lactamase in *Acinetobacter* species. These isolates were determined with the highest MIC levels overall and may be explained by their generally high intrinsic resistance against various antimicrobial agents [24]. The presence of the NDM gene in a single isolate, which was susceptible to C/T, was once again proven genetically, to exclude an eventual gene loss during storage at −80 °C. Low or no protein expression might be an explanation for the activity of C/T against this NDM-producing isolate.

Perhaps, the antipseudomonal activity of C/T could lead to potential therapy regimen in infections (mostly respiratory infections) associated with the increasing number of MDR *P. aeruginosa* strains in the last decade [25, 26], especially the global increasing rate of carbapenemase-producing strains [27]. In contrast to previous studies showing at least a certain effect of C/T against carbapenem-resistant *P. aeruginosa* [10], all three isolates (2 VIM-2 and 1 VIM-11) were highly resistant (MIC > 256 mg/L) in our study. This is due to the unstable and variable structure of the MBL [28] that tazobactam cannot inhibit in those isolates and *P. aeruginosa* activates few other mechanisms for its resistance [29]. The number of particular species tested against C/T is rather low and may not be representative for the respective species. Thus, further studies should consider higher number of such species. Ongoing clinical trials should investigate the activity of C/T with higher dosing regimens (e.g. twofold), especially for indications (e.g., nosocomial pneumonia) other than the complicated intra-abdominal and urinary tract infections.

Compared to disk diffusion and broth microdilution, the Etest shows an agreement of approximately 95% [30], and the accuracy in further studies could be increased by performing an additional method of testing.

For the therapeutic coverage of infections caused by ESBL-producing bacterial isolates, ceftolozane/tazobactam (C/T) appears to be a good alternative to other currently available antimicrobial agents, e.g., temocillin, pivmecillinam, or carbapenems. Unfortunately, this new agent does not add to our little antimicrobial arsenal against carbapenemase-producing pathogens. Therefore, if an infection with MDR bacteria is assumed, C/T should be considered as a treatment option, and therefore the routine susceptibility testing methods should include the testing for this antimicrobial agent. Nevertheless, we are in need of a thorough implementation of antibiotic stewardship programs and new solutions of encountering carbapenemase-producing isolates with only a few novel agents in the pipeline [31, 32].

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Authors' Contributions

CP and BG proposed, designed, and carried out the study, performed data analysis, and drafted the manuscript. PAN participated in critical discussion and proofreading of the manuscript. All the authors read and approved the final version.

Conflict of Interest

The authors declare that they have no competing interests.

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