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Original Article

Activity of ceftolozane/tazobactam against commonly encountered antimicrobial resistant Gram-negative bacteria in Lebanon

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Abstract

Introduction: In view of the continuous rise in Gram-negative bacterial resistance and limited treatment options, Ceftolozane/tazobactam (C/T) is a newly introduced antimicrobial agent in Lebanon for its demonstrated activity against resistant Gram-negative bacteria. However, *in vitro* data is not available about its activity against commonly isolated bacteria in this country.

Methodology: The analysis included clinical isolates, multidrug–resistant (MDR) and extended-spectrum Beta-lactamases (ESBLs), representing 124 *Escherichia coli*, 75 *Klebsiella pneumoniae* and 100 *Pseudomonas aeruginosa*, identified using the MALDI-TOF. The minimum inhibitory concentration (MIC) for C/T was determined by the Etest (Liofilchem, Roseto degli Abruzzi, Italy). In addition, the disk diffusion (DD) test was used to determine the activity of C/T and of the antimicrobials routinely used to test for such pathogens.

Results: The C/T activity against the ESBL producers *E. coli* and *K. pneumoniae* isolates were similar (MIC_{90} value of 1 and 1.5 µg/mL, respectively; susceptibility of 100% and 96%, respectively). However, the activity of C/T against the *E. coli* and *K. pneumoniae* MDR isolates was much lower (MIC_{90} value of 256 and 96 µg/mL, respectively; susceptibility of 54% for each). The C/T MIC_{90} value for the non-MDR *P. aeruginosa* isolates was 3 µg/mL and ≥ 256 µg/mL for the MDR *P. aeruginosa* isolates (susceptibility of 96% vs 42% respectively). Overall, the C/T activities show comparable or higher susceptibility to the routinely used antimicrobials.

Conclusion: The high *in vitro* activity of C/T points out its value as a possible alternative to the antimicrobials currently used for treatment of infections caused by such pathogens and would help in minimizing toxicity and bacterial resistance.

Key words: antimicrobial resistance; ceftolozane/tazobactam; Gram-negative bacteria; Lebanon.

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Introduction

Antibiotic resistance is an emerging health problem worldwide. Especially concerning are the multidrug-resistant (MDR) Gram-negative bacteria mainly *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [1,2]. One of the main mechanisms of resistance to antibiotics among these bacteria is the production of β-lactamases, which are bacterial enzymes that confer resistance to a wide variety of β-lactam antibiotics depending on the type of β- lactamase enzyme produced [1].

Pseudomonas aeruginosa pathogens, for example, are among the organisms that cause infections that are difficult to treat. This is so, because around 30% of the infections caused by *P. aeruginosa* are resistant to multiple classes of antibiotics [3]. Even though, the aminoglycosides and the polymyxins remain the most consistently active drugs against *P. aeruginosa*, both have severe adverse effects and could constitute suboptimal treatments due to pharmacokinetic limitations [4,5]. To minimize the use of such agents, β-lactam/β-lactamase inhibitor combinations were introduced. Examples include piperacillin/tazobactam and cefoperazone/sulbactam that were considered alternatives for treating MDR *P. aeruginosa* as well as the extended-spectrum beta-lactamase (ESBL) producers Enterobacteriaceae, especially *E. coli* and *K. pneumoniae*. However, their effectiveness has been compromised [6,7].

Lately, ceftolozane/tazobactam (Zerbaxa, Cubist Pharmaceuticals, Lexington, MA, USA), a novel antibiotic product combining a new cephalosporin and a widely used β- lactamase inhibitor, was introduced as an agent active against many MDR isolates of *P. aeruginosa* [3-8]. Its use also aims at minimizing the increased antimicrobial resistance especially among the carbapenems. Ceftolozane, the β-lactam component of the combination, has a similar mechanism of action to other cephalosporin antibiotics but has greater stability against AmpC beta-lactamases (AmpC) enzymes mediated resistance, which is a common resistance mechanism within *P. aeruginosa* [9,10]. When combined with tazobactam, the antibiotic regains activity against many ESBL–producing Enterobacteriaceae as well [10]. This medication is approved for treatment of complicated urinary tract infections and intraabdominal infections (in combination with metronidazole) [11-13].

In Lebanon, ceftolozane/tazobactam (C/T) was recently registered at the Lebanese Ministry of Public Health (October 2019) but was available under special requests to hospitals since September 2017. Because no local data exists about C/T *in vitro* activity against multi-resistant *P. aeruginosa, E. coli and K. pneumoniae* pathogens in Lebanon, this study is warranted to assess its activity against these common pathogens prior to its introduction. Also, such baseline data can guide treatment options and future studies targeting the C/T activity in this country.

Methodology

Bacterial isolates and their identification

Consecutive non-duplicate isolates of 124 *E. coli* (MDR = 57 and ESBLs = 67), 75 *K. pneumoniae* (MDR = 26 and ESBLs = 49) and 100 *P.* aeruginosa (MDR = 69; non-MDR = 31) recovered from different clinical specimens were submitted for investigation at the Clinical Microbiology Laboratory, Department of Pathology and Laboratory Medicine, American University of Beirut Medical Center (AUBMC) during the period between February 2017 and December 2018.

Identification of the isolates was done using the matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) system (Bruker Daltonik, GmbH, Bremen, Germany).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Etest for minimal inhibitory concentrations (MICs) determination and the disk diffusion (DD) test as reported before [2]. Both the C/T strips (concentration range ≤ 0.016 and ≥ 256 µg/mL) for MICs determination and the C/T disks (40 µg) were obtained from Liofilchem, Scozia, Italy.

The 2018 Clinical and Laboratory Standards Institute (CLSI) C/T MICs' breakpoints (µg/mL) were used to interpret the susceptibility category as susceptible, intermediate and resistant, respectively, for Enterobacteriaceae: ≤ 2, 4, ≥ 8, and for *P. aeruginosa*: ≤ 4, 8, ≥ 16. For the C/T (40 µg) DD, the susceptible, intermediate and resistant zone of inhibition (mm), respectively, were ≥ 21, 18 - 20, ≤ 17 for Enterobacteriaceae, and ≥ 21, 17 - 20, ≤ 16 for *P. aeruginosa*.

The other antimicrobial agents tested by DD are the ones routinely used for testing these pathogens, and their results were also interpreted according to the 2018 CLSI guidelines.

The categorization of bacterial resistance to antimicrobial agents was based on the definition created by a group of international experts initiated by the European Center for Disease Prevention and Control and the Centers for Disease Control and Prevention. They defined MDR as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, extensively drug- resistant (XDR) as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories and pan drug-resistant (PDR) as non-susceptibility to all agents in all antimicrobial categories [14]. In our study, resistance to cefoxitin was also included in categorizing *E. coli* and *K. pneumoniae* isolates as MDR.

The characterization of *E. coli* and *K. pneumoniae* isolates as ESBL producers was carried out as previously reported from our lab [15].

Quality Control

The quality of testing with Etest and DD test was ensured using the American Type Culture Collection (ATCC) quality control strains of *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853).

Results

The clinical sources for the MDR isolates of *E. coli*, *K. pneumoniae* and *P. aeruginosa*, respectively, were urinary: 66%, 64% and 25%, respectively, respiratory: 10%, 18%, and 52%, respectively, blood: 16%, 9%, and 3%, respectively, wound: 4%, 5%, and 9%, respectively. Other sources with very low isolate recovery (0 - 6%) included: tissue, fluid, catheter, abscess, ear swab and bone.

The distribution of the tested isolates among the three genera according to their C/T MICs range, MIC_{90}, and % susceptible strains among the ESBL, MDR and all strains for *E. coli*, *K. pneumoniae* and *P. aeruginosa* are presented in Table 1. Generally, the C/T activity against the *E. coli* and *K. pneumoniae* showed similar results among the ESBL producing isolates (MIC_{90} value of 1 and 1.5 µg/mL, respectively, and the % susceptibility of 100% and 96%, respectively). Among the MDR isolates, however, the activity was much lower (MIC_{90} value of 256 and 96 µg/mL, respectively, and the % susceptibility was 54% for each).

Table 1. Ceftolozane/tazobactam MICs values for *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

Bacterial type (No. tested)	C/T MICs (µg/mL) Range	C/T MICs (µg/mL) MIC_{90}	% Susceptible in category
E. coli			
ESBL (n = 67)	0.19 - 2	1	100
MDR (n = 57)	0.19 - ≥ 256	256	54
All (n = 124)	0.19 - ≥ 256	32	79
K. pneumoniae			
ESBL (n = 49)	0.38 - 8	1.5	96
MDR (n = 26)	0.25 - ≥ 256	96	54
All (n = 75)	0.25 - ≥ 256	8	81
P. aeruginosa			
Non MDR (n = 31)	0.5 - 24	3	96
MDR (n = 69)	0.38 - ≥ 256	≥ 256	43
All (n = 100)	0.38 - ≥ 256	≥ 256	60

C/T: Ceftolozane/tazobactam; MDR: Multi-drug-resistant; MIC: Minimum Inhibitory Concentration; ESBL: Extended-spectrum beta-lactamases.

For the *P. aeruginosa* isolates, the activity of C/T against MDR versus non-MDR isolates is also presented in Table 1. The non-MDR isolates, revealed a low MIC_{90} value (3 µg/mL) with high susceptibility activity (96%) compared to the MDR isolates which showed high MIC_{90} value (≥ 256 µg/mL) and low percentage of susceptibility (42%).

Table 2 shows the activity of C/T compared to other antimicrobial agents routinely tested against these pathogens. The C/T generally showed higher or comparable activity, except for aminoglycosides, fosfomycin and tigecycline.

Worth to note that comparison of C/T test results of the isolates between the Etest method and the DD method revealed discrepant findings between the susceptibility category of the Etest and the intermediate category of DD. This was noted among 4% of *P. aeruginosa* isolates that were shown to be susceptible by Etest and intermediate by DD. This discrepancy was also noted among 17% each of the *E. coli* and *K. pneumoniae* isolates. However, one isolate of *P. aeruginosa* showed resistance to C/T, with an MIC (24 µg/mL), but the result in the DD was intermediate (17mm). This strain showed susceptible results to all the other tested antimicrobials except for aztreonam and imipenem where it revealed intermediate results.

Discussion

To the best of our knowledge this is the first study to report on the *in vitro* activity of C/T against multi-resistant strains of *P. aeruginosa*, *E. coli* and *K. pneumoniae* from Lebanon, especially prior to its introduction in the country. The discussion to follow

Table 2. Activity of C/T versus different antimicrobial agents tested routinely against *E. coli, K. pneumoniae* and *P. aeruginosa.*

AMA	Percent susceptibility among								
	E. coli			*K. pneumoniae*			*P. aeruginosa*		
	MDR (n = 57)	ESBL (n = 67)	All (n = 124)	MDR (n = 26)	ESBL (n = 49)	All (n = 75)	MDR (n = 69)	Non MDR (n = 31)	All (n = 100)
C/T	54	100	79	54	96	81	43	96	60
Amk	96	100	98	96	100	99	52	100	67
CAZ	2	13	8	0	8	5	19	100	44
Cipro	11	18	15	27	35	32	19	87	40
Genta	65	49	56	69	51	57	52	100	67
Tazo	44	70	58	42	63	56	22	100	46
IMP	100	100	100	100	100	100	27	84	45
SXT	25	27	26	35	8	17			
Tige*	100	100	100	40	100	72			
Fosf+	89	98	94	73	83	80			
ATM							17	71	34
FEP							33	97	53

AMA: Antimicrobial agents; C/T: Ceftolozane/tazobactam; MDR: Multi-drug-resistant; MIC: Minimum Inhibitory Concentration; ESBL: Extended-spectrum beta-lactamases; Amk: Amikacin; Cipro: Ciprofloxacin; Genta: Gentamicin; Tazo: Tazocin; Tetra: Tetracycline; SXT: trimethoprim-sulphamethoxazole; Tige: Tigecycline; Fosfo: Fosfomycin; ATM: Aztreonam; FEP: Cefepime; SXT: trimethoprim-sulphamethoxazole; Tige: Tigecycline; Fosfo: Fosfomycin; ATM: Aztreonam; FEP: Cefepime; CAZ: Ceftazidime; IMP: Imipenem; *tested only for respiratory and miscellaneous isolates; + tested only for urinary isolates.

will relate findings in this study to some of those reported from regional countries and others.

The *P. aeruginosa* isolates categorized as non-MDR showed excellent susceptibility to C/T. The 96% C/T susceptibility rates among our non-MDR *P. aeruginosa* isolates were close to the high rates reported from other countries; e.g. 87% from Latin America, 95-100% from Germany, Poland**,** and USA [8,16-18]**,** and higher than those susceptibility rates reported from India (17-33%) [19]**.** However, the MDR *P. aeruginosa* isolates in our study showed low susceptibility rates to C/T (43%). This rate was higher than that reported from Kuwait (33%) but lower than those reported from other countries, for example: 57% in Turkey and Israel, 63% in Qatar, 89% in Abu Dhabi, 65% - 92% in Europe, 79% in USA and 49% - 82% in Latin America [3,8,16,20-23]. In a recent study from France by Viala *et al*., the C/T activity for the overall 42 *P. aeruginosa* was 88% while among those categorized as MDR, it ranged between 73% and 86% [24].

Concerning the ESBL-EC and ESBL-KP, a high and retained activity of C/T was revealed against these isolates in this study, 100% and 96%, respectively. The susceptibility rates for both pathogens were generally higher than those previously reported by others from different parts of the world including Israel and Turkey (93% and 74%, respectively) [8]**,** Europe (81% - 94% and 41% - 62%, respectively), USA (88% and 30%, respectively) [8]**,** Latin America (33% - 84% and 33% - 78%, respectively). Interestingly, in our study, the high susceptibility rates among our isolates in both genera were similar, while those reported from different countries around the world showed an overall higher activity of C/T against ESBL-EC compared to ESBL-KP [3-8]. A clear example is noted from what was reported from four Latin American countries where the overall activity of C/T against ESBL-EC isolates was 92% compared to 57% against ESBL-KP isolates. The explanation for this discrepancy could be due to the presence of different enzymes responsible for ESBL isolates from different countries.

In a recent study from France by by Viala *et al*., the C/T activity for the overall 62 Enterobacteriaceae resistant to third generation cephalosporins was 55%. However, among the 29 ESBL-producing isolates gathered from 9 species of Enterobacteriaceae, the C/T activity was 66% [24].

In our study, the MDR isolates that were also resistant to cefoxitin, and possibly reflect AmpC phenotype, showed low C/T activity (54%) against both EC and KP in this category. This finding was lower than what was reported for the MDR-EC isolates (76%) but close to those reported for the MDR-KP isolates (51%) from Kuwait [20].

The C/T activities against the different pathogens tested in our study, showed an overall comparable or higher activities to many of the different antimicrobials used to treat multi-resistant strains of *P. aeruginosa, E. coli* and *K. pneumoniae* (e.g. piperacillin /tazobactam, third generation cephalosporins) as noted in Table 2. Thus, in Lebanon and under many clinical conditions, C/T can be considered as an alternative to many antimicrobials with high rate of toxicity (e.g. aminoglycosides) or contributing to the rise of carbapenem–resistant Enterobacteriaceae (e.g. carbapenems use in ESBLs).

The exact mechanism(s) involved in the microbial resistance to C/T seems to be a complex one. Generally, the activity of C/T is less affected by the common resistance mechanisms identified in Gram-negative pathogens including, porin loss, efflux pumps, alteration of PBPs, membrane changes and the hyper production of the chromosomal cephalosporinase AmpC.

Regarding the production of β-lactamases, C/T shows good activity against commonly encountered ESBLs e.g. CTX-M-14 and CTX-M-15, weak activity against some SHV-type ESBLs, and essentially very compromised activity against organisms producing carbapenemases, especially metallo-beta-lactamases (MBLs) enzymes [25]. It has been shown that *P. aeruginosa* activates unstable and variable structure of the MBLs together with other mechanisms to inactivate C/T resulting in resistance to this drug [26]**.** To note, the first emergence of clinical resistance of *P. aeruginosa* to C/T was reported by Vidal *et al* [27] in a patient with wound infection due to two morphotypes of *P. aeruginosa (*MICs ≥ 32/4ug/ml and ≥ 32/5µg/ml). The clinical emergence of resistance was traced to G183D substitution in the AmpC. According to Cabot *et al*., the development of high-level resistance to C/T appears to occur efficiently only in a *P. aeruginosa* mutator background, in which multiple mutations lead to overexpression and structural modifications of AmpC [9]**.** A recent study from Qatar ascribed the resistance mechanism of *P. aeruginosa* to C/T to be due to mutations in the PDC enzymes (also known as AmpC) leading to AmpC hyperproduction and to the presence of oxacillinases genes OXA-4, OXA-10 and OXA-50 [21]. Although molecular studies to determine genes involved in the C/T resistance among the tested isolates were not entertained in this study, this is being pursued along with what was done previously for CRE isolates genes characterization [28,29].

Conclusion

The high C/T activity revealed among the multi-resistant strains of *P. aeruginosa, E. coli* and *K. pneumoniae* can represent a good valuable alternative to other currently available antimicrobial agents used for the treatment of infections caused by such pathogens in Lebanon. The study sets a baseline information for future follow up on C/T activity in this country.

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