Ceftolozane/tazobactam and ceftazidime/avibactam antimicrobial activity against clinically relevant gram-negative bacilli isolated in Mexico

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Abstract

Introduction: There is limited information on the effectiveness of ceftolozane/tazobactam and ceftazidime/avibactam combinations on clinically relevant strains isolated in Mexico. **Objective:** To determine the antimicrobial profile of both antibiotic combinations in our community. **Method:** The present research study was prospective, descriptive and cross-sectional. Clinically relevant strains isolated from pure-strain cultures were included during the period from August 2018 to January 2019 in Mexicali, Baja California, Mexico. **Results:** 74 enterobacteriaceae and 19 *Pseudomonas aeruginosa* strains were analyzed; the percentage of sensitivity of ceftazidime/avibactam was 100 % for enterobacteriaceae and 72.7 % for *Pseudomonas aeruginosa*; the percentage of sensitivity of ceftolozane/tazobactam for enterobacteriaceae was 90.5 % and 72.7 % for *Pseudomonas aeruginosa*. **Conclusions:** The ceftolozane/tazobactam and ceftazidime/avibactam combinations offer good antimicrobial sensitivity in vitro, both for ESBL-producing enterobacteriaceae and *Pseudomonas aeruginosa*. More data are required to assess clinical response in patients receiving these antibiotic combinations.

KEY WORDS: Cephalosporins. Beta-lactamase inhibitors. Multi-drug resistance.

Actividad antimicrobiana de ceftolozano-tazobactam y ceftazidima-avibactam contra bacilos gramnegativos clínicamente relevantes aislados en México

Resumen

Introducción: Existe poca información acerca de la efectividad de las combinaciones ceftolozano/tazobactam y ceftazidima/avibactam en cepas clínicamente relevantes aisladas en México. **Objetivo:** Determinar el perfil antimicrobiano de ambos antibióticos en nuestra comunidad. **Método:** El presente estudio de investigación fue prospectivo, descriptivo y transversal. Se incluyeron cepas clínicamente relevantes aisladas a partir de cultivos de cepa pura durante el periodo de agosto de 2018 a enero de 2019 en Mexicali, Baja California, México. **Resultados:** Se analizaron 74 cepas de enterobacterias y 19 cepas de *Pseudomonas aeruginosa*; el porcentaje de sensibilidad de ceftazidima/avibactam fue de 100 % contra enterobacterias y de 72.7 % contra *Pseudomonas aeruginosa*; el porcentaje de sensibilidad de ceftolozano/tazobactam fue de 90.5 % para enterobacterias y de 72.7 % para *Pseudomonas aeruginosa*. **Conclusiones:** Las combinaciones ceftolozano/tazobactam y ceftazidima/avibactam ofrecen buena sensibilidad antimicrobiana in vitro, tanto contra enterobacterias productoras de betalactamasas de espectro extendido como contra *Pseudomonas aeruginosa*. Se requieren más datos para valorar la respuesta clínica en pacientes que reciben esas combinaciones de antibióticos.

PALABRAS CLAVE: Cefalosporinas. Inhibidores de betalactamasas. Resistencia a múltiples fármacos.

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Introduction

Gram-negative bacteria antimicrobial resistance has become one of the main causes of morbidity and mortality and a serious public health problem in the world.¹ The increasing prevalence of extended-spectrum beta-lactamase (ESBL)- and carbapenemase-producing strains in recent years constitutes a challenge due to resistance mechanisms easy dispersal, difficulty for choosing an appropriate antimicrobial treatment, and increased hospital stay time.^{2,3}

Ceftolozane/tazobactam (C/T) and ceftazidime/avibactam (CZ/A) are two new cephalosporin combinations with antimicrobial spectrum against *Pseudomonas aeruginosa*, as well as against ESBL- and carbapenemase-producing enterobacteriaceae of clinical interest. In 2014, both antibiotic combinations received approval from the United States Food and Drug Administration and, in 2017, in Mexico, by the Federal Commission for the Protection against Sanitary Risks, for the treatment of complicated urinary and intra-abdominal infections.⁴⁻⁶

There is little information regarding the effectiveness of these antibacterial drugs against strains isolated in Mexico;^{7,8} therefore, the purpose of this research project was to determine the susceptibility profile of both antibiotic combinations in our community.

Method

This research study was prospective, descriptive and cross-sectional. Ninety-three clinically relevant strains isolated from pure strain cultures were included between August 2018 and January 2019, with the participation of the MD Microbiología Clínica, S. de R.L. de C.V. laboratory (Mexicali, Baja California, Mexico) and the Clinical Microbiology laboratory of the Faculty of Medicine of the Autonomous University of Baja California, Mexicali campus. The origin of the samples for microbiological analysis was multiple, including urine, bronchial secretion, blood, secretions from wounds and abscesses, synovial fluid, soft tissue and bone biopsies, peritoneal fluid and cerebrospinal fluid.

Processing of the biological samples was carried out according to the American Society for Microbiology clinical microbiology procedures manual.⁹ In all types of samples, 5 % ram blood agar and MacConkey agar were inoculated; methylene blue eosin agar and trypticasein soy agar were added to the urine cultures;

cerebrospinal fluid and blood cultures had chocolate agar added. Tissue biopsy samples were macerated in a mortar and a ram brain-heart infusion broth was inoculated to subsequently add blood agar, MacConkey agar and chocolate agar. The cultures were incubated at 37 °C for 24 to 72 hours under aerobic conditions and with 5.5 % carbon dioxide (Shellab® incubator, model SCO6AD, Shelolab); no incubation was carried out under anaerobic conditions.

Once the strains were developed, they were identified using Biomérieux Vitek 2® Compact automated system, which uses identification cards for Gram-negative bacilli and Gram-positive cocci; the Gram-negative identification card consists of 47 biochemical tests and one negative control well. The procedure for filling out the card is the following:

- 3 mL of sterile saline (0.45-0.50 % NaCl with pH 4.5 to 7.0) are aseptically transferred to a 12 x 75 mm polystyrene tube.
- With a sterile microbiological inoculating loop, a sufficient number of colonies are collected to prepare a homogeneous suspension equivalent to 0.5 McFarland units.
- The tube with the suspension and the card are placed in a rack that is specially designed to be introduced in the automated equipment.

To measure CZ/A and C/T antimicrobial activity, the disk diffusion technique was used in Mueller-Hinton agar, as stipulated by the procedures in document M100-S28¹⁰ of the United States Institute of Clinical and Laboratory Standards; for the antibiogram of the rest of the antibiotics, Vitek 2® Compact system cards were used for Gram-negative bacilli AST-XN05 (ampicillin/sulbactam, aztreonam, cefepime, cefexime, ceftriaxone, cefuroxime, chloramphenicol, colistine, ESBL detection, levofloxacin, meropenem, minocycline, moxifloxacin, piperacillin, tetracycline, ticarcillin/clavulanic acid, tigecycline, trimethoprim) and AST-N285 tests (amikacin, amoxicillin/clavulanic acid, ampicillin, cefazolin, ceftazidime, ceftriaxone, ciprofloxacin, doripenem, ertapenem, ESBL detection, gentamicin, imipenem, meropenem, nitrofurantoin, piperacillin/tazobactam, trimethoprim/sulfamethoxazole). The automated antibiogram procedure is as follows: from a bacterial suspension with a turbidity of 0.5 McFarland units, 14 µL are transferred to a polystyrene tube with 3 mL of saline; subsequently, the corresponding antibiogram card is inserted in the rack with the tube and introduced into the equipment.

Table 1. Enterobacteriaceae and *Pseudomonas aeruginosa* sensitivity to CZ/A and C/T

Microorganism	Type of sample	No. of samples	Sensitivity (%)	
			CZ/A	C/T
Enterobacteriaceae				
Escherichia coli	Urine	58	100	88
	Others	4	100	100
Klebsiella spp.	Urine	4	100	100
	Others	1	100	100
Salmonella	Urine	0	NT	NT
	Others	2	100	100
Enterobacter aerogenes	Urine	0	NT	NT
	Others	1	100	100
Morganella morganii	Urine	0	NT	NT
	Others	1	100	100
Proteus mirabilis	Urine	1	100	100
	Others	1	100	100
Serratia marcescens	Urine	0	NT	NT
	Others	1	100	100
Pseudomonas	Bronchial secretion	11	72.72	72.72
	Others	8	75	75

NT = not tested, CZ/A = ceftazidime/avibactam, C/T = ceftolozane/tazobactam.

Interpretation of the automated microdilution and disk diffusion results was based on document M100-S28 cutoff points.

For statistical analysis, susceptibility and resistance percentages were calculated. McNemar test was used to compare antibiotics' effectiveness, with a p-value < 0.05 being regarded as significant.

Results

Seventy-four enterobacteriaceae and 19 *Pseudomonas aeruginosa* strains were analyzed. CZ/A sensitivity percentage was 100 % for enterobacteriaceae and 72.7 % for *Pseudomonas aeruginosa* (Table 1); as for C/T, sensitivity percentage was 90.5 % for enterobacteriaceae and 72.7 % for *P. aeruginosa*. The most common sample of infectious origin was urine, with 68.8 % of total samples (Table 1). Twenty-five ESBL-producing and one carbapenemase-producing enterobacteriaceae were found. Tables 2 and 3 show the sensitivity percentages of the strains for the rest of the tested antibiotics.

Discussion

CZ/A and C/T are new antibiotic combinations that have been introduced to the market with promising results against multidrug resistant bacteria.¹¹ Current problem of bacterial resistance¹² has turned into an important public health issue. The results show that these new combinations of cephalosporin with beta-lactamase inhibitors are good options when making a therapeutic decision in our setting, and for this reason we decided to submit the information so far obtained as a brief communication.

CZ/A showed a higher sensitivity for ESBL-producing enterobacteriaceae in comparison with C/T (100 % vs. 80 %). Both drug combinations showed superior antimicrobial activity in comparison with commonly-used antibiotics in enterobacteriaceae, such as quinolones (100 % vs. 56 %), cephalosporins (100 % vs. 63.4 %), and penicillins (100 % vs. 24 %). In *Pseudomonas* isolates, CZ/A and C/T showed higher activity (73.6 % sensitivity) in comparison with carbapenem antibiotics

Table 2. Enterobacteriaceae sensitivity to clinically used antibiotics

Antibiotic	Sensitivity	<i>Escherichia coli</i> (62)	<i>Klebsiella</i> spp. (5)	<i>Salmonella</i> (2)	<i>Enterobacter aerogenes</i> (1)	<i>Morganella morganii</i>	<i>Proteus mirabilis</i>	<i>Serratia marcescens</i>
AMP	No. of strains	49	4	NT	NT	NT	2	NT
	Sensitivity	24 %	0 %	NT	NT	NT	50 %	NT
AMC	No. of samples	27	4	NT	NT	NT	NT	NT
	Sensitivity	44 %	50 %	NT	NT	NT	NT	NT
SAM	No. of samples	34	1	2	1	1	2	NT
	Sensitivity	38.23 %	0 %	100 %	100 %	100 %	50 %	NT
CXM	No. of samples	34	2	2	1	NT	2	NT
	Sensitivity	57.14 %	0 %	0 %	0 %	NT	50 %	NT
CXM axetil	No. of samples	32	1	2	1	NT	2	NT
	Sensitivity	56 %	0 %	0 %	0 %	NT	50 %	NT
CRO	No. of samples	62	5	2	1	1	1	NT
	Sensitivity	64.51 %	40 %	100 %	100 %	100 %	100 %	NT
FEP	No. of samples	35	3	2	1	1	2	NT
	Sensitivity	68.57 %	33 %	100 %	100 %	100 %	50 %	NT
ETP	No. of samples	51	5	NT	NT	1	2	NT
	Sensitivity	98 %	100 %	NT	NT	100 %	100 %	NT
MEM	No. of samples	62	5	2	1	1	2	NT
	Sensitivity	96.77 %	100 %	100 %	100 %	100 %	100 %	NT
LVX	No. of samples	62	5	2	1	1	2	NT
	Sensitivity	56.45 %	80 %	100 %	100 %	0 %	50 %	NT
CIP	No. of samples	58	4	2	1	1	2	NT
	Sensitivity	56.89 %	100 %	100 %	100 %	0 %	50 %	NT
AMK	No. of samples	55	3	NT	NA	1	2	NT
	Sensitivity	98.18 %	100 %	NT	NA	100 %	100 %	NT
GEN	No. of samples	49	4	NT	NA	1	2	NT
	Sensitivity	72.00 %	75 %	NT	NA	0 %	50 %	NT
SXT	No. of samples	51	4	2	1	1	2	NT
	Sensitivity	65 %	25 %	100 %	100 %	0 %	100 %	NT
NIT	No. of samples	53	4	NT	NT	NA	2	NT
	Sensitivity	84.90 %	25 %	NT	NT	NA	50 %	NT
FOS	No. of samples	54	4	NT	NT	NA	2	NT
	Sensitivity	94.44 %	25 %	NT	NT	NA	100 %	NT

NT = not tested, AMP = ampicillin, AMC = amoxicillin/clavulanic acid, SAM = ampicillin/sulbactam, CXM = cefuroxime, CRO = ceftriaxone, FEP = cefepime, ETP = ertapenem, MEM = meropenem, LVX = levofloxacin, CIP = ciprofloxacin, AMK = amikacin, GEN = gentamicin, SXT = trimethoprim/sulfamethoxazole, NIT = nitrofurantoin, FOS = fosfomicin.

Table 3. *Pseudomonas aeruginosa* sensitivity to anti-pseudomonas antibiotics

Antibiotics	Sensitivity	<i>Pseudomonas</i> (n = 19)
Ticarcillin/AC	No. of strains	16
	Sensitivity	12.5
Piperacillin/T	No. of samples	19
	Sensitivity	52.63
Ceftazidime	No. of samples	17
	Sensitivity	64.7
Cefepime	No. of samples	18
	Sensitivity	61.11
Imipenem	No. of samples	18
	Sensitivity	38.88
Meropenem	No. of samples	19
	Sensitivity	42.1
Levofloxacin	No. of samples	19
	Sensitivity	36.84
Ciprofloxacin	No. of samples	19
	Sensitivity	31.57
Amikacin	No. of samples	17
	Sensitivity	58.82
Colistin	No. of samples	18
	Sensitivity	94.44

AC = clavulanic acid, T = tazobactam.

(meropenem 42.1 %, imipenem 38.8 %) and anti-pseudomonas such as amikacin (58.82 %), piperacillin/tazobactam (52.3 %), ceftazidime (64.7 %) and cefepime (61.1 %), which places them as the most sensitive antibiotics, only after colistin (94.4 %).

In addition to the comparison that was made with the new drugs of interest, this study shows at the same time the elevated resistance of strains to several antibiotics considered first-choice for Gram-negative bacteria such as cefuroxime, ceftriaxone, levofloxacin and ciprofloxacin, with approximately 40 % resistance of *Escherichia coli* and 50 % of *Pseudomonas aeruginosa* to carbapenems, 40 % to anti-pseudomonas penicillins and around 30 % resistance to aminoglycosides. These resistance patterns observed in our community are similar to those reported in recent publications¹² and should be considered by the

clinician at the time of making a therapeutic decision.

In conclusion, the CZ/A and C/T combinations have a good response in vitro, especially with regard to ESBL-producing enterobacteriaceae and *Pseudomonas aeruginosa*. CZ/A and C/T, when compared with antibiotics that are conventionally used in clinical practice for infections caused by enterobacteriaceae, did not show a statistically significant difference with regard to meropenem ($p = 1$ vs. $p = 0.625$, respectively), but were clearly superior to ceftriaxone ($p < 0.01$ vs. $p < 0.01$). As for *Pseudomonas aeruginosa*, due to the reduced number of strains, it is not pertinent to claim statistical superiority of CZ/A and C/T in comparison with other anti-pseudomonas antibiotics such as colistin and meropenem.

Although CZ/A and C/T can be quite useful in the management of highly resistant Gram-negative microorganisms, currently available data to support their clinical efficacy in Mexico are still limited. Another aspect to consider is the economic cost, which can be almost double when compared to other antibiotics that showed similar antimicrobial activity, such as carbapenems and aminoglycosides.

We strongly advise judicious use of the susceptibility data obtained with this research, since the frequency of pathogenic strains isolation, as well as bacterial resistance can considerably vary between different geographical regions, even between hospitals in the same city. It is essential to know the specific epidemiological situation of the place we are located in, the most commonly isolated bacteria in the community, as well as their resistance patterns, which will allow guiding antibiotic treatment with less margin of error.¹³

One limitation of the present investigation was that the reagents to perform CZ/A and C/T microdilution were not available, which is why comparing in vitro strength between both antibiotics against the identified strains was not possible.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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