Clinical Microbiology and Infection 26 (2020) 1264–1265

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Letter to the Editor

Emergence of ceftazidime/avibactam resistance in KPC-8–producing *Klebsiella pneumoniae* in South America

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ARTICLE INFO

Article history:
Received 13 January 2020
Received in revised form
6 March 2020
Accepted 13 March 2020
Available online 23 March 2020

Editor: L. Leibovici

Klebsiella pneumoniae is one of the so-called ESKAPE pathogens. These organisms are the main cause of nosocomial infections worldwide, causing life-threatening infections amongst critically ill and immunocompromised individuals. They are characterized by drug resistance mechanisms. *Klebsiella pneumoniae* carbapenemase (KPC)-producing isolates display resistance to multiple antimicrobial agents, usually including last-resort alternative options, leading to an urgent need to develop new drugs or combinations. In Argentina sequence type (ST) 258 harbouring bla_{KPC-2} emerged in 2010 and remained prevalent until the last few years, when the emergence of different STs such as ST25, ST11 and ST307 appeared likely to change the local epidemiology [1].

Ceftazidime/avibactam (CZA) is a novel β-lactam/β-lactamase inhibitor combination that inactivates KPC and OXA-48 carbapenemases, AmpC and extended-spectrum β-lactamases (ESBL), but it is not active against metallo-β-lactamases. In the last 2 years, CZA was introduced in some nosocomial centres in Argentina with promising expectations. The combination of enhanced expression of KPC, porin mutations and the increase of ceftazidime (CAZ) hydrolysis in some variants has been reported as the main resistance mechanisms to this antibiotic combination [2,3].

Three CZA-resistant *K. pneumoniae* isolates harbouring KPC-8 were obtained from urine samples of patients who had previously been treated with multiple antimicrobials but had not received CZA therapy in a hospital in Buenos Aires during the period June to November 2019.

Identification was performed by MALDI-TOF MS and susceptibility testing by Phoenix System (NMIC-406 panel) and broth microdilution for CZA and colistin (Med Chem Express). The isolates were characterized phenotypically and genotypically by PCR using specific primers for the detection of bla_{CTX-M}, bla_{KPC}, bla_{OXA}, bla_{NDM}, bla_{VIM} and bla_{IMP} and were confirmed by sequencing (Macrogen). Repetitive-element PCR and multilocus sequence typing were also performed. Plasmid conjugation assay was performed in lysogeny broth with *Escherichia coli* J53 AzR as the recipient strain and *KpB1* as the donor strain.

KpB1, *KpB2* and *KpB3* were resistant to all β-lactams, including CZA (MIC: 16 mg/L) and to amikacin, gentamicin, trimethoprim/sulfamethoxazole and fluoroquinolones. They remained susceptible to colistin (MIC: 1 mg/L), fosfomycin and tigecycline.

The three isolates generated indistinguishable repetitive-element PCR fingerprints, and *KpB1* belonged to ST11 according to multilocus sequence typing. The phenotypic synergy test was positive for ESBL detection ceftriaxone, amoxicillin clavulanic acid and ceftazidime (CRO-AMC-CAZ). Double-disc synergy tests for carbapenemases using imipenem, phenylboronic acid and meropenem (IMI-PBA-MER), and imipenem, EDTA and meropenem (IMI-EDTA-MER) were performed. KPC production was confirmed, whereas the test was negative for the presence of metallo-β-lactamases. Genotypic analysis confirmed that the isolates harboured bla_{KPC-8} and $bla_{CTX-M-15}$. The conjugation experiment performed on *KpB1* revealed the plasmid location of the KPC-8 coding gene, a result confirmed by the colourimetric Blue-Carba Test, by the

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https://doi.org/10.1016/j.cmi.2020.03.013

Table 1
Antimicrobial drug susceptibility for *KpB1*, *Escherichia coli* J53 AzR and *KpB1*TC

Antimicrobial agent	MIC (mg/L)		
	KpB1	*E. coli* J53 AzR	*KpB1*TC
Ampicillin	>16	≤4	>16
Ampicillin/sulbactam	>16/8	≤4/2	>16/8
Piperacillin/tazobactam	>64/4	≤4/4	>64/4
Cefazolin	>8	≤2	>8
Cefoxitin	16	≤4	≤4
Ceftriaxone	>4	≤1	>4
Ceftazidime	128	≤1	64
Cefepime	>16	≤1	16
Ertapenem	8	≤0.25	0,5
Imipenem	4	≤0.25	1
Meropenem	4	≤0.5	1
Amikacin	>32	≤8	>32
Gentamicin	>8	≤2	>8
Trimethoprim/sulfamethoxazole	>2/38	≤0.5/9.5	>2/38
Colistin	≤1	≤1	≤1
Ciprofloxacin	>2	≤0.125	≤0.125
Levofloxacin	>4	≤1	≤1
Fosfomycin	≤16	≤16	≤16
Tigecycline	2	≤1	≤1
Ceftazidime/avibactam	16	0.125	1

KpB1, *KpB2* and *KpB3* showed the same susceptibility profile.

phenylboronic acid synergy test and by PCR, on the transconjugant (*KpB1*TC). The antimicrobial susceptibility data of *KpB1* and *KpB1*TC are listed in Table 1. Both *KpB1* and *KpB1*TC displayed resistance to extended-spectrum cephalosporins, including CAZ, the resistance of which was inhibited by adding avibactam). This effect was observed in *KpB1*TC with avibactam at ≥4 mg/L, compared to *KpB1* with avibactam ≥10 mg/L (data not shown). The *KpB1*TC MIC to CZA was eightfold higher (1 mg/L) than *E. coli* J53 AzR.

Among acquired carbapenemases reported in Argentina, KPC-2 is absolutely prevalent and to a lesser extent KPC-3, while KPC-8 was not previously detected [1]. KPC-8 differs from KPC-2 by two amino acid substitutions (V240G and H274Y) and from KPC-3 by one amino acid substitution (V240G). Mutations within the KPC Ω loop (R164 to D179) have been described in KPC-2 and KPC-3. These substitutions have been related to enhanced affinity towards CAZ, prevention of binding to avibactam and reversion of carbapenem resistance [2,4]. Mutations distantly located from the Ω loop in the regions covering the amino acids 240–243 (close to the hinge loop) and 263–277 (in the vicinity of the X loop and the hinge loop) have been also described [5,6]. Substitution V240G, as observed in KPC-8, may enhance backbone flexibility, so that larger substrates might be accommodated in the active site. KPC-8–producing isolates presented an 80-fold increase in CAZ MICs compared to KPC-2 due as a result of the enhanced CAZ catalytic efficiency of KPC-8, in addition to the protein expression level and its stability and solubility [7].

An increase in CZA MICs in KPC-8–producing *K. pneumoniae* isolates was reported in 2017 by Shields et al. [2]. In our study the fact that the addition of avibactam restored CAZ activity suggests, as was observed by Shields et al., that the resistance to CZA in these KPC variants may be related to the high CAZ MIC value. Shields et al. described the emergence of mutations in plasmid-borne bla_{KPC-3} in isolates recovered after 10 to 19 days' CZA treatment with respect to baseline isolates. All these isolates belonged to ST258, while KPC-8–producing isolates recovered in the present study corresponded to ST11. The presence of this ST was reported in our region to be initially associated with the production of CTX-M-15 and more recently with KPC-2 [1]. Moreover, decreased CZA susceptibility was reported in 2017 by Shen et al. [8] in KPC-2–producing clinical isolates corresponding to ST11. These isolates displayed an increased gene expression of bla_{KPC-2} and the coproduction of ESBLs, which confers higher CAZ hydrolysis activity, added to the loss of OmpK35.

KPC-8 is a KPC variant with low prevalence worldwide and in our region, but there is a potential risk that the increasing use of CZA may exert selective pressure in favour of its dissemination.

In conclusion, we reported three CZA-resistant *K. pneumoniae* harbouring KPC-8 clinical isolates. At the time of this writing, there were no previous reports of CZA resistance in South America in carbapenem-resistant *Enterobacteriaceae*.

Transparency Declaration

Funding was received by UBACyT 20020150100174BA (to Marcela Radice) and UBACyT 20020130100167BA (to Angela Famiglietti), Argentina. All authors report no conflicts of interest relevant to this letter.

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