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Short Communication

Carbapenemase detection testing in the era of ceftazidime/avibactam-resistant KPC-producing Enterobacterales: A 2-year experience

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ABSTRACT

Objectives: The aim of this study was to investigate the prevalence of ceftazidime/avibactam (CZA) resistance among carbapenemase-producing Enterobacterales (CPE) blood culture isolates as well as the performance of the main carbapenemase phenotypic detection methods to identify KPC variants associated with CZA resistance.

Methods: Non-duplicate CPE strains isolated from blood cultures during 2018–2020 were tested for antimicrobial susceptibility. Molecular testing was used to identify carbapenemase-producers. Strains harbouring bla_{KPC} and with a CZA minimum inhibitory concentration (MIC) ≥8 mg/L were investigated by sequencing. Subsequentially, five phenotypic carbapenemase detection methods were evaluated on these strains, namely the modified carbapenem inactivation method (mCIM), Rapidec® Carba NP, the disk diffusion synergy test, NG-Test CARBA® 5 and RESIST-5 O.O.K.N.V.

Results: Overall, the CZA resistance rate was high (13.7%) and remained relevant (5.9%) excluding metallo-β-lactamases-producers. All isolates harbouringbla_{KPC} mutants (n = 8) were associated with reduced carbapenem MICs and negative results by all detection methods based on revelation of enzyme activity. Lateral flow immunoassays failed to detect KPC-31 (n = 4) and KPC-33 (n = 2) but correctly identified KPC-14 (n = 2). Conversely, isolates harbouring wild-type KPC genes (n = 3) were associated with high-level CZA resistance and carbapenem resistance and tested positive by all of the evaluated methods.

Conclusion: In the era of CZA-based therapies, molecular bla_{KPC} identification followed by a carbapenem hydrolysis-based phenotypic assay could be the most reasonable diagnostic algorithm to detect all KPC-producers and to identify mutants associated with impaired carbapenemase activity and CZA resistance.

1. Introduction

The emergence of carbapenemase-producing Enterobacterales (CPE) has become a major public-health concern over the last decade owing to the dissemination of complex multidrug-resistant phenotypes [1]. In this scenario, rapid and accurate detection of carbapenemases is of paramount importance both for epidemiological and infection control purposes. Additionally, rapid characterisation of carbapenemase types can help to guide antibiotic therapy, as various carbapenemase classes confer different resistance spectra to novel β-lactam/inhibitor combinations such as ceftazidime/avibactam (CZA). Indeed, avibactam inhibits the activity of Ambler class A, class C and some class D β-lactamases, including carbapenemases (e.g. KPC, OXA-48), but remains ineffective towards Ambler class B enzymes [metallo-β-lactamases (MBLs)] such as NDM, VIM and IMP [2]. Despite the fact that CZA has been recently introduced, several reports have described in vivo selection of KPC-producing Enterobacterales resistant to CZA following prolonged treatment. The majority of these CZA-resistant strains harbour new KPC variants exhibiting single amino acid substitutions between positions 164–179 in their omega loop, with Asp179Tyr (D179Y) being particularly common. KPC D179Y variants are characterised by loss of carbapenemase activity and restoration of carbapenem susceptibility together with a concomitant reduced binding to avibactam [3–5]. Moreover, KPC variants encoding mutations outside the omega loop region and associated with CZA resistance (e.g. KPC-41, KPC-23, KPC-14, KPC-8 and KPC-50) have recently been isolated from patients with or without a history of CZA treatment [6–10].

As recently shown, molecular testing is capable of detecting all KPC mutants, whereas phenotypic methods based on carbapenem hydrolysis [i.e. Carba NP test and its commercial derivatives,

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matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS)-based assays and the carbapenem inactivation method] or disk diffusion synergy tests may be unable to detect KPC variants with reduced carbapenemase activity [11–13]. The inability of lateral flow immunoassays (LFIAs) to detect KPC variants encoding D179Y mutations has also been reported [11–14].

Therefore, the choice of the most suitable carbapenemase detection method depends on the carbapenemase epidemiological context, including the prevalence of isolates harbouring KPC variants not detectable by the most commonly used phenotypic methods.

In Italy, CZA has been available since February 2018 and recent reports attest to the emergence of CZA-resistant Enterobacterales strains [15,16].

In this study, we analysed the prevalence of CZA resistance among CPE strains isolated from blood cultures collected at the University Hospital 'Città della Salute e della Scienza di Torino' (Turin, Italy) in the period 2018–2020. Subsequently, we assessed the performance of five phenotypic carbapenemase detection methods on KPC-producing Enterobacterales showing resistance or reduced susceptibility to CZA.

2. Materials and methods

2.1. Characterisation of carbapenemase-producing isolates

Non-duplicate CPE strains isolated from blood cultures were prospectively collected from February 2018 to August 2020. Isolates were identified by MALDI-TOF/MS (Bruker Daltonik GmbH, Bremen, Germany). Antimicrobial susceptibility was determined by a commercially available microdilution assay (Panel NMDR, MicroScan® WalkAway® 96 Plus; Beckman Coulter, Switzerland), and CZA minimum inhibitory concentrations (MICs) were confirmed by Etest (bioMérieux, France). Susceptibility data were interpreted according to current European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (version 11.0, 2021; https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_11.0_Breakpoint_Tables.pdf). A commercial molecular assay (Xpert® Carba-R; Cepheid, Sunnyvale, CA, USA) was used to characterise carbapenemase-producers when the meropenem MIC was >0.12 mg/L (EUCAST screening cut-off value for CPE) or the ceftazidime/avibactam MIC was ≥8 mg/L.

2.2. Evaluation of phenotypic methods for the detection of KPC

Among isolates harbouring the bla_{KPC} gene and with a CZA MIC ≥ 8 mg/L, carbapenemase production was investigated in duplicate by five phenotypic methods, namely the modified carbapenem inactivation method (mCIM), Rapidec® Carba NP (bioMérieux), the disk diffusion synergy test (KPC, MBL and OXA-48 Confirm Kit; Rosco Diagnostica A/S) and two LFIAs [NG-Test CARBA® 5 (NG Biotech, France) and RESIST-5 O.O.K.N.V (Coris Bioconcept, Belgium)]. Assays were performed on overnight subcultures from blood agar following Clinical and Laboratory Standards Institute (CLSI) guidelines (M100, 27th ed; http://www.iacld.ir/DL/public/96/CLSI-2017.pdf) or the manufacturer's instructions, as appropriate.

2.3. Characterisation of bla_{KPC} genes

On KPC-producing strains with a CZA MIC ≥ 8 mg/L, bla_{KPC} genes were amplified by PCR using the following primers: KPC-fw, TGT CAC TGT ATC GCC GTC TAG; and KPC-rev, TTA CTG CCC GTT GAC GCC CAA TCC. Amplicons were capillary sequenced using an Applied Biosystems 3130xl Genetic Analyzer (Life Technologies, Carlsbad, CA, USA) and were compared with available sequences in the GenBank database.

3. Results

3.1. Prevalence of ceftazidime/avibactam resistance

A total of 168 CPE strains were collected, of which 89.9% (n = 151) produced KPC, 7.1% (n = 12) produced VIM, 2.4% (n = 4) produced OXA-48-like and 0.6% (n = 1) produced both VIM and KPC (Table 1).

Overall, the CZA resistance rate was 13.7% (n = 23) and the MIC_{50} and MIC_{90} values were 2 mg/L and 256 mg/L, respectively.

Among the CZA-resistant isolates, 52.2% (n = 12) were VIM MBL-producing Enterobacterales, 43.5% (n = 10) were KPC-producing *Klebsiella pneumoniae* and 4.3% (n = 1) was a VIM/KPC-co-producing *K. pneumoniae*. MICs ranged from 16 mg/L to >256 mg/L (Table 1). Excluding Enterobacterales strains expressing VIM MBLs, which are intrinsically non-susceptible to the activity of avibactam, the CZA resistance rate was 5.9% (n = 10) and the MIC_{50} and MIC_{90} values were 1.5 mg/L and 6 mg/L, respectively. All CZA-resistant KPC-producing *K. pneumoniae* isolates were collected from blood cultures of patients treated with CZA.

Table 1
Characterisation of carbapenemase-producing Enterobacterales isolated from blood cultures (2018–2020).

Carbapenemase status	Species	No. of isolates	MIC range (mg/L)[a]	
			MEM	CZA
CZA-susceptible (MIC ≤ 8 mg/L)				
bla_{KPC}	*Klebsiella pneumoniae*	138	≤0.12 to >32	0.125–8
	Klebsiella oxytoca	1	16	0.5
	Serratia marcescens	1	>8	0.125
	Enterobacter cloacae	1	2	0.5
$bla_{OXA-48-like}$	*K. pneumoniae*	3	1–16	0.125–0.5
	Escherichia coli	1	8	0.125
CZA-resistant (MIC > 8 mg/L)				
bla_{VIM}	*K. pneumoniae*	2	1–8	>256
	E. cloacae	7	2–32	>256
	E. coli	2	1–2	>256
	Morganella morganii	1	2	>256
bla_{VIM} + bla_{KPC}	*K. pneumoniae*	1	>32	>256
bla_{KPC}	*K. pneumoniae*	10	≤0.12 to >32	16 to >256

MIC, minimum inhibitory concentration; MEM, meropenem; CZA, ceftazidime/avibactam.
[a] CZA MICs were determined by Etest, whereas MEM MICs were determined by a commercial microdilution system. MICs were interpreted following current European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints.

3.2. Characterisation of bla_{KPC} and performance of carbapenemase detection assays

Among the KPC-producing Enterobacterales isolates, 11 of the 151 were included in the evaluation of the mCIM, Rapidec® Carba NP, disc diffusion synergy test, NG-Test CARBA® 5 and RESIST-5 O.O.K.N.V assays. Ten of them showed CZA resistance with MICs ranging from 16 mg/L to >256 mg/L, whereas one *K. pneumoniae* isolate was susceptible to CZA with an MIC of 8 mg/L. Characterisation of the isolates and the results obtained by the five carbapenemase detection methods are detailed in Table 2.

Data sequencing revealed that eight *K. pneumoniae* strains carried a mutated bla_{KPC} gene (four bla_{KPC-31}, two bla_{KPC-33} and two bla_{KPC-14}). All mutated KPC variants were associated with increased CZA MICs (ranging from 8 mg/L to >256 mg/L) and carbapenem susceptibility. Unlike molecular testing that was able to detect all mutated KPC variants, methods based on carbapenem hydrolysis (Rapidec® Carba NP test and mCIM) or boronic acid synergy (disc diffusion synergy test) achieved negative results. Of note, both LFIAs were capable detecting bla_{KPC-14} but not bla_{KPC-31} and bla_{KPC-33}.

Conversely, the three isolates harbouring wild-type KPC genes (two bla_{KPC-3} and one bla_{KPC-2}) were associated with high-level CZA resistance (MICs > 256 mg/L), carbapenem resistance and tested positive for carbapenemase production by all of the evaluated methods.

4. Discussion

In the era of multidrug-resistant Gram-negative bacteria, rapid diagnostic tests detecting carbapenemase activity or genes are increasingly used for epidemiological and infection control purposes as well as to predict antimicrobial resistance. In fact, analysis of resistance markers together with knowledge of local epidemiology may allow the prediction of antibiotic-resistant phenotypes and to optimise empirical antibiotic therapy early when conventional antimicrobial susceptibility testing results are not yet available.

CZA represents an optimal choice for the treatment of infections caused by serine carbapenemase-producing Gram-negative bacteria. However, recent reports highlighted important limitations of the main carbapenemase detection methods in detecting KPC variants associated with CZA resistance.

In this study, we analysed the prevalence of CZA resistance among CPE strains isolated from blood cultures of patients admitted to a reference university hospital of Northwest Italy since the beginning of CZA use. Subsequently, we evaluated the ability of the main phenotypic carbapenemase detection methods to detect KPC variants harboured by isolates with increased CZA MICs.

Overall, the CZA resistance rate among CPE isolates was high (13.7%) and remained relevant (5.9%) excluding intrinsically-resistant MBL-producers compared with a more recent Italian report [17].

Although KPC variants encoding the D179Y mutation represent the most common mechanism associated with CZA resistance, we observed two cases of bloodstream infection by *K. pneumoniae* strains carrying KPC-14, a KPC-2 Δ242-GT-243 variant rarely reported in the clinical setting to date [8,18]. Additionally, 3 of 11 *K. pneumoniae* isolates were resistant to CZA and harboured no mutation in bla_{KPC}, suggesting the presence of other resistance mechanisms.

All KPC mutants were associated with reduced carbapenem MICs owing to the lack of carbapenemase activity, which consequently led to negative results by all of the methods based on enzyme activity (i.e. Rapidec® Carba NP test, mCIM and disc diffusion synergy test). Conversely, LFIAs failed to detect KPC-2- and KPC-3-D179Y but correctly identified KPC-14, suggesting a specific role of this amino acid substitution in the lack of binding affinity to the LFIA antibodies. Interestingly, 3 of 11 KPC-producing *K. pneumoniae* strains showed a meropenem MIC ≤ 0.125 mg/L, which is lower than the screening carbapenemase cut-off defined by EUCAST (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf).

These findings outline the limitations and challenges faced by diagnostic laboratories to correctly identify strains producing various KPC variants. Genotypic information is the most revealing, while phenotypic assays often used yield conflicting results that do not help with the diagnosis of a KPC-producer. Indeed, the majority of avibactam-resistant KPC variants are associated with restoration of carbapenem susceptibility, expression of an extended-spectrum β-lactamase (ESBL) phenotype and negative results by the most used phenotypic carbapenemase detection methods. This evidence could legitimise the use of carbapenems, missing the implementation of infection control measures. However, since various

Table 2
Characterisation of KPC-producing strains showing CZA MICs ≥8 mg/L and performance of different carbapenemase detection assays.

Isolate	bla_{KPC} variant (amino acid mutation)	MIC (mg/L) (EUCAST interpretation)[a]			Carbapenemase detection assay				
		CZA	MEM	IPM	NG-Test CARBA® 5	RESIST-5 O.O.K.N.V.	Rapidec® Carba NP	mCIM	Disk diffusion synergy test (KPC, MBL and OXA-48 Confirm Kit)
KpBC01	bla_{KPC-14} (Δ242-GT-243)	64 [R]	≤0.12 [S]	≤1 [S]	Pos.	Pos.	Neg.	Neg.	Neg.
KpBC02	bla_{KPC-14} (Δ242-GT-243)	>256 [R]	2 [S]	≤1 [S]	Pos.	Pos.	Neg.	Neg.	Neg.
KpBC03	bla_{KPC-33} (D179Y)	8 [S]	≤0.12 [S]	≤1 [S]	Neg.	Neg.	Neg.	Neg.	Neg.
KpBC04	bla_{KPC-33} (D179Y)	>256 [R]	8 [I]	≤1 [S]	Neg.	Neg.	Neg.	Neg.	Neg.
KpBC05	bla_{KPC-31} (D179Y)	>256 [R]	4 [I]	≤1 [S]	Neg.	Neg.	Neg.	Neg.	Neg.
KpBC06	bla_{KPC-31} (D179Y)	32 [R]	4 [I]	≤1 [S]	Neg.	Neg.	Neg.	Neg.	Neg.
KpBC07	bla_{KPC-31} (D179Y)	>256 [R]	8 [I]	≤1 [S]	Neg.	Neg.	Neg.	Neg.	Neg.
KpBC08	bla_{KPC-31} (D179Y)	16 [R]	≤0.12 [S]	≤1 [S]	Neg.	Neg.	Neg.	Neg.	Neg.
KpBC09	bla_{KPC-2}	>256 [R]	>32 [R]	>8 [R]	Pos.	Pos.	Pos.	Pos.	Pos.
KpBC10	bla_{KPC-3}	>256 [R]	>32 [R]	>8 [R]	Pos.	Pos.	Pos.	Pos.	Pos.
KpBC11	bla_{KPC-3}	>256 [R]	>32 [R]	>8 [R]	Pos.	Pos.	Pos.	Pos.	Pos.

CZA, ceftazidime/avibactam; MIC, minimum inhibitory concentration; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MEM, meropenem; IPM, imipenem; S, susceptible; I, intermediate; R, resistant; Pos., positive; Neg., negative.

[a] CZA MICs were determined by Etest, whereas MEM and IPM MICs were determined by a commercial microdilution system. MICs were interpreted following current EUCAST breakpoints.

subpopulations of KPC-producing Enterobacterales with different resistance patterns can co-occur following treatment with CZA [19], a drug-driven selection of carbapenem-resistant subpopulations should be also considered. Indeed, Shields et al. highlighted meropenem resistance selection in CZA-resistant and meropenem-susceptible *K. pneumoniae* isolates during in vitro passage at subinhibitory meropenem concentrations [20].

In conclusion, use of rapid testing should be judicious and considered according to local epidemiology and clinical information of recent CZA therapy. In the era of CZA-based therapies, molecular bla_{KPC} detection followed by a carbapenem hydrolysis-based phenotypic assay could be the most reasonable diagnostic algorithm to detect all KPC-producers and to presumptively differentiate mutants associated with CZA resistance and impaired carbapenemase activity. However, since more resistance mechanisms could be involved in CZA resistance, traditional antimicrobial testing remains necessary.

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None.

Competing interests

None declared.

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki. Formal ethical approval was obtained by the institutional review board of our centre [Protocol No. 0029345].

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