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Klebsiella pneumoniae carbapenemase variants: the new threat to global public health

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SUMMARY *Klebsiella pneumoniae* carbapenemase (KPC) variants, which refer to the substitution, insertion, or deletion of amino acid sequence compared to wild *bla*$_{KPC}$ type, have reduced utility of ceftazidime-avibactam (CZA), a pioneer antimicrobial agent in treating carbapenem-resistant *Enterobacterales* infections. So far, more than 150 *bla*$_{KPC}$ variants have been reported worldwide, and most of the new variants were discovered in the past 3 years, which calls for public alarm. The KPC variant protein enhances the affinity to ceftazidime and weakens the affinity to avibactam by changing the KPC structure, thereby mediating bacterial resistance to CZA. At present, there are still no guidelines or expert consensus to make recommendations for the diagnosis and treatment of infections caused by KPC variants. In addition, meropenem-vaborbactam, imipenem-relebactam, and other new β-lactam-β-lactamase inhibitor combinations have little discussion on KPC variants. This review aims to discuss the clinical characteristics, risk factors, epidemiological characteristics, antimicrobial susceptibility profiles, methods for detecting *bla*$_{KPC}$ variants, treatment options, and future perspectives of *bla*$_{KPC}$ variants worldwide to alert this new great public health threat.

KEYWORDS *bla*$_{KPC}$ variants, *Klebsiella pneumoniae*, ceftazidime-avibactam

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INTRODUCTION

Antimicrobial resistance has become one of the most severe threats to global public health. Infections caused by carbapenem-resistant *Enterobacterales* (CRE) are associated with a particularly significant economic burden and mortality rate (1), which is often two to three times higher than carbapenem-susceptible *Enterobacterales* (2). Furthermore, CRE isolates are resistant to most of the antimicrobial agents available, limiting the choice of antimicrobial agents in clinical practice (2–4). In 2017, the World Health Organization published a priority list of antibiotic-resistant bacteria that listed CRE, carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), and carbapenem-resistant *Acinetobacter baumannii* (CRAB) as the most urgent threats, suggesting that there is an urgent need to develop new antimicrobial agents to counteract the rapid acquisition of antimicrobial resistance (5).

The most important mechanism underlying carbapenem resistance in carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and carbapenem-resistant *Escherichia coli* is the production of carbapenemases, including class A carbapenemases [mainly *Klebsiella pneumoniae* carbapenemase (KPC)], class B metallo-β-lactamases [mainly New Delhi metallo-β-lactamase (NDM)] , and some class D OXA-48-like carbapenemases (mainly OXA-181, OXA-232, and OXA-163) (6). Based on those, novel β-lactam-β-lactamase inhibitor combinations were developed continually to cope with infections caused by carbapenemase-producing *Enterobacterales*, including ceftazidime-avibactam (CZA), meropenem-vaborbactam, and imipenem-relebactam. CZA, launched in the USA in 2015 and in China in 2019, displayed potent *in vitro* activity against KPC-producing *Enterobacterales* and is a pioneer antimicrobial agent in treating infections caused by KPC-producing *Enterobacterales* (7–9). Unlike traditional enzyme inhibitors such as tazobactam and sulbactam, avibactam does not contain a β-lactam structure. Avibactam acts by covalent acylation of its β-lactamase targets in a reversible process in which the structure of avibactam is restored by deacylation (without hydrolysis), and intact avibactam is released to provide long-lasting enzyme inhibition effect (10). Most importantly, avibactam has a broader spectrum, inhibiting class A carbapenemases (particularly KPC-2), extended-spectrum β-lactamases (ESBLs), class C cephalosporinases, and some class D carbapenemases. Therefore, since its introduction into clinical use, CZA has been considered one of the most effective antimicrobial agents for the treatment of infections caused by KPC-producing strains, especially *K. pneumoniae* (9).

Nevertheless, the widespread clinical use of CZA has forced CRE to mutate to adapt to the increasing antibiotic pressure. The bla_{KPC} variant derived from bla_{KPC-2} or bla_{KPC-3} mutation has been reported (11–13). The bla_{KPC} variant usually refers to the substitution, insertion, or deletion of one or more amino acids compared to wild bla_{KPC} type (such as bla_{KPC-2} and bla_{KPC-3}), and those leading to modifications in the amino acid sequence with the carbapenemase active site, which are of greatest concern. So far, more than 145 bla_{KPC} variants have been reported worldwide, and most of the new variants were discovered in the past 3 years. A crucial phenotypic feature of KPC variants is their resistance to CZA compared to the wild-type (WT) gene product that is notably susceptible. This has led to new challenges in appropriate therapeutic selection (11, 14). In addition to posing a challenge for antimicrobial therapy, bla_{KPC} variants may challenge the performance of some classical carbapenemase detection methods used by clinical laboratories (14).

This review aims to discuss the clinical characteristics, risk factors, epidemiological characteristics, antimicrobial susceptibility profiles, methods for detecting bla_{KPC} variants, treatment options, and future perspectives of bla_{KPC} variants worldwide to alert this new significant public health threat.

EFFICACY OF CEFTAZIDIME-AVIBACTAM IN THE TREATMENT INFECTIONS CAUSED BY KPC-PRODUCING *ENTEROBACTERALES*

A multicenter, prospective, observational study was conducted between January 2018 and March 2019 to evaluate the outcomes and predictors of mortality in patients with KPC- or OXA-48-*K. pneumoniae* infections treated with CZA, with a focus on KPC-*K. pneumoniae* bloodstream infections (BSIs) (15). The study included 147 patients, 140 isolates were KPC-positive and 7 isolates were OXA-48-positive. Of these patients, 68 (46.3%) received CZA and 79 (53.7%) received CZA in combination with at least one other active agent, such as colistin, aminoglycosides, colistin plus tigecycline, tigecycline, trimethoprim/sulfamethoxazole, fosfomycin, etc. The 14- and 28-day mortality rates were 9% and 20%, respectively. The clinical characteristics of 71 patients with KPC-*K. pneumoniae* BSIs treated with a regimen containing CZA (cases) and of 71 patients, matched by propensity score, whose KPC-*K. pneumoniae* BSIs were treated with regimens not containing ceftazidime/avibactam (controls) were collected in this study. The results showed that 28-day mortality in the patients with KPC-*K. pneumoniae* BSIs treated with CZA was significantly lower than that observed in the matched patients whose KPC-*K. pneumoniae* BSIs were treated with regimens without CZA (18.3% vs 40.8%; $P = 0.005$). A retrospective, observational, multicenter study of 577 adults with BSIs or non-bacteremic infections primarily involving the urinary tract, lower respiratory tract, and intra-abdominal structures was conducted to evaluate the outcomes and mortality in patients with KPC-*K. pneumoniae* infections treated with CZA monotherapy and CZA combination regimens (16). The results showed that the 30-day all-cause mortality rate was 25% (146/577). There was no significant difference in mortality between patients treated with CZA alone and those treated with combination regimens (26.1% vs 25.0%, $P = 0.79$). Another retrospective multicenter study of 138 Italian patients with KPC-*K. pneumoniae* infections (KPC-*K. pneumoniae* bacteremic, KPC-*K. pneumoniae* non-bacteremic), all of whom received CZA as salvage therapy, was conducted to document the clinical characteristics and outcomes of these cases and to specifically investigate the outcomes and predictors of mortality in patients with KPC-*K. pneumoniae* bacteremia (17). All KPC-*K. pneumoniae* isolates were susceptible to CZA *in vitro*. The overall 30-day mortality rate was 34.1% (47/138), and 8.7% (12/138) of patients experienced recurrent KPC-*K. pneumoniae* infection after discontinuation of CZA treatment. In addition, the 30-day mortality rate of 109 patients with KPC-*K. pneumoniae* bacteremia receiving CZA was significantly lower than that of the control group (36.5% vs 55.7%, $P = 0.005$). This suggests that CZA-based therapy is an effective treatment option for infections caused by KPC-producing Gram-negative bacteria.

In the context of increasing carbapenem resistance in *Enterobacterales*, the introduction of CZA, with its broad spectrum of enzyme inhibition and low side effects, has provided a major boost to the clinical treatment of CRE infections (7, 9). CZA is the preferred treatment option for KPC-producing infections outside of the urinary tract (18). However, as CZA becomes more widely used in clinical practice, KPC-producing strains develop resistance to CZA through mutation under conditions of antimicrobial pressure and the presence of various other factors, leading to treatment failure (19, 20).

EPIDEMIOLOGICAL CHARACTERISTICS OF KPC-TYPE CARBAPENEMASE

First identified in 1996 in the northeastern United States, KPCs are the most common and widely distributed carbapenemases (21). KPCs can hydrolyze most β-lactams, including carbapenems, cephalosporins, monobactams, and cephamycins. They have been identified in many Gram-negative bacteria, including *Enterobacterales* and nonfermenting bacteria (e.g., *P. aeruginosa* and *A. baumannii*). *K. pneumoniae* is the most predominant KPC-producing species (22, 23). The strains producing KPC-2 or KPC-3 showed diverse susceptibility to imipenem and meropenem but were usually resistant to ertapenem. With the widespread use of carbapenems, KPC-producing bacteria have spread internationally. The dissemination of bla_{KPC} involves clonal spread, horizontal transfer, and plasmid-mediated spread. The prevalent bla_{KPC} variants vary

with geographic location, e.g., a predominance of bla_{KPC-2} in China but a predominance of bla_{KPC-3} and bla_{KPC-2} in the Americas and Europe (24–28). The international spread of KPC-producing *K. pneumoniae* is primarily associated with a single multilocus sequence type, sequence type 258 (ST258), and its variants (29). *K. pneumoniae* ST258 is the culprit in over 77% of KPC-producing *K. pneumoniae* infections in the USA and 90% in Israel (25, 26). The profile of sequence types (STs) was different between regions. ST512 is the most prevalent ST in Italy (28), whereas ST11 and ST437 predominate in China and Brazil, respectively (30, 31). Transmission of bla_{KPC} genes is mainly mediated by horizontal transfer of plasmids or mobility of small genetic elements (mainly Tn*4401* transposon) (32). The bla_{KPC} in *K. pneumoniae* had been reported on various plasmid types, such as IncF, IncI2, IncX, IncA/C, IncR, and ColE1, but the predominant plasmid type is IncF with FII K replicon (29, 33, 34).

Sporadic variants of bla_{KPC} were identified in 2006–2018 (≤7 reported per year) (35). However, since 2019, reports of bla_{KPC} variants have increased dramatically. The number reported between 2019 and 2020 exceeds the sum of reported bla_{KPC} variants in the previous 17 years (Fig. 1 and 2). The emergence of bla_{KPC-2} or bla_{KPC-3} mutants, such as bla_{KPC-33}, bla_{KPC-31}, etc., as the most prevalent cause of bacterial resistance to CZA during therapy has been attributed to the mechanism that altered the Ω-loop hydrogen

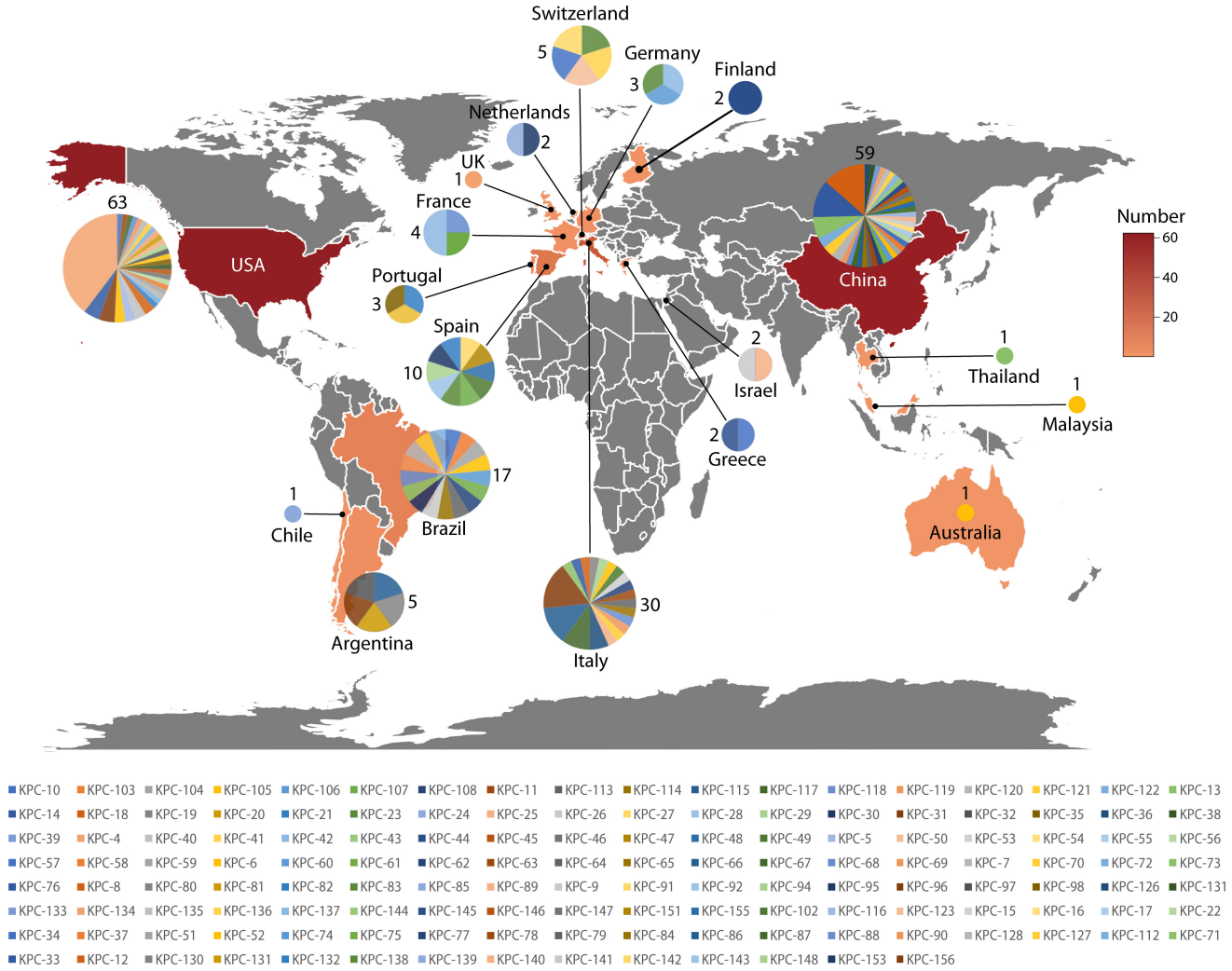

FIG 1 Distribution of *Klebsiella pneumoniae* carbapenemase variants based on KPC-2 and KPC-3 mutations. The data are calculated based on the number of *Klebsiella pneumoniae* carbapenemase variants uploaded to the National Center for Biotechnology Information each year in each region (https://www.ncbi.nlm.nih.gov/).

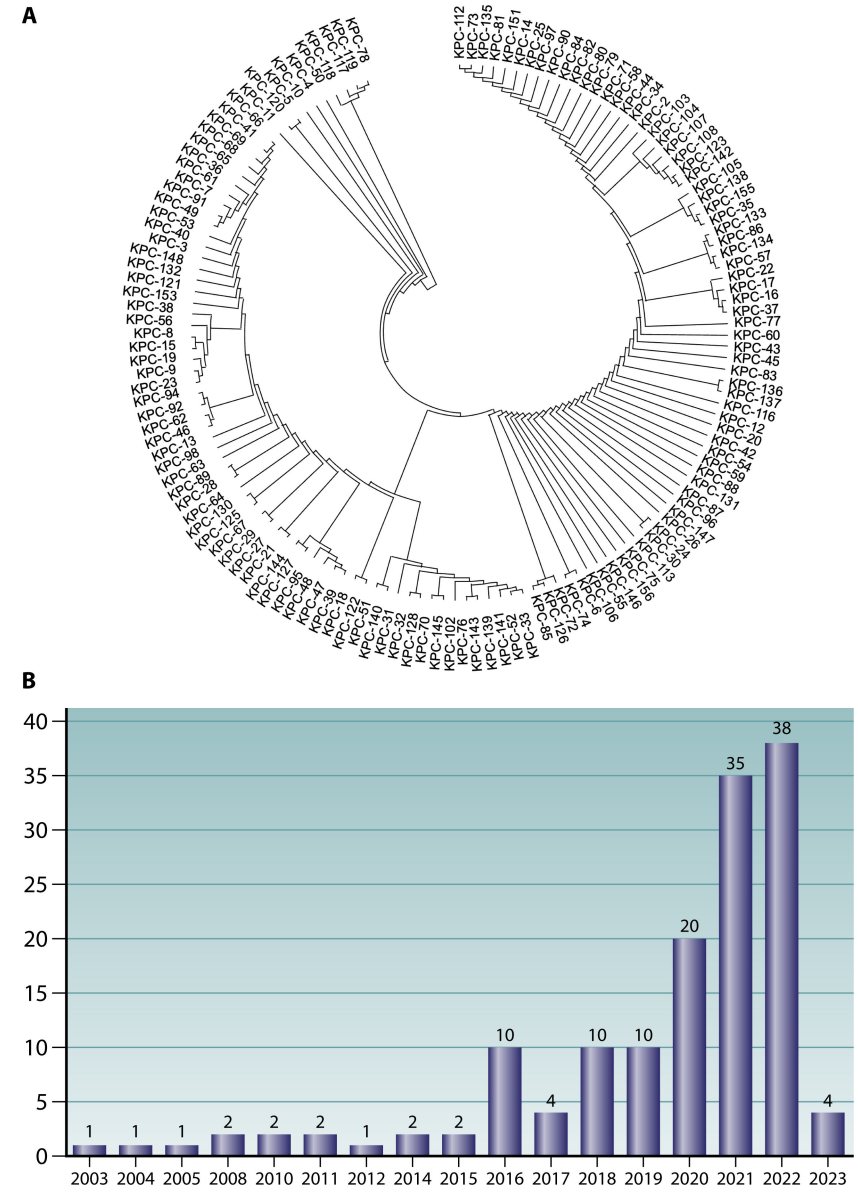

FIG 2 KPC variants evolutionary tree analysis chart (A) and the number of new variants reported in PubMed over the years (B) (up to March 2023).

bonding structure of KPC (Fig. 3) (36, 37). Compared to the wild type, KPC variant protein presented reduced catalytic ability to carbapenems, meanwhile responding poorly to avibactam inhibition (38–40). The crystallographic analysis of the D179N KPC-2 variant and the D179Y variant revealed the depth mechanism of CZA resistance conferred by D179N/Y variants of KPC-2 (40). The D179N KPC-2 structure showed that the change of the carboxyl to an amide moiety at position 179 disrupted the salt bridge with R164 present in KPC-2. Additional interactions were disrupted in the Ω-loop, causing a decrease in the melting temperature. Shifts originating from N179 were also transmitted toward the active site, including ~1-Å shifts of the deacylation water and interacting residue N170 (40). The structure of the D179Y KPC-2 (KPC-33) revealed more drastic changes, as this variant exhibited disorder of the Ω-loop, with other flanking regions also being disordered (40). On the contrary, the crystal structure of D179N KPC-2 in complex with vaborbactam revealed wild-type KPC-2-like vaborbactam-active site interactions,

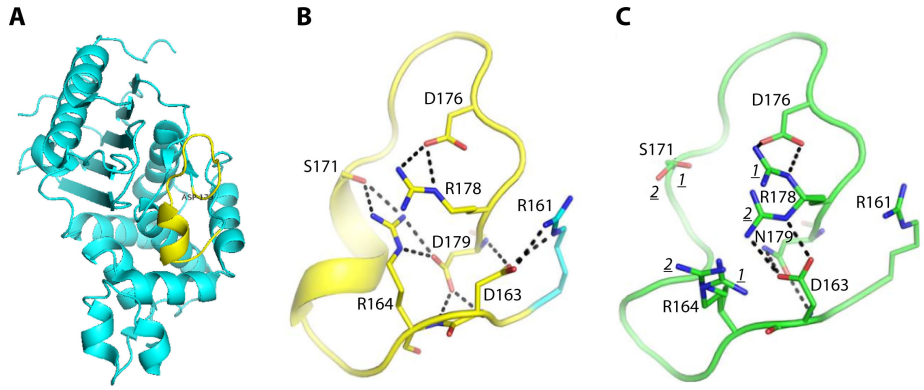

FIG 3 Ω-Loop destabilization as a mechanism of resistance to ceftazidime-avibactam. (A) Structure of KPC-2 (Protein Data Bank accession number 5UL8), Ω-Loop was shown in yellow; (B) hydrogen bonding network involving Ω-loop Arg and Asp residues in the WT KPC-2 (40); (C) Hydrogen bonding network involving Ω-loop Arg and Asp residues in D179N KPC-2 (40).

with D179N change in KPC-2 that does not perturb the binding mode of vaborbactam significantly (40).

By March 2023, 145 bla_{KPC} variants are registered in the National Center for Biotechnology Information (NCBI) database, all derived from mutations of bla_{KPC-2} or bla_{KPC-3}. Overall, bla_{KPC-4} (n = 26), bla_{KPC-33} (n = 9), bla_{KPC-12} (n = 8), bla_{KPC-6} (n = 5), bla_{KPC-71} (n = 4), bla_{KPC-10} (n = 3), bla_{KPC-76} (n = 3), bla_{KPC-44} (n = 3), bla_{KPC-25} (n = 2), bla_{KPC-36} (n = 2), bla_{KPC-5} (n = 2), and bla_{KPC-90} (n = 2) were mutants from bla_{KPC-2}. These KPC-2 variants were mainly identified from the USA (n = 35), China (n = 20), and Italy (n = 3). In contrast, bla_{KPC-31} (n = 8), bla_{KPC-66} (n = 4), bla_{KPC-67} (n = 3), bla_{KPC-18} (n = 2), bla_{KPC-29} (n = 2), bla_{KPC-40} (n = 2), bla_{KPC-49} (n = 2), bla_{KPC-61} (n = 2), and bla_{KPC-70} (n = 2) were mutants from bla_{KPC-3}. These bla_{KPC-3} variants were mainly reported from Italy (n = 15), the USA (n = 8), and France (n = 1). We aggregated the number of bla_{KPC} variants uploaded to NCBI each year by region, and the global distribution of specific variants is shown in Fig. 1. The USA and China ranked first and second with 63 and 59 detected cases, respectively. It is worth noting that China showed a sudden increase in bla_{KPC} variants in 2020 after ceftazidime-avibactam was approved for clinical use in 2019. The threat of bla_{KPC} variants in China may be more worthy of vigilance. The bla_{KPC} variants gene have been identified in various Gram-negative bacilli, including *K. pneumoniae* (73.8%), *Enterobacter hormaechei* (7.1%), *E. coli* (6%), and *Enterobacter cloacae* (5.5%), among which *K. pneumoniae* is predominant (Tables 1 and 2).

The most frequently reported bla_{KPC-2} variant, bla_{KPC-33}, was derived from bla_{KPC-2} with a single amino acid substitution. It was first reported in Italy in October 2020 and subsequently reported in China and Greece (41, 42). In these studies, bla_{KPC-33} was harbored by *K. pneumoniae*, which was predominantly isolated from rectal swabs and sputum samples. Although the ST distribution of KPC-33-producing *K. pneumoniae* varies in different regions, ST11 in China, ST1685 in Italy, and ST39 in Greece, these strains share the same feature that all bla_{KPC-33} are carried by Tn*4401*-like transposons (30, 37, 42). The most frequently reported bla_{KPC-3} variant, bla_{KPC-31}, derived from bla_{KPC-3} with a single amino acid substitution (D179Y), was first reported in Italy in August 2019 and subsequently mainly reported in Italy, France, and Spain (12, 36, 43–45). The *K. pneumoniae* strain producing bla_{KPC-31} is primarily detected in blood specimens, and the corresponding STs included ST512, ST307, ST101, and ST2502. Like other bla_{KPC} variants, all bla_{KPC-31} are located on the genetic element of Tn*4401*-like transposons (12, 36, 43–45).

THE CLINICAL CHARACTERISTICS AND RISK FACTORS OF bla_{KPC} VARIANTS

In 2017, Shields et al. (46) first reported the evolution of bla_{KPC} in three patients with infection due to KPC-producing *K. pneumoniae*, resulting in therapy failure. bla_{KPC-3} was

TABLE 1 Distribution of KPC variants based on bla_{KPC-2}

KPC-2-like enzyme	Country and year of first publication	Geographical distribution	Bacteria	Divergence from KPC-2	Plasmids	Clonal dissemination	Genetic environment	Accession number
KPC-4	USA, 2013	USA	*Enterobacter cancerogenus, Enterobacter cloacae, Serratia marcescens, K. pneumoniae*	P104R/V240G	IncL/M, IncN, IncHI2	*K. pneumoniae*: ST258, ST834, ST964, ST307; *Enterobacter cloacae*: ST171	Tn*4401* variants	JQ837276
KPC-5	USA, 2013	USA	*Pseudomonas aeruginosa, K. pneumoniae*	Pro103→Arg	IncX	*K. pneumoniae*: ST429	Tn*5563* and IS*6100A*, Tn*4401* variants	NG_049259
KPC-6	USA, 2012	USA	*K. pneumoniae*	Val240Gly			Tn*4401* variants	EU555534
KPC-10	Puerto Rico, 2010	Puerto Rico	*Acinetobacter calcoaceticus-baumannii*	H272Y				GQ140348
KPC-12	China, 2021	China	*K. pneumoniae*	L169M	IncFII, IncR		Downstream of IS*Kpn28* and upstream of IS*Kpn6*	CP036400
KPC-14	France, 2019	France, USA, Italy	*K. pneumoniae*	D242-GT-243 deletion	IncN	*K. pneumoniae*: ST16, ST1685	Tn*4401b*	CP045022
KPC-15	China, 2014	China	*K. pneumoniae*	119 Leucine and 146 lysine			IS*Kpn6*-like transposon	KC433553
KPC-16	China, 2016	China	*K. pneumoniae*	P202S and F207L		*K. pneumoniae*: ST11		NG_049249
KPC-17	China, 2016	China	*K. pneumoniae*	F207L		*K. pneumoniae*: ST11		KC465200
KPC-21	Portugal, 2018	Portugal	*E. coli*	Trp105Arg		*E. coli*: ST131	Upstream a partial IS*Kpn6* and downstream a truncated Tn*3* transposon	MH133192
KPC-24	Chile, 2016	Chile	*K. pneumoniae*	R6P		*K. pneumoniae*: ST1161	Tn*4401a*	KR052099
KPC-33	Italy, 2020	Italy, China, Greece	*K. pneumoniae*	D179Y	IncFII-R	*K. pneumoniae*: ST11, ST1685, ST39		MT550691
KPC-35	USA, 2019	USA	*K. pneumoniae*	L169P	IncF	*K. pneumoniae*: ST258		MH404098
KPC-35	China, 2019	China	*K. pneumoniae*	L169P		*K. pneumoniae*: ST11		CP050279.1
KPC-36	Italy, 2020	Italy	*K. pneumoniae*	D163E		*K. pneumoniae*: ST1519	Tn*4401a*	MK976712
KPC-44	Greece, 2021	Greece, Finland	*K. pneumoniae*	276ins YTRAPNKDDKH-SEAV	IncFIB	*K. pneumoniae*: ST39	Tn*4401a*	SAMN14734455
KPC-51	China, 2020	China	*K. pneumoniae*	D179N, Y241H, H274N				MN725731
KPC-52	China, 2020	China	*K. pneumoniae*	D179Y, valine insertion after 262 position				MN725732
KPC-55	South Korea, 2020	South Korea	*K. pneumoniae*	T794A	IncX3	*K. pneumoniae*: ST307	Tn*4401a*	MT028409
KPC-57	Greece, 2021	Greece	*K. pneumoniae*	D179V	IncFIB	*K. pneumoniae:* ST39	Tn*4401a*	MT358626
KPC-71	China, 2022	China	*K. pneumoniae*	Ser182dup	pKpQIL	*K. pneumoniae:* ST11	Tn*4401a*	OK315339
KPC-74	China, 2021	China	*K. pneumoniae*	G239_V240del	IncFII-R	*K. pneumoniae:* ST11	Tn*As1*-IS*Kpn6*-like-bla_{KPC-74}-IS*Kpn27*-Tn*iA*-IS*26*	NG_070742
KPC-82	USA, 2021	USA	*Citrobacter koseri*	Ala267-Ser275	IncN		Tn*4401* variants	MW485086
KPC-90	China, 2021	China	*Pseudomonas aeruginosa*	180ins Tyr-Thr		*Pseudomonas aeruginosa*: ST463	IS*26*-IS*Kpn6*-bla_{KPC-90}-IS*26*	MZ570431

(*Continued on next page*)

TABLE 1 Distribution of KPC variants based on bla_{KPC-2} (*Continued*)

KPC-2-like enzyme	Country and year of first publication	Geographical distribution	Bacteria	Divergence from KPC-2	Plasmids	Clonal dissemination	Genetic environment	Accession number
KPC-93	China, 2022	China	*K. pneumoniae*	267ins Pro-Asn-Asn-Arg-Ala	IncFII-R	*K. pneumoniae:* ST11	Tn*1721*-ΔIS*Kpn6*- bla_{KPC-93}-IS*Kpn8*-IS*26*	MZ569034
KPC-112	China, 2022	China	*K. pneumoniae*	del166Leu/167Asn and 242Gly/243Thr	IncFII-R	*K. pneumoniae:* ST15	Tn*1721*-ΔIS*Kpn6*- $bla_{KPC-112}$-IS*Kpn8*-IS*26*	OM177660
KPC-113	China, 2023	China	*Pseudomonas aeruginosa*	266ins Gly	Type I	*P. aeruginosa:* ST3903	IS*6100*-IS*Kpn27*-$bla_{KPC-113}$-ΔIS*Kpn6*-Tn*1403*	OM317762
KPC-123	China, 2022	China	*Citrobacter koseri*	ins179_TY and ins270_DDKHSEA			IS*Kpn27*-bla_{KPC}-IS*Kpn6*	ON012820

TABLE 2 Distribution of KPC variants based on bla_{KPC-3}

KPC-3-like enzyme	Country and year of first publication	Geographical distribution	Bacteria	Divergence from KPC-3	Plasmids	Clonal dissemination	Genetic environment	Accession number
KPC-23	Greece, 2019	Greece	*K. pneumoniae*	V240A	IncFIIk-FIB	*K. pneumoniae*: ST258	Tn*4401a*	AWU66461
KPC-28	France, 2019	France	*E. coli*	Δ242-GT-243 and H274Y				KY282958
KPC-29	Italy, 2021	Italy	*K. pneumoniae*	D272insKDD	pKpQIL	*K. pneumoniae*: ST512		WP_096807439
KPC-31	Italy, 2019	Italy	*K. pneumoniae*	D179Y	FIA(HI1)-R	*K. pneumoniae*: ST512, ST307, ST101, ST2502	Tn*4401* variants	MAPH01000113
KPC-39	Italy, 2020	Italy, France	*K. pneumoniae*	A172T	FIA(HI1)-R	*K. pneumoniae*: ST101		
KPC-40	USA, 2019	USA	*Enterobacter hormaechei*	L167_E168dup	IncN	*Enterobacter hormaechei:* ST407		WP_115470049
KPC-41	Switzerland, 2019	Switzerland,	*K. pneumoniae*	269-Pro-Asn-Lys-270	IncFII	*K. pneumoniae*: ST395		MK497255
KPC-48	Spain, 2020	Spain	*K. pneumoniae*	L169P-A172T				MN422013
KPC-49	Spain, 2021	Spain, Italy	*E. coli, K. pneumoniae*	Arg-163-Ser	IncF	*E. coli:* ST131, *K. pneumoniae:* ST512	Tn*4401a*	MN619655
KPC-50	Switzerland, 2020	Switzerland	*K. pneumoniae*	276-Glu-Ala-Val-277	IncFIB	*K. pneumoniae:* ST258		MN654342
KPC-94	Spain, 2022	Spain	*K. pneumoniae*	LN169-170H		*K. pneumoniae:* ST512		
KPC-95	Spain, 2022	Spain	*K. pneumoniae*	D179Y and A172T		*K. pneumoniae:* ST512		
KPC-115	Argentina, 2022	Argentina	*K. pneumoniae*	del168Leu/169Asn and Ser170Pro		*K. pneumoniae:* ST11	Tn*4401a*	OM714909
KPC-121	Italy, 2022	Italy	*K. pneumoniae*	179ins Ser		*K. pneumoniae:* ST512		SAMN27596901
KPC-125	Italy, 2022	Italy	*K. pneumoniae*	Asp179Ala		*K. pneumoniae:* ST512		ON012820

TABLE 3 Summary of KPC variant cases

No.	Sex/age, years	Infection	Organism	Wild-type	KPC variant	ST	Therapy before the KPC variant was detected	Therapy	Outcome	Pathogen clearance	Reference
1	Male/59	Pneumonia	*K. pneumoniae*	KPC-3	KPC-31	ST2502	CZA, 10 d	Meropenem 2 g tid 3 h	Cure	No	(45)
2	Male/68	Bloodstream infection	*K. pneumoniae*	KPC-3	KPC-31	ST512	CZA,14 d	Meropenem-vaborbactam 1/1 g q8	Cure	Yes	(44)
3	Male/40	Bloodstream infection, pneumonia	*K. pneumoniae*	KPC-3	KPC-31	ST307	Meropenem, tigecycline, polymyxin B, CZA	Meropenem + polymyxin B	Failure	No	(51)
4	Female/50+	Subphrenic abscess	*K. pneumoniae*	KPC-3	KPC-8, KPC-31	ST258	CZA, 19 d	Piperacillin/tazobactam, gentamicin	NA	No	(46)
5	Female/70+	Pneumonia	*K. pneumoniae*	KPC-3	KPC-31	ST258	CZA, 15 d	Meropenem + polymyxin B	Cure	Yes	(46)
6	Female/NA	Bloodstream infection, pneumonia	*K. pneumoniae*	KPC-3	KPC-31	ST1519	CZA, 14 d	Meropenem (increased dose, extended infusion time) + gentamicin	Failure	No	(50)
7	Female/60	Pneumonia, wound infection	*K. pneumoniae*	KPC-3	KPC-31	ST101	CZA, 14 d	CZA + tigecycline	Failure (death)	No	(48)
8	Male/47	Intra-abdominal infection	*K.pneumoniae*	KPC-3	KPC-31/KPC-39/ KPC-47/KPC-48	NA	CZA, 12 d	Imipenem, tigecycline, gentamicin	Cure	No	(53)
9	Male/67	Bloodstream infection, pneumonia	*K. pneumoniae*	KPC-3	KPC-3 (A177E, D179Y)	ST258	CZA, 15 d	Meropenem 1 g q12, 12 d	Cure	Yes	(52)
10	Female/40+	Pneumonia, urinary tract infection	*K. pneumoniae*	KPC-3	KPC-32	ST258	CZA, 14 d	Meropenem	Failure	No	(46)
11	Male/24	Bloodstream infection, pneumonia	*K. pneumoniae*	KPC-2	KPC-33	NA	CZA, 33 d	Polymyxin B + gentamicin + tigecycline, meropenem- vaborbactam 25 d	Cure	Yes	(49)
12	Male/42	Pneumonia	*K. pneumoniae*	KPC-2	KPC-33	ST11	CZA, 16 d	Imipenem + amoxicillin/clavulanate + polymyxin B	Failure	No	(11)
13	NA	Bloodstream infection	*K. pneumoniae*	KPC-2	KPC-44	ST39	CZA + tigecycline, 14 d	Colistin + trimethoprim/sulfamethoxazole	Cure	Yes	(47)
14	Female/40+	Bloodstream infection	*E. coli*	KPC-3	KPC-49	ST131	CZA, 10 d	Amikacin 500 mg/24 h i.v.	Cure	Yes	(54)
15	NA	Bloodstream infection	*Citrobacter koseri*	NA	KPC-82	NA	CZA, 28 d	Meropenem-vaborbactam	Cure	Yes	(56)
16	Male/22	Pneumonia	*K. pneumoniae*	KPC-2	KPC-33	ST11	CZA, 41 d	Imipenem + CZA	Cure	Yes	(57)
17	Female/52	Pneumonia	*K. pneumoniae*	KPC-2	KPC-79, KPC-76	ST11	CZA, 9 d	CZA + amikacin, meropenem + tigecycline + fosfomycin	Failure	No	(55)
18	Male/50	Pneumonia	*K. pneumoniae*	KPC-2	KPC-35, KPC-78, KPC-33	ST859	CZA, 9 d	CZA + tigecycline + fosfomycin + meropenem	Failure	No	(55)

mutated to bla_{KPC-31} and bla_{KPC-32} during CZA treatment for 10 to 19 days. By searching the PubMed database, up to August 2022, 18 cases of infections caused by KPC variant-producing *Enterobacterales* have been reported internationally. These infections were reported in 11 males, 5 females, and 2 patients of unknown sex. *K. pneumoniae* was the pathogen in 16 cases, *E. coli* and *C. koseri* in one case each (Table 3) (11, 44–55). Limited data showed that bla_{KPC-31} (44.4%, 8/18) and ST 258 (22.2%, 4/18) were the dominant genotypes and ST type of bla_{KPC} variants. All patients were treated with CZA (for 9–41 days) before KPC variant-producing *Enterobacterales* strains were identified, suggesting that CZA treatment may be an independent risk factor for inducing mutations of bla_{KPC}. In the 18 patients infected with a KPC variant-producing CRE strain, the treatment failure rate was up to 38.9% (7/18), and 50% (8/16) of the patients were older than 50. Four patients continued to receive CZA in combination with other antimicrobial agents (including meropenem, tigecycline, amikacin, or imipenem) for anti-infective treatment after CZA-resistant bla_{KPC} variant strains were identified. Among them, three patients were not successfully cured after receiving the above anti-infection regimens, while one was cured after receiving CZA in combination with imipenem. Eleven patients discontinued CZA therapy and were treated with other antimicrobial agents, to which the bacterial isolates were susceptible, including tigecycline, polymyxin B, colistin, meropenem, and amikacin. Among them, seven patients' infection symptoms were effectively controlled. Three patients were successfully treated with meropenem-vaborbactam, based on the bacteria being *in vitro* susceptible to meropenem-vaborbactam. However, more real-world research data are still needed to confirm the effectiveness of meropenem-vaborbactam in treating infection caused by the bla_{KPC} variant.

A retrospective, observational research was conducted with all patients admitted to the intensive care unit (ICU) dedicated to coronavirus disease patients at the City of Health & Sciences in Turin, between May 2021 and January 2022, with the primary endpoint to study strains with resistance to CZA. This study enrolled 17 patients with colonization or invasive infection caused by CZA-resistant *K. pneumoniae* which were susceptible to meropenem. It is worth noting that 76.5% of patients did not receive therapy with CZA. Cluster analysis showed that 16 KPC-33-producing *K. pneumoniae* isolates belonged to a single clone, indicating that there was a risk of clonal transmission of this novel bla_{KPC} variant (58).

KPC-2 or KPC-3 carbapenemase-producing *K. pneumoniae* are susceptible to mutations during treatment leading to treatment failure, and the occurrence of such mutations is not easily recognized clinically and may be missed, putting infected patients at high risk. Available studies suggest that the use of ceftazidime-avibactam is an important factor in the development of mutations in KPCase-producing *K. pneumoniae*. As there are no current guidelines for the treatment of infections caused by KPCase-producing mutants of *K. pneumoniae*, it is recommended that clinical monitoring for changes in antimicrobial susceptibility to antimicrobial agents, particularly carbapenems and ceftazidime-avibactam, be performed at regular intervals (e.g., 3–5 days) to monitor for the occurrence of mutations.

GENETIC SEQUENCES SURROUNDING bla_{KPC} CARBAPENEMASE GENES

Transposable elements play an essential role in bacteria's genetic variation and evolution. In most countries and regions, such as Europe (33), the USA (59), and Brazil (31, 60), the bla_{KPC} is mainly located on transposable elements like Tn*4401*, Tn*3*-Tn*4401* chimera CTB, and IS*26*. Tn*4401* and Tn*3*-Tn*4401* chimera CTB, belonging to the Tn*3* family, can mobilize bla_{KPC-2} at a high transposition frequency. Tn*4401*, a 10 kb transposon, has been reported as the genetic structure mediating original bla_{KPC} acquisition, with the gene order of Tn*4401-tnpR*, Tn*4401-tnpA*, IS*Kpn7*, bla_{KPC}, and IS*Kpn6* (61). Owing to the diversity in the intervening sequence between the *ISKpn7* and bla_{KPC} genes, a total of eight unique Tn*4401* isoforms (a to h) have been characterized, with Tn*4401a* and Tn*4401b* being the most widespread (62). In Asia, bla_{KPC-2} is predominantly located on different variants of Tn*1721* and IS*26 (63–65)*. However, the structural sequences

surrounding the *bla*$_{KPC}$ variants genes are infrequently described. Scattered studies have shown that some KPC variants, similar to *bla*$_{KPC-2}$ and *bla*$_{KPC-3}$, are located within the Tn*4401* transposon (56). The core structure of IS*Kpn6*-*bla*$_{KPC}$- IS*Kpn28* was identified in *bla*$_{KPC-12}$ and *bla*$_{KPC-74}$ (Tables 1 and 2). Therefore, we collected 52 *bla*$_{KPC}$ variant gene sequences from NCBI databases to analyze the genetic environment surrounding the *bla*$_{KPC}$ gene. The result showed the core structure of the *tnpR-tnpA*-IS*Kpn7*-*bla*$_{KPC}$-IS*Kpn6* was identified in the strains carrying *bla*$_{KPC-31}$, *bla*$_{KPC-32}$, *bla*$_{KPC-33}$, *bla*$_{KPC-14}$, *bla*$_{KPC-56}$, *bla*$_{KPC-29}$, *bla*$_{KPC-68}$, *bla*$_{KPC-27}$, *bla*$_{KPC-49}$, or *bla*$_{KPC-115}$. The core structure of the IS*Kpn27*-*bla*$_{KPC}$-IS*Kpn6* was identified in the strains producing *bla*$_{KPC-71}$, *bla*$_{KPC-79}$, *bla*$_{KPC-93}$, *bla*$_{KPC-123}$, *bla*$_{KPC-51}$, or *bla*$_{KPC-52}$ (Fig. 4).

SUSCEPTIBILITY PROFILE OF THE STRAINS PRODUCING KPC VARIANTS

The *bla*$_{KPC}$ variants can cause a seesaw effect. On the one hand, under the pressure of CZA, the *bla*$_{KPC}$ sequence mutates, including *bla*$_{KPC-8}$ (66), *bla*$_{KPC-30}$, *bla*$_{KPC-31}$ (36), *bla*$_{KPC-32}$, *bla*$_{KPC-40}$ (67), *bla*$_{KPC-41}$ (68), *bla*$_{KPC-50}$ (69), and *bla*$_{KPC-57}$ (42), resulting in changes in the protein structure and weakening its ability to bind to avibactam, thus making the bacteria resistant to ceftazidime-avibactam [minimum inhibitory concentration (MIC) range 16–>128 mg/L]. On the other hand, the bacteria regained their susceptibility to carbapenems (especially imipenem, Table 4). In recent years, with the increasing public attention to *bla*$_{KPC}$ variants, studies have found that some *bla*$_{KPC}$ variants play avibactam-resistant ESBL profile, including *bla*$_{KPC-14}$ (70), *bla*$_{KPC-28}$ (71), *bla*$_{KPC-33}$ (36), *bla*$_{KPC-46}$, *bla*$_{KPC-51}$ (72), *bla*$_{KPC-52}$ (72), *bla*$_{KPC-53}$ (73), and *bla*$_{KPC-66}$ (36). The ESBL phenotype-positive KPCs can be inhibited by the classic enzyme inhibitor clavulanic acid; ESBL phenotype confirmatory tests that routinely use clavulanic acid as an inhibitor often report positive results, misleading the clinicians to consider the strains as ESBL producers rather than carbapenemase producers. KPC variants producing strains tended to be inhibited by meropenem-vaborbactam. Therefore, meropenem-vaborbactam is considered a salvage therapy after failure of CZA treatment (49). It is important to note that CRE strains carrying *bla*$_{KPC}$ variants with decreased susceptibility to CZA can also show cross-resistance to the siderophore cephalosporin cefiderocol (74). This may be related to the fact that cefiderocol and ceftazidime are very similar in structure.

KPC VARIANTS POSE NEW CHALLENGES FOR LABORATORY TESTING

Rapid detection of *bla*$_{KPC}$ is essential in treating CRE infections. Standard methods for detecting carbapenemases include both phenotypic and genotypic assays. Phenotypic assays include the Carba NP assay, modified carbapenem inactivation method (mCIM), EDTA-modified carbapenem inactivation method (eCIM), 3-aminophenylboronic acid (APB)/EDTA method, and time-of-flight mass spectrometry (86–88). Genotypic detection methods based on nucleic acid detection techniques, include GeneXpert Carba R assay (Cepheid, Sunnyvale, CA, USA), Verigene Gram-negative blood culture test (Nanosphere, Northbrook, IL, USA), and FilmArray system (bioMérieux, Marcy l'Étoile, France) (89–92). Phenotypic assays are mainly based on the ability of carbapenemases to hydrolyze carbapenems. The sensitivity and specificity of Carba NP assay, mCIM, eCIM, and APB/EDTA method are higher than 90% in detecting carbapenemases [including KPC, NDM, Verona metallo-β-lactamase (VIM), São Paulo metallo-β-lactamase (SPM)] (93–97). Moreover, phenotypic assays have been highly favored due to their low cost. However, the emergence of bla$_{KPC}$ variant genes poses significant challenges to such phenotypic assays in detecting *bla*$_{KPC}$ variant genes among CZA-resistant CRE (14). Since KPC-variant producing strains are often susceptible or intermediate to imipenem or meropenem, carbapenemase phenotypic assays are prone to reporting false-negative results (81). NG-Test Carba 5 and RESIST-5 O.O.K.N.V are two of the most commonly used enzyme-linked immunochromatography-based test strips. Ding et al. (14) showed that NG-Test Carba 5 effectively detected *bla*$_{KPC-35}$ (n = 3), *bla*$_{KPC-78}$ (n = 1), and *bla*$_{KPC-79}$ (n = 1), but false-negative results were observed for *bla*$_{KPC-33}$ (n = 5), *bla*$_{KPC-71}$ (n = 1), and *bla*$_{KPC-76}$ (n = 8). A similar result was obtained by Bianco et al. in which NG-Test Carba 5 and RESIST-5

TABLE 4 Antimicrobial susceptibility profiles and carbapenemase assay results of KPC variant-producing strains[a]

Organism	Variants	MIC (mg/L)								Carbapenemase assay						Reference
		ETP	IPM	MEM	CZA	MEV	IMR	COL	TGC	mCIM	Rapid Carba NP	NG-Test Carba 5	RESIST-5 O.O.K.N.V	GeneXpert	RAPIDEC Carba NP	
K. pneumoniae	KPC-12	256(R)	16(R)	64(R)	4(S)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	(75)
NA	KPC-14	ND	ND	ND	ND	ND	ND	ND	ND	NEG	NEG	POS	ND	POS	NEG	(71)
K. pneumoniae	KPC-14	ND	≤1(S)	≤0.125(S)	64(R)	ND	ND	ND	ND	NEG	NEG	POS	POS	ND	ND	(12)
K. pneumoniae	KPC-23	ND	512(R)	512(R)	16(R)	4(S)	ND	1(S)	4(I)	ND	ND	ND	ND	ND	ND	(76)
E. coli	KPC-28	ND	ND	ND	ND	ND	ND	ND	ND	NEG	NEG	POS	ND	POS	NEG	(71)
K. pneumoniae	KPC-31	>1(R)	<0.25(S)	2(I)	>8(R)	0.125(S)	ND	0.5(S)	0.5(S)	ND	ND	ND	ND	ND	ND	(44)
K. pneumoniae	KPC-31	ND	≤1(S)	4(R)	>256(R)	ND	ND	ND	ND	NEG	NEG	NEG	NEG	ND	ND	(12)
K. pneumoniae	KPC-31	ND	0.25(S)	4(R)	>64(R)	1(S)	ND	0.5(S,PB)	2(S)	ND	ND	ND	ND	ND	ND	(41)
K. pneumoniae	KPC-33	32(R)	0.5(S)	4(R)	>64(R)	2(S)	ND	ND	ND	NEG	ND	ND	ND	POS	ND	(14)
K. pneumoniae	KPC-35	16(R)	1(S)	4(R)	64(R)	2(S)	ND	ND	ND	NEG	ND	POS	ND	POS	ND	(14)
K. pneumoniae	KPC-36	>32(R)	>256(R)	>256(R)	16(R)	2(S)	ND	ND	2(S)	ND	ND	POS	ND	POS	ND	(77)
K. pneumoniae	KPC-39	ND	>16(R)	>16(R)	>16(R)	16(R)	8(R)	0.5(S)	1(S)	ND	ND	ND	ND	ND	ND	(53)
K. pneumoniae	KPC-41	4(R)	4(R)	1(S)	>128(R)	ND	ND	ND	ND	ND	POS	ND	ND	ND	ND	(68)
K. pneumoniae	KPC-47	ND	>16(R)	>16(R)	>16(R)	16(R)	16(R)	1(S)	1(S)	ND	ND	ND	ND	ND	ND	(53)
K. pneumoniae	KPC-48	ND	1(S)	1(S)	>16(R)	1(S)	1(S)	1(S)	1(S)	ND	ND	ND	ND	ND	ND	(53)
E. coli	KPC-49	ND	2(I)	0.5(S)	16(R)	ND	ND	≤2(S)	≤0.5(S)	ND	ND	ND	ND	ND	ND	(54)
K. pneumoniae	KPC-50	1(I)	16(R)	2(I)	>256(R)	<0.125(S)	2(S)	ND	ND	ND	POS	ND	ND	ND	ND	(69)
K. pneumoniae	KPC-51	ND	1(S)	4(R)	2048(R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	(72)
K. pneumoniae	KPC-52	ND	2(I)	2(I)	256(R)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	(72)
K. pneumoniae	KPC-53	ND	2(I)	4(R)	64(R)	2(S)	ND	>8(R)	1(S)	ND	ND	ND	POS	ND	ND	(73)
K. pneumoniae	KPC-55	ND	1(S)	2(I)	0.125(S)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	(78)
K. pneumoniae	KPC-70	18 mm (R)	28 mm (S)	21 mm (I)	>256(R)	ND	ND	ND	22 mm (S)	ND	ND	ND	ND	ND	ND	(79)
K. pneumoniae	KPC-71	16(R)	0.5(S)	2(I)	>64(R)	0.5(S)	ND	ND	ND	NEG	ND	NEG	ND	POS	ND	(14)
K. pneumoniae	KPC-74	16(R)	0.5(S)	1(S)	128(R)	ND	ND	ND	8(R)	ND	ND	NEG	ND	ND	ND	(80)
K. pneumoniae	KPC-76	32(R)	2(I)	4(R)	>64(R)	2(S)	ND	ND	ND	NEG	ND	NEG	ND	POS	ND	(14)
K. pneumoniae	KPC-78	32(R)	0.5(S)	2(I)	>64(R)	1(S)	ND	ND	ND	NEG	ND	POS	ND	POS	ND	(14)
K. pneumoniae	KPC-79	32(R)	4(R)	8(R)	64(R)	4(S)	ND	ND	ND	NEG	ND	POS	ND	POS	ND	(14)
Citrobacter koseri	KPC-82	ND	4(R)	2(I)	128(R)	0.125(S)	ND	ND	ND	ND	ND	ND	ND	POS	ND	(56)
K. pneumoniae	KPC-94	>2(R)	≤0.5(S)	2(I)	>16(R)	ND	ND	0.5(S)	1(S)	ND	ND	ND	ND	POS	ND	(81)
K. pneumoniae	KPC-95	>2(R)	1(S)	1(S)	>16(R)	ND	ND	0.5(S)	0.5(S)	ND	ND	ND	ND	POS	ND	(81)
K. pneumoniae	KPC-112	ND	0.5(S)	1(S)	>128(R)	ND	ND	ND	2(I)	ND	ND	ND	ND	ND	ND	(82)
K. pneumoniae	KPC-115	0.5(S)	≤0.25(S)	≤0.5(R)	24(R)	ND	ND	ND	ND	ND	ND	POS	ND	ND	ND	(83)
K. pneumoniae	KPC-121	≥256(R)	8(R)	16(R)	≥256(R)	32(R)	8(R)	0.125(S)	ND	ND	ND	ND	ND	ND	ND	(84)

(*Continued on next page*)

TABLE 4 Antimicrobial susceptibility profiles and carbapenemase assay results of KPC variant-producing strains[a] (*Continued*)

Organism	Variants	MIC (mg/L)								Carbapenemase assay						Reference
		ETP	IPM	MEM	CZA	MEV	IMR	COL	TGC	mCIM	Rapid Carba NP	NG-Test Carba 5	RESIST-5 O.O.K.N.V	GeneXpert	RAPIDEC Carba NP	
Citrobacter koseri	KPC-123	≤0.5(S)	≤0.5(S)	≤0.5(S)	64(R)	ND	ND	1(S)	≤0.25(S)	ND	ND	ND	ND	ND	ND	(85)

[a]MIC, minimum inhibitory concentration; KPN, *K. pneumoniae*; ECO, *E. coli*; CKO, *Citrobacter koseri*; ETP, ertapenem; IPM, imipenem; MEM, meropenem; CZA, ceftazidime-avibactam; MEV, meropenem-vaborbactam; IMR, imipenem-relebactam; COL, colistin; PB, polymyxin B; TGC, tigecycline; mCIM, modified carbapenem inactivation method; POS, positive; NEG, negative; ND, not detected.

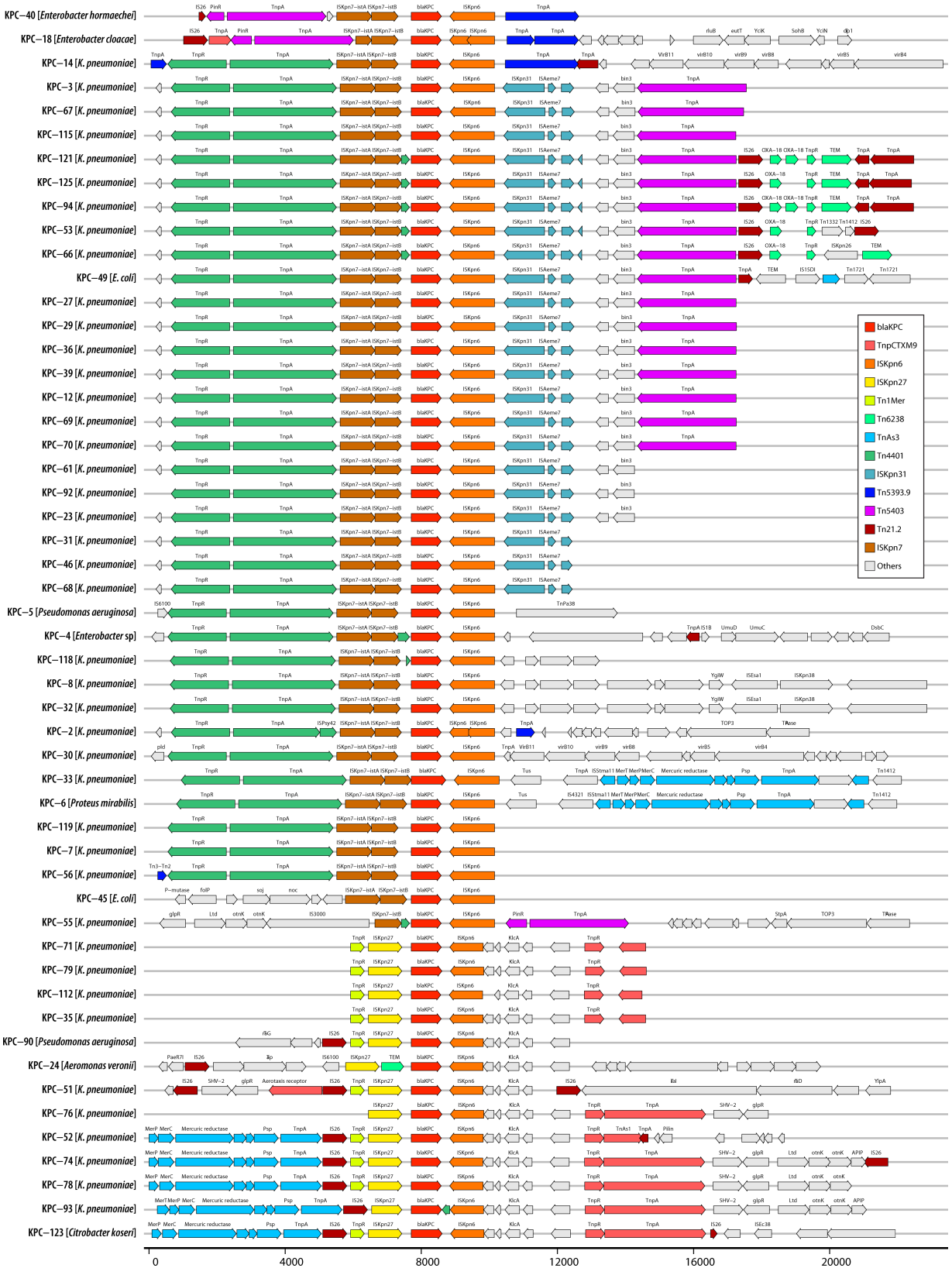

FIG 4 Genetic sequences surrounding *bla*$_{KPC}$ carbapenemase genes.

O.O.K.N.V could detect *bla*$_{KPC-14}$ (n = 2), but could not detect *bla*$_{KPC-31}$ (n = 4) and *bla*$_{KPC-33}$ (n = 2) (12). The false-negative results of enzyme immunochromatography in detecting *bla*$_{KPC}$ variants may be related to the mutation site, which changes the target site of carbapenemase binding to the antibody and thus fails to bind to the antibody, resulting in false-negative results.

KPC variants may challenge the performance of some classical carbapenemase detection methods used by clinical laboratories. PCR assay may be suitable to detect *bla*$_{KPC}$ variant. The GeneXpert Carba R assay is a qualitative, *in vitro* real-time PCR assay designed to detect five arbapenemases gene families, including *bla*$_{IMP}$, *bla*$_{KPC-2\ or\ 3}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, and *bla*$_{VIM}$, with more than 96% sensitivity and specificity (90, 92, 98). Ding et al. (14) showed that GeneXpert Carba R has potential advantage in detecting *bla*$_{KPC}$ variant due to its detection principle, which is not affected by gene locus variation and can effectively detect *bla*$_{KPC-33}$ (n = 5), *bla*$_{KPC-35}$ (n = 3), *bla*$_{KPC-71}$ (n = 1), *bla*$_{KPC-76}$ (n = 8), *bla*$_{KPC-78}$ (n = 1), and *bla*$_{KPC-79}$ (n = 1). Several studies showed that the presence of the novel *bla*$_{KPC-46}$, *bla*$_{KPC-66}$, *bla*$_{KPC-92}$, *bla*$_{KPC-94}$, and *bla*$_{KPC-95}$ were confirmed by the X-pert Carba R assay (81, 99). A similar study compared three carbapenemase detection methods, including GeneXpert Carba R, NG-Test Carba 5, and colloidal gold immunoassay test, to evaluate the performance of these methods in detecting KPC variants protein. The result showed that GeneXpert Carba R can confirm the presence of all 13 types of KPC variant protein, including KPC-2, KPC-3, KPC-25, KPC-33, KPC-35, KPC-51, KPC-52, KPC-71, KPC-76, KPC-77, KPC-78, KPC-93, and KPC-123 (100). There are other methods for detecting carbapenemases based on PCR amplification, such as Verigene Gram-negative blood culture test (Nanosphere, Northbrook, IL, USA), FilmArray system (bioMérieux, Marcy l'Étoile, France), etc., but there are no published data on the performance of these methods in detecting KPC variants (91). Different methods for detecting KPC variants may get different results. The laboratories need to pay attention to this special phenomenon. When the conventional carbapenemase detection method shows negative results, but the bacteria show resistance to CZA, further molecular testing is required to determine whether bacteria produce carbapenemase.

The emergence of KPC variants disrupts conventional laboratory thinking about carbapenemase detection and changes the practice of inferring bacterial susceptibility to CZA from carbapenemase detection results (most automated susceptibility testing systems do not yet include CZA). The reporting of false-negative carbapenemase results is likely to mislead clinicians in their anti-infective treatment. Based on the above information, we believe that CZA susceptibility testing should be performed concurrently with carbapenemase testing, and KPC variants can be identified early by combining the antimicrobial susceptibility phenotype and carbapenemase results to provide more information early to start precision treatment of infections caused by KPC variants.

CURRENTLY AVAILABLE TREATMENT OPTIONS

Carbapenems

Since several KPC-variant producing strains regained susceptibility to imipenem or meropenem while remaining resistant to CZA, this suggests the potential value of carbapenems in the treatment of infections caused by KPC-variant strains. Sporadic cases of treatment success have been reported for meropenem alone (increased dose, extended infusion time) or in combination with other antimicrobial agents in managing infections caused by KPC variant-producing *K. pneumoniae* (45, 52). However, meropenem or other antimicrobial combinations have also shown a high failure rate in treating such resistant strains in some cases when the *bla*$_{KPC}$ variant reverts to the original *bla*$_{KPC-2}$ during treatment, allowing the bacteria to regain carbapenem resistance (11, 51, 57). Carbapenem therapy was associated with a 50% all-cause mortality rate in patients infected with KPC variant producer (usually related to clinical failure), which is much higher than that observed in patients with ESBL-producing *K. pneumoniae* infections and similar to that observed in KPC-2/3-producing *K. pneumoniae* infections not treated

with CZA (53). The limited data currently available suggest that most bla_{KPC} variants occur during CZA treatment. As previously described, CZA inhibits KPC-2 or KPC-3, while meropenem or imipenem provides potent activity against most KPC variants. Since the bacteria may revert to classical bla_{KPC} phenotypes such as bla_{KPC-2} or bla_{KPC-3} producer in response to environmental changes when treated with carbapenems alone to which the bacterial isolates were susceptible, is it possible that an antimicrobial regimen of carbapenems combined with CZA could effectively cover both the KPC-2/3 and KPC variants producing strains, thus preventing the chance of clonal transformation of bacteria? Should we try this approach to meet the clinical needs? However, such combinations in treating KPC variant-producing bacterial infections are rarely reported (57). The efficacy and safety must also be validated by further data from *in vitro* or *in vivo* studies.

New β-lactam-β-lactamase inhibitor combinations

Meropenem-vaborbactam

Vaborbactam is a non-β-lactam serine β-lactamase inhibitor based on a cyclic boronic acid pharmacophore (101). Meropenem-vaborbactam inhibits the activity of class A serine enzymes (including ESBLs and KPC) and AmpCs, but not OXA-48 carbapenemases and metallo-β-lactamases (102). Meropenem-vaborbactam was approved by the U.S. Food and Drug Administration in 2017 for treating complicated urinary tract infections (cUTIs) in adults and by the European Medicines Agency in 2018 for treating cUTIs, complicated intra-abdominal infections, or hospital-acquired pneumonia and bacteremia occurring in association (or suspected association) with any of these infections (103). In the European Union, meropenem-vaborbactam is also indicated for treating infections caused by aerobic Gram-negative bacteria in adults with limited options. In a study by Lapuebla et al. evaluating the activity of meropenem-vaborbactam against KPC-producing *Enterobacterales*, the combination inhibited 98.5% (131/133) of isolates at 1/8 mg/L (104). A global multicenter epidemiological study in 2014 included 10,426 *Enterobacterales* strains, ≤2/8 mg/L of meropenem-vaborbactam inhibited 99.3% of the strains tested (105). Subsequent studies on 11,559 *Enterobacterales* strains collected in 2015 found that meropenem-vaborbactam inhibited 99.5% of KPC-producing *Enterobacterales* at a concentration of 4/8 mg/L (106). Studies have also shown potent *in vitro* activity of meropenem-vaborbactam against the *K. pneumoniae* strains producing KPC variants, including KPC-14, KPC-28, KPC-31, KPC-33, KPC-35, KPC-39, KPC-50, KPC-71, KPC-76, KPC-78, or KPC-79 (14, 107, 108). A retrospective study collected 12 KPC variant-producing *K. pneumoniae* isolates from ICU patients between November 2020 to January 2021, including KPC-62 (n = 11) and KPC-31 (n = 1) (109). The result showed that all strains that carried KPC variant were resistant to CZA (MIC ≥64 mg/L), but susceptible to meropenem-vaborbactam (MIC range, 0.25/8 mg/L ~ 2/8 mg/L) (109). Case reports have shown that meropenem-vaborbactam can successfully cure bacteremia caused by KPC-31 producers, suggesting that meropenem-vaborbactam may be a new option for treating infections caused by KPC variant-producing *Enterobacterales* strains (49). Similarly, meropenem-vaborbactam has successfully treated infections caused by KPC-82-producing *Citrobacter koseri* (56).

Imipenem-relebactam

Relebactam is a new β-lactamase inhibitor with a diazabicyclooctane core, similar to avibactam (110). Imipenem-relebactam inhibits class A serine enzymes (including ESBLs and KPC) and AmpCs, but not OXA-48 carbapenemase, metallo-β-lactamase, or class A carbapenemase GES-20 (111). The Study for Monitoring Antimicrobial Resistance Trends reported that relebactam restored susceptibility to imipenem in 80.5%, 100%, and 74.1% of imipenem-nonsusceptible *Pseudomonas aeruginosa*, *Enterobacteriaceae*, and *K. pneumoniae*, in 2015 (112). A study by Papp-Wallace et al. showed that all *Enterobacterales* strains were highly susceptible to imipenem-relebactam (MIC ≤2 mg/L) (113). A

study by Carpenter et al. also showed similar results, with imipenem-relebactam being the most active combination against CRE ($MIC_{50/90}$ ≤0.25/0.5 mg/L) (114). *In vitro* studies have also shown excellent activity of imipenem-relebactam against the *K. pneumoniae* strains producing KPC variants, including KPC-31, KPC-33, KPC-35, and KPC-62 (109, 115). Therefore, it may be speculated that imipenem-relebactam has good *in vitro* antimicrobial activity against KPC variants. At the same time, imipenem-relebactam has good antimicrobial activity against KPC-2- or KPC-3-producing *Enterobacterales*, so in theory, using imipenem-relebactam in treating KPC variant infection can effectively prevent the emergence of KPC-2/3-producing strains.

Aztreonam-avibactam

The infections caused by metallo-β-lactamase-producing strains remain a major challenge in clinical treatment because avibactam, vaborbactam, and relebactam can not inhibit the activity of metallo-β-lactamases. However, the aztreonam-avibactam combination is highly anticipated because metallo-β-lactamases do not hydrolytically destroy the antimicrobial activity of aztreonam, and so aztreonam-avibactam can inhibit the *Enterobacterales* strains producing either class A serinease, metallo-β-lactamase, or class D OXA-48 carbapenemase (116). Interestingly, some KPC variant-producing strains regained susceptibility to carbapenems while simultaneously regaining susceptibility to aztreonam. KPC-31, KPC-33, KPC-49, and KPC-94 producers showed high susceptibility to aztreonam (MIC range: ≤0.5–4 mg/L), but KPC-95-, KPC-82-, KPC-55-, and KPC-14-producing strains remained resistant to aztreonam (MIC >16 mg/L) (54, 70, 81). Our results showed (Li Ding, et al. data unpublished) that the *K. pneumoniae* strains producing KPC variant (including KPC-33, KPC-35, KPC-71, KPC-76, KPC-78, KPC-79, and KPC-112) were highly susceptible to aztreonam-avibactam (MIC ranges, 2/4–4/4 mg/L).

Other antimicrobial agents

Tigecycline

Tigecycline belongs to a new class of glycylcycline antibiotics. It has been touted as one of the last lines of defense in treating complex infections caused by multi-drug resistant Gram-negative and Gram-positive bacteria. Binding to bacterial 30S ribosomes prevents the entry of transfer RNA. It prevents amino acids from integration into peptide chains, ultimately blocking bacterial protein synthesis and limiting bacterial growth. It has great *in vitro* antimicrobial activity against class A, B, C, and D β-lactamase-producing *Enterobacterales (117)*. Scattered studies have shown that tigecycline has high *in vitro* antimicrobial activity against KPC variant-producing *Enterobacterales* (55, 82). But an excess mortality risk was demonstrated in comparative clinical trials (118). While it is generally not recommended for treating bacteremia because of its low steady-state concentrations in serum following the current dosing recommendation, tigecycline is primarily used in combination regimens when treating carbapenem-resistant Gram-negative infections (119). However, the emergence of tigecycline-resistant strains has recently been reported (120). Overexpression of resistance-nodulation-division efflux pumps such as AcrAB is an essential molecular mechanism underlying tigecycline resistance (121). Additionally, *tet*(X) gene variants are newly emerging mechanisms of tigecycline resistance (122, 123).

Polymyxin

Polymyxins are an "old" class of lipopeptide antibiotics approved in the late 1950s (124). Polymyxins have regained public attention due to their excellent activity against CRE, CRPA, and CRAB. The two clinically available polymyxins, colistin and polymyxin B, demonstrate comparable spectra of antibacterial activity, mechanism of action, and resistance profile because of their similar structures (125). CHINET report in 2018 showed that polymyxin B had excellent *in vitro* activity against 272 clinical isolates of CRKP

(93.8% susceptible) (126). However, nephrotoxicity and heteroresistance are two major limitations of polymyxins (125, 127).

Modification of lipid A portion of lipopolysaccharide (LPS) is the main mechanism of *Enterobacterales* resistant to polymyxin, which can be caused by chromosomal mutations in genes of the two-component system involved in LPS modification, namely *PhoPQ*, *PmrAB*, and *CrrAB*, and *MgrB (128*). Additionally, plasmid-mediated polymyxin resistance gene *mcr-1* can also medicate strains resistant to polymyxin (129). Several studies showed that KPC variants did not affect the susceptibility of polymyxin, suggesting that polymyxin still has potential advantages in treating KPC variant infections (41, 55).

FUTURE OUTLOOK

To address the challenges posed by the global spread of bla_{KPC} variants, multiple studies need to be conducted to curb the infections caused by these bacteria. Firstly, countries need to establish a collaborative surveillance network for the bla_{KPC} to survey the spread of KPC variant producers in real-time and carry out proactive hospital infection control measures to curb the spread. Secondly, more effective antimicrobial agents need to be developed continuously to deal with infections and be used rationally to avoid the emergence of the resistant strain. Countries should open fast-track approval channels for new drugs to be used to save patients as soon as possible. Thirdly, clinical microbiology laboratories should strengthen the routine detection and inform bla_{KPC} variant-positive isolates. *In vitro* diagnostic companies need to develop methods that can accurately and timely detect new bla_{KPC} variants. The manufacturers of automated antimicrobial susceptibility testing systems should add carbapenemase testing and report the result of KPC carbapenemases in advance based on clinical need. Rapid whole-genome sequencing should be applied to predict the resistance profile mediated by KPC variants. In the context of limited treatment options currently available, there is an urgent need to develop treatment guidelines for infections caused by KPC variant producers. Finally, ARC (avibactam-resistant carbapenemase) is classified as a class A enzyme. The designation of KPC variants is currently confusing. Some KPC variants are designated as ESBLs and some KPC variants are named as carbapenemases. More KPC variants are being reported, and more are expected in the future. Although the sequence difference between KPC variants and KPC wild type is not significant, the corresponding antimicrobial susceptibility profile and detection techniques are very different. The traditional KPC inhibitor APB cannot inhibit the activity of ARC. Such ARC enzymes should be named separately to attract attention.

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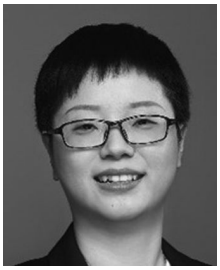

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