AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

Mutations in bla_{KPC-3} That Confer Ceftazidime-Avibactam Resistance Encode Novel KPC-3 Variants That Function as Extended-Spectrum β-Lactamases

Ghady Haidar,[a] Cornelius J. Clancy,[b,c,d] Ryan K. Shields,[b,c] Binghua Hao,[c] Shaoji Cheng,[b] M. Hong Nguyen[a,b,c]

University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA[a]; University of Pittsburgh, Pittsburgh, Pennsylvania, USA[b]; XDR Pathogen Laboratory, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA[c]; VA Pittsburgh Healthcare System, Pittsburgh, Pennsylvania, USA[d]

ABSTRACT We identified four bla_{KPC-3} mutations in ceftazidime-avibactam-resistant clinical *Klebsiella pneumoniae* isolates, corresponding to D179Y, T243M, D179Y/T243M, and EL165-166 KPC-3 variants. Using site-directed mutagenesis and transforming vectors into *Escherichia coli*, we conclusively demonstrated that mutant bla_{KPC-3} encoded enzymes that functioned as extended-spectrum β-lactamases; mutations directly conferred higher MICs of ceftazidime-avibactam and decreased the MICs of carbapenems and other β-lactams. Impact was strongest for the D179Y mutant, highlighting the importance of the KPC Ω-loop.

KEYWORDS KPC, ceftazidime-avibactam, drug resistance mechanisms, site-directed mutagenesis

Carbapenem-resistant *Enterobacteriaceae* (CRE) have emerged as major pathogens worldwide. In the United States, production of *Klebsiella pneumoniae* carbapenemases (KPCs) is the predominant mechanism of carbapenem resistance (1–3). Until recently, treatment options against infections due to CRE in general, and carbapenemase-producing *Enterobacteriaceae* (CPE) in particular, were limited (4, 5). Ceftazidime-avibactam was approved in 2015 by the Food and Drug Administration for the treatment of complicated intra-abdominal infections and complicated urinary tract infections (6). Avibactam, a non-β-lactam β-lactamase inhibitor, inhibits extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and class A carbapenemases (including KPC) but not metallo-β-lactamases (7).

We recently demonstrated that the outcomes of CPE-infected patients treated with ceftazidime-avibactam were comparable to those previously reported for salvage regimens that included two active agents and that toxicity rates were lower (7). Unfortunately, ceftazidime-avibactam resistance emerged within 10 to 19 days in three patients treated for KPC-producing *K. pneumoniae* infections. We further showed that ceftazidime-avibactam-resistant isolates had a variety of bla_{KPC-3} mutations that resulted in variant KPC-3 enzymes (8). Meropenem MICs were reduced ≥4-fold against ceftazidime-avibactam-resistant *K. pneumoniae* isolates (8). In fact, ceftazidime-avibactam-resistant isolates were identified as extended-spectrum β-lactamase (ESBL) producers by our clinical microbiology laboratory rather than as KPC-producing *K. pneumoniae* isolates (6, 8). It is unclear if variant KPC-3 enzymes functioned as ESBLs or if ESBL phenotypes were due to other resistance determinants carried by the isolates. To demonstrate conclusively that KPC-3 variants conferred ceftazidime-avibactam resistance, carbapenem susceptibility, and an ESBL

Received 28 November 2016 **Returned for modification** 23 January 2017 **Accepted** 7 February 2017

Accepted manuscript posted online 21 February 2017

Citation Haidar G, Clancy CJ, Shields RK, Hao B, Cheng S, Nguyen MH. 2017. Mutations in bla_{KPC-3} that confer ceftazidime-avibactam resistance encode novel KPC-3 variants that function as extended-spectrum β-lactamases. Antimicrob Agents Chemother 61:e02534-16. https://doi.org/10.1128/AAC.02534-16.

Address correspondence to Cornelius J. Clancy, cjc76@pitt.edu.

S.C. and M.H.N. contributed equally to this article.

phenotype, we introduced mutations to bla_{KPC-3} by site-directed mutagenesis and measured antibiotic MICs against isogenic *Escherichia coli* expressing mutant genes.

We first cloned wild-type bla_{KPC-3} and flanking regions (600 bp upstream, 240 bp downstream) into pET30a (Novagen, Madison, WI) (8). We then created codon changes in bla_{KPC-3} using the Q5 site-directed mutagenesis kit (New England BioLabs, MA). The following codon changes were based on the bla_{KPC-3} mutations observed in ceftazidime-avibactam-resistant clinical *K. pneumoniae* isolates from our center: (i) a tyrosine for aspartic acid substitution at Ambler amino acid position 179 (D179Y), (ii) a methionine for threonine substitution at position 243 (T243M), (iii) a T243M/D179Y double substitution, or (iv) a glutamic acid and leucine insertion between positions 165 and 166 (EL165-166). The first three mutations were observed in isolates from our three previously reported patients (8), and the fourth mutation was detected in a previously unreported clinical isolate. PCR primers are listed in Table S1 in the supplemental material. pET30a plasmids (Novagen, Madison, WI) containing wild-type or mutant bla_{KPC-3} were transformed into *E. coli* DH5α (New England BioLabs, MA) by heat shock, and successful gene transfer was confirmed by PCR and sequencing.

E. coli transformed with bla_{KPC-3} were susceptible to ceftazidime-avibactam (MIC = 0.5 μg/ml) but resistant to meropenem, imipenem, ertapenem, ceftazidime, ceftriaxone, cefepime, ceftaroline, ampicillin-sulbactam, piperacillin-tazobactam, and aztreonam (Table 1). Ceftazidime-avibactam MICs were higher against each of the *E. coli* isolates transformed with mutated bla_{KPC-3} (MIC range, 4 to 16 μg/ml) than against *E. coli* transformed with wild-type bla_{KPC-3}. In rank order by KPC-3 variant, the increase in ceftazidime-avibactam MICs was as follows: D179Y/T243M (32-fold) > D179Y or T243M (16-fold) > EL165-166 (8-fold). In contrast, carbapenem MICs were significantly lower against *E. coli* harboring mutated bla_{KPC-3} (meropenem MIC range, 0.06 to 0.125 μg/ml; imipenem MIC range, 0.25 to 2 μg/ml; ertapenem MIC range, 0.06 to 1 μg/ml) than they were against wild-type bla_{KPC-3}. In rank order, the decrease in carbapenem MICs was as follows: D179Y/T243M (meropenem, 64-fold; imipenem, 32-fold; ertapenem, 1,024-fold) > D179Y (meropenem, 64-fold; imipenem, 32-fold; ertapenem, 512-fold) > EL165-166 (meropenem, 32-fold; imipenem, 32-fold; ertapenem, 256-fold) > T243M (meropenem, 32-fold; imipenem, 4-fold; ertapenem, 64-fold).

E. coli isolates with D179Y/T243M and D179Y KPC-3 variants exhibited ≥128-fold, ≥512-fold, ≥256-fold, and 8- to 256-fold reductions in the MICs of ampicillin-sulbactam, piperacillin-tazobactam, aztreonam, and other β-lactams, respectively, compared to those of *E. coli* isolates with wild-type KPC-3. Ampicillin, ampicillin-sulbactam, and ceftriaxone MICs against *E. coli* isolates harboring T243M, EL165-166, or wild-type KPC-3 did not differ significantly (≤2-fold differences for each antibiotic). However, compared to wild-type KPC-3, T243M and EL165-166 variants were associated with reductions of ≥128-fold in cefepime MIC, 8-fold in ceftaroline MIC, and ≥64-fold in piperacillin-tazobactam MIC. The aztreonam MIC was reduced 256-fold against *E. coli* harboring EL165-166 variant KPC-3 but was unchanged against *E. coli* harboring the T243M variant.

E. coli isolates transformed with mutated bla_{KPC-3} manifested ESBL phenotypes (Tables 2 and 3) (9). For transformants with each mutated bla_{KPC-3}, disk diffusion zone size differences were ≥14 mm between ceftazidime and ceftazidime-clavulanate and ≥5 mm between cefotaxime and cefotaxime-clavulanate. *E. coli* isolates with D179Y/T243M, D179Y, and T243M KPC-3 variants exhibited ≥5 twofold (i.e., ≥32-fold) decreases in cefotaxime MICs in combination with clavulanate when compared to cefotaxime alone. *E. coli* isolates with variant KPC-3 enzymes were susceptible to cefotetan, cefoxitin, and piperacillin-tazobactam (Tables 1 and 3).

In testing isogenic *E. coli* isolates that harbored wild-type or mutated bla_{KPC-3} generated by site-directed mutagenesis, we studied the impact of KPC-3 variants on antibiotic susceptibility without the influence of background resistance mechanisms, such as other β-lactamases or porin mutations. Our findings confirm that the bla_{KPC-3} mutations that emerged during ceftazidime-avibactam treatment of *K. pneumoniae*

TABLE 1 Susceptibility to β-lactam agents and carbapenems among isogenic *E. coli* strains harboring KPC-3 or KPC-3 variants[a]

Antibiotic agent[b]	Susceptibility	Wild type	D179Y	T243 M	D179Y/T243 M	EL165-166
Ceftazidime	MIC (μg/ml)	256	256	64	512	128
	Fold change vs wild-type KPC-3		None	↓4	↑2	↓2
	Susceptibility pattern	R	R	R	R	R
Ceftazidime-avibactam	MIC (μg/ml)	0.5	8	8	16	4
	Fold change vs wild-type KPC-3		↑16	↑16	↑32	↑8
	Susceptibility pattern	S	I	I	R	S
Meropenem	MIC (μg/ml)	4	0.06	0.125	0.06	0.125
	Fold change vs wild-type KPC-3		↓64	↓32	↓64	↓32
	Susceptibility pattern	R	S	S	S	S
Imipenem	MIC (μg/ml)	8	0.25	2	0.25	0.25
	Fold change vs wild-type KPC-3		↓32	↓4	↓32	↓32
	Susceptibility pattern	R	S	S	S	S
Ertapenem	MIC (μg/ml)	64	0.125	1	0.06	0.25
	Fold change vs wild-type KPC-3		↓512	↓64	↓1,024	↓256
	Susceptibility pattern	R	S	S	S	S
Ampicillin	MIC (μg/ml)	512	8	512	8	512
	Fold change vs wild-type KPC-3		↓64	None	↓64	None
	Susceptibility pattern	R	S	R	S	R
Ampicillin-sulbactam	MIC (μg/ml)	512	4	512	2	512
	Fold change vs wild-type KPC-3		↓128	None	↓256	None
	Susceptibility pattern	R	S	R	S	R
Piperacillin-tazobactam	MIC (μg/ml)	512	0.5	8	1	1
	Fold change vs wild-type KPC-3		↓1,024	↓64	↓512	↓512
	Susceptibility pattern	R	S	S	S	S
Ceftriaxone	MIC (μg/ml)	64	8	32	8	64
	Fold change vs wild-type KPC-3		↓8	↓2	↓8	None
	Susceptibility pattern	R	S	I	S	S
Cefepime	MIC (μg/ml)	512	4	4	2	2
	Fold change vs wild-type KPC-3		↓128	↓128	↓256	↓256
	Susceptibility pattern	R	DDS	DDS	S	S
Ceftaroline	MIC (μg/ml)	512	16	64	16	64
	Fold change vs wild-type KPC-3		↓32	↓8	↓32	↓8
	Susceptibility pattern	R	R	R	R	R
Aztreonam	MIC (μg/ml)	512	2	512	1	2
	Fold change vs wild-type KPC-3		↓256	None	↓512	↓256
	Susceptibility pattern	R	S	R	S	S

[a]Downward arrows indicate fold reduction in MIC while upward arrows indicate fold increase in MIC. S, susceptible; I, intermediate; R, resistant; DDS, dose-dependent susceptible.

[b]Antibiotics were obtained from the University of Pittsburgh Medical Center pharmacy. Avibactam was supplied by AstraZeneca. Susceptibilities were determined by broth microdilution (ceftazidime, ceftazidime-avibactam [fixed avibactam concentration of 4 μg/ml], imipenem, and meropenem), Etest methods (ampicillin, ampicillin-sulbactam, ceftriaxone, cefepime, ceftaroline, piperacillin-tazobactam), and disk diffusion (cefotetan, cefoxitin, piperacillin-tazobactam). Clinical and Laboratory Standards Institute (CLSI) interpretive breakpoints were used (9). An isolate was defined as carbapenem resistant if it was resistant to either ertapenem, imipenem, or meropenem (MICs of ≥2, ≥4, or ≥4 μg/ml, respectively), as recommended by the CLSI and Centers for Disease Control (3, 9).

infections directly conferred reduced susceptibility to ceftazidime-avibactam, reduced carbapenem and other β-lactam MICs, and encoded enzymes that functioned as ESBLs.

We found that the D179Y/T243M KPC-3 variant exerted the greatest impact on ceftazidime-avibactam and carbapenem MICs compared to that of wild-type KPC-3; this was followed by the D179Y variant and then the T243M or EL165-166 variants. D179Y and T243M single substitutions, ceftazidime-avibactam resistance, and restored carbapenem susceptibility were generated previously in KPC-3-producing *K. pneumoniae* by *in vitro* selection, but the mutations were not validated as the causes of altered MICs (10). In another study, the introduction of substitutions at KPC-2 amino acid positions 179

TABLE 2 Extended-spectrum β-lactamase assays for isogenic *E. coli* strains harboring KPC-3 or KPC-3 variants for cephalosporin versus cephalosporin-clavulanate

KPC-3 or variant	ESBL testing results									
	Disk diffusion (zone size in mm)			Disk diffusion (zone size in mm)			Etest MIC (μg/ml)			
	Ceftazidime	Ceftazidime-clavulanate	Zone size difference	Cefotaxime	Cefotaxime-clavulanate	Zone size difference	Cefotaxime	Cefotaxime-clavulanate	No. 2-fold (actual fold) decrease in MICs	ESBL?[a]
Wild-type	10	15	5	14	18	4	>16	>1	NA[c]	
D179Y	No zone[b]	23	17	23	31	8	4	0.06	Six 2-fold (or 64-fold)	Yes
T243M	No zone	20	14	19	24	5	4	0.06	Six 2-fold (or 64-fold)	Yes
D179Y/T243M	No zone	23	17	28	34	6	4	0.125	Five 2-fold (or 32-fold)	Yes
EL165-166	No zone	23	17	13	32	19	>16	>1	NA	Yes

[a]Isolates were identified as ESBL producers by CLSI criteria (9), i.e., a difference of ≥5 mm between zone diameters of a ceftazidime or cefotaxime disk and the respective clavulanate-containing disk or a ≥3 twofold (i.e., ≥8-fold) decrease in cefotaxime MIC in combination with clavulanate versus cefotaxime alone (as determined using an Etest strip).
[b]When no zone was present, the zone size difference was calculated by subtracting 6 mm (the size of the disk) from the zone size around ceftazidime-clavulanate.
[c]NA, not applicable.

and 243 by site-directed mutagenesis also resulted in ceftazidime-avibactam resistance in *E. coli* (11). The precise mechanisms by which the mutations reported here mediate changes in ceftazidime-avibactam, carbapenem, and other β-lactam susceptibilities are unclear. The relative importance of D179Y compared to EL165-166 or T243M suggests a central role for KPC Ω-loop stability. The Ω-loop is maintained by a salt bridge between D179 and arginine at position 164 (11, 12). Position 179 KPC variants with Ω-loop instability demonstrate enhanced ceftazidime affinity, which is postulated to restrict the binding of avibactam (11). This may not be the sole mechanism of ceftazidime-avibactam resistance, however, as KPC variants did not protect *E. coli* against ceftazidime-clavulanate. The decreases that we observed in meropenem, imipenem, and ertapenem MICs against KPC-3 variants are likely due to reduced carbapenemase activity as is well-recognized to occur following a variety of stepwise bla_{KPC} mutations (13). Determining the crystal structure of novel KPC-3 variants, their interactions with β-lactams, and enzyme kinetics is a future research priority.

Our findings suggest that the ESBL phenotypes reported by the clinical microbiology lab for the original ceftazidime-avibactam-resistant *K. pneumoniae* isolates were caused in part by the variant KPC-3 enzymes. It has been previously proposed that KPC-2 variants with substitutions at amino acid position 179 behave as ESBLs (11). Although *E. coli* with mutant bla_{KPC-3} fulfilled Clinical and Laboratory Standards Institute (CLSI) criteria for the presence of ESBL (9), consensus definitions have not been established (14–20). A commonly used definition is that ESBLs are β-lactamases that confer resistance to penicillins, first-, second-, and third-generation cephalosporins, and often aztreonam (but not carbapenems) and that are inhibited by β-lactamase inhibitors like clavulanate (14). Susceptibility to cephamycins like cefoxitin and cefotetan, as observed for the KPC-3 variants here, is not included in the formal CLSI definition but also supports an ESBL phenotype (14–20). Moreover, piperacillin-tazobactam susceptibility is well-recognized among certain ESBL-producing *Enterobacteriaceae* (21). Many KPC-2- and KPC-3-producing *Enterobacteriaceae* fulfill CLSI criteria for ESBL production,

TABLE 3 Cephamycin susceptibility testing

KPC-3 or variant	Kirby-Bauer test result[a]			
	Cefotetan (zone size in mm)	Cefotetan susceptibility pattern	Cefoxitin (zone size in mm)	Cefoxitin susceptibility pattern
Wild-type	14	I	16	I
D179Y	26	S	32	S
T243M	17	S	22	S
D179Y/T243M	24	S	29	S
EL165-166	18	S	25	S

[a]S, sensitive; I, intermediate; R, resistant.

but the enzymes are not considered ESBLs due to their carbapenemase activity (22). With increased use of ceftazidime-avibactam and other new agents with activity against CPE, it is likely that a wide range of bla_{KPC} mutations will emerge that result in KPC variants with differing antibiotic affinities and hydrolytic properties. In this regard, the evolution of KPCs is likely to resemble that of ESBLs, among which a wide array of TEM, SHV, and CTX variants have arisen. Our experience suggests that molecular probes for bla_{KPC} detection may be less useful as a clinical tool than phenotypic assays and sequencing approaches.

The optimal treatment of infections caused by *Enterobacteriaceae* expressing variant KPCs is unclear. Reversion to carbapenem susceptibility and identification of ESBL phenotypes imply a potential role for carbapenems. However, the efficacy of this drug class in these settings is unproven, and the stability of restored carbapenem susceptibility is unknown. The use of both ceftazidime-avibactam and a carbapenem for treatment of CPE infections is intriguing; avibactam may protect the carbapenem against hydrolysis by carbapenemase, and there would be carbapenem counterselection against bla_{KPC-3} mutations that lead to ceftazidime-avibactam resistance. There are currently no data to support dual ceftazidime-avibactam and carbapenem therapy, and it is unknown if other mechanisms of resistance, such as porin mutations, would develop in this setting. New agents in development, including novel combinations of β-lactamase inhibitors with carbapenems or other agents, merit investigation. In the past, the use of β-lactamase/β-lactamase inhibitor combinations against infections by ESBL-producing organisms has generated conflicting efficacy data (23–26). Better understanding of isolate genetics may help define the roles of various agents.

In conclusion, we have shown that novel bla_{KPC-3} mutations result in variant KPC-3 enzymes that significantly reduce ceftazidime-avibactam susceptibility and generally behave like ESBLs. With the increased use of ceftazidime-avibactam, it is expected that resistance will continue to emerge and plasmids carrying mutant genes may disseminate by horizontal gene transfer. We advocate routine ceftazidime-avibactam susceptibility testing of *Enterobacteriaceae*, even in the absence of prior drug exposure, and screening of resistant isolates for bla_{KPC} carriage or mutations.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02534-16.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

This work was funded, in part, by grants from the National Institutes of Health (UM1AI104681 and R21AI288338 to M.H.N., R21AI111037 to C.J.C., and K08AI114883 to R.K.S.).

We declare no conflicts of interest.

REFERENCES

1. Haidar G, Alkroud A, Cheng S, Churilla TM, Churilla BM, Shields RK, Doi Y, Clancy CJ, Nguyen MH. 2016. Association between the presence of aminoglycoside-modifying enzymes and *in vitro* activity of gentamicin, tobramycin, amikacin, and plazomicin against *Klebsiella pneumoniae* carbapenemase- and extended-spectrum-beta-lactamase-producing *Enterobacter* species. Antimicrob Agents Chemother 60:5208–5214. https://doi.org/10.1128/AAC.00869-16.
2. Ahn C, Syed A, Hu F, O'Hara JA, Rivera JI, Doi Y. 2014. Microbiological features of KPC-producing *Enterobacter* isolates identified in a U.S. hospital system. Diagn Microbiol Infect Dis 80:154–158. https://doi.org/10.1016/j.diagmicrobio.2014.06.010.
3. Centers for Disease Control and Prevention. FAQs about choosing and implementing a CRE definition. Centers for Disease Control and Prevention, Atlanta, GA. https://www.cdc.gov/hai/organisms/cre/definition.html.
4. Qureshi ZA, Paterson DL, Potoski BA, Kilayko MC, Sandovsky G, Sordillo E, Polsky B, Adams-Haduch JM, Doi Y. 2012. Treatment outcome of bacteremia due to KPC-producing *Klebsiella pneumoniae*: superiority of combination antimicrobial regimens. Antimicrob Agents Chemother 56:2108–2113. https://doi.org/10.1128/AAC.06268-11.
5. Tzouvelekis LS, Markogiannakis A, Piperaki E, Souli M, Daikos GL. 2014. Treating infections caused by carbapenemase-producing *Enterobacteriaceae*. Clin Microbiol Infect 20:862–872. https://doi.org/10.1111/1469-0691.12697.
6. Forest Pharmaceuticals, Inc. 2016. Avycaz (ceftazidime-avibactam) prescribing information. Forest Pharmaceuticals, Inc., Cincinnati, OH.
7. Shields RK, Potoski BA, Haidar G, Hao B, Doi Y, Chen L, Press EG, Kreiswirth BN, Clancy CJ, Nguyen MH. 2016. Clinical outcomes, drug toxicity, and emergence of ceftazidime-avibactam resistance among patients treated for carbapenem-resistant *Enterobacteriaceae* infections. Clin Infect Dis 63:1615–1618. https://doi.org/10.1093/cid/ciw636.
8. Shields RK, Chen L, Cheng S, Chavda KD, Press EG, Snyder A, Pandey R,

Doi Y, Kreiswirth BN, Nguyen MH, Clancy CJ. 28 December 2016. Emergence of ceftazidime-avibactam resistance due to plasmid-borne bla_{KPC-3} mutations during treatment of carbapenem-resistant *Klebsiella pneumoniae* infections. Antimicrob Agents Chemother https://doi.org/10.1128/AAC.02097-16.

9. Clinical and Laboratory Standards Institute. 2015. Performance standards for antimicrobial susceptibility testing; 25th informational supplement. CLSI M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.
10. Livermore DM, Warner M, Jamrozy D, Mushtaq S, Nichols WW, Mustafa N, Woodford N. 2015. *In vitro* selection of ceftazidime-avibactam resistance in *Enterobacteriaceae* with KPC-3 carbapenemase. Antimicrob Agents Chemother 59:5324–5330. https://doi.org/10.1128/AAC.00678-15.
11. Winkler ML, Papp-Wallace KM, Bonomo RA. 2015. Activity of ceftazidime/avibactam against isogenic strains of *Escherichia coli* containing KPC and SHV beta-lactamases with single amino acid substitutions in the omega-loop. J Antimicrob Chemother 70:2279–2286. https://doi.org/10.1093/jac/dkv094.
12. Levitt PS, Papp-Wallace KM, Taracila MA, Hujer AM, Winkler ML, Smith KM, Xu Y, Harris ME, Bonomo RA. 2012. Exploring the role of a conserved class A residue in the omega-loop of KPC-2 beta-lactamase: a mechanism for ceftazidime hydrolysis. J Biol Chem 287:31783–31793. https://doi.org/10.1074/jbc.M112.348540.
13. Wolter DJ, Kurpiel PM, Woodford N, Palepou MF, Goering RV, Hanson ND. 2009. Phenotypic and enzymatic comparative analysis of the novel KPC variant KPC-5 and its evolutionary variants, KPC-2 and KPC-4. Antimicrob Agents Chemother 53:557–562. https://doi.org/10.1128/AAC.00734-08.
14. Paterson DL, Bonomo RA. 2005. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 18:657–686. https://doi.org/10.1128/CMR.18.4.657-686.2005.
15. Bush K, Jacoby GA. 2010. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother 54:969–976. https://doi.org/10.1128/AAC.01009-09.
16. Giske CG, Sundsfjord AS, Kahlmeter G, Woodford N, Nordmann P, Paterson DL, Canton R, Walsh TR. 2009. Redefining extended-spectrum beta-lactamases: balancing science and clinical need. J Antimicrob Chemother 63:1–4. https://doi.org/10.1093/jac/dkn444.
17. Rupp ME, Fey PD. 2003. Extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*: considerations for diagnosis, prevention and drug treatment. Drugs 63:353–365. https://doi.org/10.2165/00003495-200363040-00002.
18. Livermore DM. 2008. Defining an extended-spectrum beta-lactamase. Clin Microbiol Infect 14(Suppl):S3–S10. https://doi.org/10.1111/j.1469-0691.2007.01857.x.
19. Lee CH, Su LH, Tang YF, Liu JW. 2006. Treatment of ESBL-producing *Klebsiella pneumoniae* bacteraemia with carbapenems or flomoxef: a retrospective study and laboratory analysis of the isolates. J Antimicrob Chemother 58:1074–1077. https://doi.org/10.1093/jac/dkl381.
20. Harris PN, Tambyah PA, Paterson DL. 2015. Beta-lactam and beta-lactamase inhibitor combinations in the treatment of extended-spectrum beta-lactamase producing *Enterobacteriaceae*: time for a reappraisal in the era of few antibiotic options? Lancet Infect Dis 15:475–485. https://doi.org/10.1016/S1473-3099(14)70950-8.
21. Retamar P, Lopez-Cerero L, Muniain MA, Pascual A, Rodriguez-Bano J, ESBL-REIPI/GEIH Group. 2013. Impact of the MIC of piperacillin-tazobactam on the outcome of patients with bacteremia due to extended-spectrum-beta-lactamase-producing *Escherichia coli*. Antimicrob Agents Chemother 57:3402–3404. https://doi.org/10.1128/AAC.00135-13.
22. Stachyra T, Levasseur P, Pechereau MC, Girard AM, Claudon M, Miossec C, Black MT. 2009. *In vitro* activity of the β-lactamase inhibitor NXL104 against KPC-2 carbapenemase and *Enterobacteriaceae* expressing KPC carbapenemases. J Antimicrob Chemother 64:326–329. https://doi.org/10.1093/jac/dkp197.
23. Perez F, Bonomo RA. 2015. Editorial commentary: bloodstream infection caused by extended-spectrum beta-lactamase-producing Gram-negative bacteria: how to define the best treatment regimen? Clin Infect Dis 60:1326–1329.
24. Tamma PD, Han JH, Rock C, Harris AD, Lautenbach E, Hsu AJ, Avdic E, Cosgrove SE, Antibacterial Resistance Leadership Group. 2015. Carbapenem therapy is associated with improved survival compared with piperacillin-tazobactam for patients with extended-spectrum beta-lactamase bacteremia. Clin Infect Dis 60:1319–1325.
25. Rodriguez-Bano J, Navarro MD, Retamar P, Picon E, Pascual A, Extended-Spectrum Beta-Lactamases–Red Española de Investigación en Patología Infecciosa/Grupo de Estudio de Infección Hospitalaria Group. 2012. Beta-lactam/beta-lactam inhibitor combinations for the treatment of bacteremia due to extended-spectrum beta-lactamase-producing *Escherichia coli*: a post hoc analysis of prospective cohorts. Clin Infect Dis 54:167–174. https://doi.org/10.1093/cid/cir790.
26. Perez F, Bonomo RA. 2012. Can we really use beta-lactam/beta-lactamase inhibitor combinations for the treatment of infections caused by extended-spectrum beta-lactamase-producing bacteria? Clin Infect Dis 54:175–177. https://doi.org/10.1093/cid/cir793.