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Activity of imipenem/relebactam against carbapenemase-producing Enterobacteriaceae with high colistin resistance

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Objectives: Imipenem/relebactam, an investigational β-lactam/β-lactamase inhibitor combination for treatment of Gram-negative infections, and comparators including ceftazidime/avibactam, piperacillin/tazobactam and colistin were tested for activity against representative carbapenemase-producing Enterobacteriaceae (CPE) isolates.

Methods: MICs of the antimicrobial agents were determined using standard broth microdilution methodology for CPE isolates collected from Indiana patients, primarily during the time frame of 2013–17 (*n*=199 of a total of 200 isolates). Inhibitors were tested at 4 mg/L in all combinations.

Results: Of the CPE in the study, 199 produced plasmid-encoded KPC class A carbapenemases; 1 *Serratia marcescens* isolate produced the SME-1 chromosomal class A carbapenemase. MIC_{50}/MIC_{90} values of imipenem/relebactam were ≤0.25/0.5 mg/L, whereas MIC_{50}/MIC_{90} values of ceftazidime/avibactam were 1/2 mg/L. Resistance to colistin was observed in 54% (*n*=97) of 180 non-*Serratia* isolates tested (MIC_{50} of 4 mg/L). Colistin resistance mechanisms included production of a plasmid-encoded *mcr-1*-like gene (*n*=2) or an inactivated *mgrB* gene.

Conclusions: Imipenem/relebactam was the most potent agent tested against CPE in this study and may be a useful addition to the antimicrobial armamentarium to treat infections caused by these pathogens.

Introduction

Global dissemination of carbapenemase-producing Enterobacteriaceae (CPE) is on the rise and is designated an urgent antibiotic resistance threat.[1] Resistance to carbapenems is most often attributed to carbapenemases, including the serine carbapenemases (e.g. KPC, SME or OXA) and MBLs (e.g. NDM, IMP or VIM).[2] Therapeutic options for infections caused by CPE include recently approved β-lactam/β-lactamase inhibitor combinations and non-β-lactam agents such as colistin. These options become more restricted for cases of CPE with additional resistance to colistin.[3]

Relebactam is an investigational diazabicyclooctane β-lactamase inhibitor of class A and C β-lactamases.[4] In a recent Phase 3 clinical trial comparing imipenem/relebactam with a combination of colistin plus imipenem, imipenem/relebactam was associated with a higher favourable clinical response (71.4% versus 40%).[5] Mortality was lower in patients treated with imipenem/relebactam (9.5%) than those treated with colistin/imipenem (30%).[5] Because of these results, imipenem/relebactam was tested for restorative carbapenem activity against a recent and unique set of CPE.

Methods

A central clinical microbiology laboratory, servicing 5 Indiana hospitals and 14 healthcare centres, provided the CPE included in this study. Two hundred unique single-patient CPE isolates were selected from a collection of >500 carbapenem-resistant Enterobacteriaceae isolates, based on antimicrobial susceptibility profiles. Species in the collection included: *Klebsiella* spp. (83.5%), *Serratia marcescens* (10%), *Escherichia coli* (3.5%), *Enterobacter cloacae* (2.5%) and *Citrobacter freundii* (0.5%) (Table 1). Isolates were

Table 1. Carbapenemase identification in 200 CPE from Indiana

Organism	Carbapenemase	Number of isolates
C. freundii	KPC-3[a]	1
E. cloacae	KPC-3	5
E. coli	KPC-2	1
	KPC-3	6
Klebsiella ozaenae	KPC-3	1
K. pneumoniae	KPC-2	7
	KPC-3	159
S. marcescens	KPC-3	19
	SME-1	1

[a]KPC-3 carbapenemases include a set of KPC-3-like enzymes that lacked three to five amino acids at the N-terminus of the native enzyme due to incomplete translation of the *bla* sequence using the reverse PCR primer.

collected between 2013 and 2017, with one *E. coli* isolate collected in 2010. Resistance to carbapenems was initially determined using the VITEK® 2 system (bioMérieux). Carbapenemase activity was detected in isolates with imipenem MICs >1 mg/L first using the modified Hodge test and later confirmed by a modified carbapenem inactivation method (mCIM).[6] MBL producers were phenotypically identified and excluded from the collection based on the results from a modification of the mCIM assay in which bacteria were incubated with a 10 μg meropenem disc and EDTA (2 mg/L) to look for enhancement of the meropenem zone size in the mCIM test. Imipenem and relebactam were supplied by Merck Sharp & Dohme Corp., while all other antibiotics were purchased from the United States Pharmacopeia. Antimicrobial susceptibility testing was conducted using broth microdilution according to CLSI guidelines.[7] Because of intrinsic resistance to colistin in *S. marcescens* isolates, only the 180 non-*Serratia* isolates were tested for colistin susceptibility.

Plasmid DNA was extracted from each CPE using the QIAprep® Spin Miniprep Kit (QIAGEN). Genomic DNA was extracted using the GenElute™ Bacterial Genomic DNA Kit (Sigma–Aldrich). All organisms were screened for the carbapenemase genes bla_{KPC} and bla_{OXA-48} using conventional PCR and subsequent Sanger sequencing.[8,9] Occasional sequencing of bla_{KPC} products resulted in truncated KPC-3 sequences ('KPC-3-like' enzymes) lacking the N-terminal three to five amino acids. Later studies indicated that these were due to incomplete reads of the PCR products, not due to truncated genes. *S. marcescens* isolates were also screened for bla_{SME}.[10] Selected isolates with colistin-resistant phenotypes were investigated further for genetic resistance mechanisms. All colistin-resistant isolates were screened for the plasmid-encoded *mcr-1* gene.[11] In order to explain the resistance mechanisms in isolates with the highest colistin MICs (>16 mg/L), these isolates were examined for an inactivated *mgrB* gene.[12] All sequencing results were analysed by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results and discussion

Of the 200 CPE isolates, 95.5% (*n*=191) produced a KPC-3 or KPC-3-like carbapenemase, while KPC-2 was produced by 4% (*n*=8) of the isolates, and only in *E. coli* and *Klebsiella pneumoniae*. Of 20 *S. marcescens* isolates, 19 (95%) produced KPC-3 and 1 produced a chromosomally encoded SME-1 carbapenemase (Table 1). No isolates produced OXA-48 or MBLs.

All the CPE isolates in the study were non-susceptible to imipenem and piperacillin/tazobactam (Table 2). Susceptibility to imipenem (defined as an MIC of ≤1 mg/L using CLSI breakpoints[6]) was restored by 4 mg/L relebactam in 96.5% of isolates tested; in the presence of relebactam, 82.5% of isolates had an imipenem MIC of ≤0.25 mg/L, while 93% had an imipenem MIC of ≤0.5 mg/L (Table 2). All seven CPE with imipenem/relebactam MICs ≥2 mg/L were *S. marcescens*, including the SME-1-producing *S. marcescens* isolate with an imipenem/relebactam MIC of 4 mg/L, the highest MIC observed in the study for this combination. In the case of ceftazidime/avibactam, only 9% of the isolates (18/200) had an MIC of <0.5 mg/L, with maximum MICs of 4 mg/L reported (100% susceptible).

In the current study, the number of colistin-resistant CPE was unusually high. Of the 180 non-*Serratia* isolates tested against colistin, 54% (*n*=97) were resistant. This represents a much higher percentage than the 16% colistin resistance rate reported by Han *et al.*[13] in a 2017 study of carbapenem-resistant *K. pneumoniae* isolates from long-term acute care hospitals in the USA. All colistin-resistant CPE were *Klebsiella* spp., with the exception of one *E. cloacae* isolate, an observation similar to that observed by Bradford *et al.*,[14] who identified *E. cloacae* and *K. pneumoniae* as the most common species with colistin-resistant phenotypes. Notably, the imipenem/relebactam MIC_{90} for colistin-resistant CPE was ≤0.25 mg/L, lower than the MIC_{90} for all 200 isolates (Table 2). No imipenem/relebactam MIC was >1 mg/L for the colistin-resistant isolates.

Colistin resistance within CPE is increasingly being reported. In a global surveillance study published in 2016, strong association was shown between an increased resistance to colistin and the presence of a carbapenemase in Enterobacteriaceae.[14] In non-CPE, resistance to colistin was observed in 6% of isolates, whereas a 6-fold increase in colistin resistance (to 38%) was seen in CPE.

Colistin resistance involves both chromosomal and plasmid-mediated mechanisms. The PhoP/PhoQ signalling system that includes MgrB as a negative feedback regulator[15] is responsive to various environmental conditions, including the presence of cationic peptides such as colistin.[15] Insertional inactivation of *mgrB* results in the up-regulation of the system and subsequent acquisition of colistin resistance.[16] Other alterations, such as deletions, point mutations and nonsense mutations in the *mgrB* gene, have also been reported in clinical strains of colistin-resistant carbapenemase-producing *K. pneumoniae*.[12] In addition to chromosomally mediated resistance, transferable resistance via MCR-1, first reported from China in 2016, has since been identified in samples from animals, humans, food and environmental sources across the globe.[11,17]

Of the 97 colistin-resistant isolates screened in this study, two *K. pneumoniae* were found to contain an *mcr-1*-like gene. The *mcr-1*-like carriers from two patients in different hospitals were isolated in 2013 and 2016, with colistin MICs of 4 and 8 mg/L, respectively. Both isolates also encoded bla_{KPC-3}. Highly resistant strains, with colistin MICs of >16 mg/L, were further investigated for alterations of *mgrB*. A nonsense mutation was detected in the *mgrB* coding sequence of one *K. pneumoniae* isolate, resulting in premature termination. In accordance with previous reports, the position of the mutation within *mgrB* was identified as c64t, with nucleotide number one as first base of the GTG codon (accession number AVFC01000053, region 155512–155655).[12]

Based on *in vitro* results, imipenem/relebactam is a promising potential agent for treatment of CPE infections, including those with additional resistance to colistin. These data also demonstrate why colistin should be used with caution when treating CPE, as this

Table 2. MIC distributions of imipenem/relebactam and comparators for CPE isolates from Indiana

Isolates (*n*)	Agent	MIC (mg/L)												MIC_{50} (mg/L)	MIC_{90} (mg/L)	Percentage susceptible[a]
		≤0.25	0.5	1	2	4	8	16	>16	32	64	128	>128			
All (*n*=200)	IPM				7	24	102	39	28					8	>16	0
	IPM/REL	165	21	7	4	3								≤0.25	0.5	NA[b]
	CAZ					1		8		15	14	57	105	>128	>128	0.5
	CZA	18	20	98	54	10								1	2	100
	TZP									3	13	17	167	>128	>128	0
Non-*Serratia* (*n*=180)	CST		4	40	39	61	19	14	3					4	8	46.1
Colistin-resistant non-*Serratia* (*n*=97)	IPM				3	11	50	23	10					8	16	0
	IPM/REL	89	5	3										≤0.25	≤0.25	NA
	CAZ									2	5	29	61	>128	>128	0
	CZA	2	5	54	33	3								1	2	100
	TZP									1	1	5	90	>128	>128	0
	CST					61	19	14	3					4	16	0

IPM, imipenem; IPM/REL, imipenem/relebactam; CAZ, ceftazidime; CZA, ceftazidime/avibactam; TZP, piperacillin/tazobactam; CST, colistin.
[a]Susceptibility determined according to CLSI breakpoints except for colistin where EUCAST breakpoints were used.
[b]Not applicable; no breakpoint has been assigned.

rate of colistin-resistant CPE is high for the USA, but comparable to what has been reported in isolated European outbreaks (38%–66% colistin resistance in CPE *K. pneumoniae* isolates).[18,19] While ceftazidime/avibactam has been shown to be effective for the treatment of infections caused by CPE, resistance has emerged since its release in 2015.[20] Although it is impossible to evade antimicrobial resistance completely, it is important to have additional therapeutic options such as imipenem/relebactam available for the treatment of MDR Gram-negative infections.

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