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Independent Emergence of Colistin-Resistant *Enterobacteriaceae* Clinical Isolates without Colistin Treatment[▽]

As mentioned in some reports, with the spread of *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria, colistin use has reemerged as a treatment of last resort despite its severe nephrotoxicity and neurotoxicity (7). We conducted a surveillance study of carbapenem-resistant *Enterobacteriaceae* isolates from Huashan Hospital (Fudan University, Shanghai, China) and performed colistin antimicrobial susceptibility testing. We noticed independent emergence of colistin resistance in KPC-producing carbapenem-resistant *Enterobacteriaceae* (CRE) isolates without clinical treatment with colistin.

From April 2009 to February 2010, 82 CRE isolates, including 68 isolates of *Klebsiella pneumoniae* and 14 other CRE isolates, were collected, and each isolate was identified at species level by using a Vitek 2 compact instrument (bioMérieux, France). Antimicrobial susceptibility testing was performed using the agar dilution method, and the results were interpreted following the CLSI criteria (3). The MICs of colistin and tigecycline were interpreted following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (≤2 and ≥4 mg/liter for susceptible and resistant, respectively) (4) and the U.S. Food and Drug Administration criteria (≤2 and ≥8 mg/liter for susceptible and resistant, respectively) (6), respectively. β-Lactamase genes, including carbapenemase genes, in these isolates were detected by PCR, and all positive products were sequenced. The genetic homology of the isolates was determined by pulsed-field gel electrophoresis (PFGE) according to previously described procedures (10), and outer membrane proteins (OmpK35 and OmpK36) were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (5). Retrospective case studies were undertaken simultaneously.

In our study, 69 (84.1%) of the 82 CRE isolates were producing KPC-2-type carbapenemase and were highly resistant to carbapenems, as well as cephalosporins. The susceptibility rates for colistin and tigecycline were 92.7% and 85.4%, respectively; for minocycline and doxycycline, they were 79.3% and 61.0%, respectively. Although colistin was found to be most active against CRE isolates, 4 isolates showed high resistance to colistin, with MICs of >64 μg/ml for 3 isolates and 4 μg/ml for 1 isolate (Table 1). The 4 colistin-resistant isolates were reconfirmed by 16S rRNA sequencing, and the genotypic results matched the phenotypic results identified with the Vitek 2 compact. PFGE fingerprinting of 68 *Klebsiella pneumoniae* isolates resulted in 18 types, and the 3 colistin-resistant *Klebsiella pneumoniae* isolates exhibited unrelated genotypes. Of all isolates, 81.7% (67/82) had a loss of or decrease in outer membrane protein expression. Recently, some reported colistin resistance has resulted from antibiotic selective pressure (8). However, clinical history materials showed that although the patients had received one or more antibiotics, including carbapenem, cephalosporin, quinolones, and aminoglycosides, none of the patients had received treatment with colistin.

As colistin is the last line of defense against these troublesome carbapenemase-producing CRE isolates, the development of resistance to colistin is a serious concern (1). Although colistin susceptibility among KPC-producing bacteria ranges from 90 to 100% (2), some cases of colistin resistance have been reported, but the resistance mechanisms behind its development are not clear (9).

It is still necessary to evaluate the efficacy of combination therapy with colistin, as well as conduct surveillance and resistance mechanism investigation, to avoid the emergence of more colistin-resistant CRE isolates in health care-associated facilities.

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TABLE 1. Laboratory and clinical characteristics of 4 colistin-resistant *Enterobacteriaceae* isolates

Species, strain	Specimen source	MIC (μg/ml)[a]					Resistance mechanism				Clinical characteristics of patients		
		MEM	ETP	IMP	CLO	TGC	Carbapenemase	ESBL(s)	Porin expression		Antibiotic therapy	Underlying disease	Outcome
									OmpK35	OmpK36			
Enterobacter cloacae													
09-1210	Urine	64	128	32	>64	1	KPC-2	CTX-M-99	Loss	Loss	None	None	Improved
Klebsiella pneumoniae													
09-1999	Sputum	2	4	16	>64	8	None	None	Loss	Decreased	Cephalosporin	None	Uncured
09-3091	Sputum	>256	>256	256	>64	1	KPC-2	SHV-12, CTX-M-15	Normal	Loss	Quinolones, aminoglycoside	None	Improved
09-3011	Sputum	32	128	32	4	2	KPC-2	SHV-12, CTX-M-99	Decreased	Loss	Carbapenem, cephalosporin, quinolones, aminoglycoside	None	Improved

[a] Breakpoints for *Enterobacteriaceae* when testing tigecycline are those of the U.S. Food and Drug Administration (≤2 and ≥8 mg/liter for susceptible and resistant, respectively). MICs of colistin were interpreted following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (≤2 and ≥4 mg/liter for susceptible and resistant, respectively) (3, 6). MEM, meropenem; ETP, ertapenem; IMP, imipenem; CLO, colistin; TGC, tigecycline.

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