Short Communication

Carbapenemase-Producing *Enterobacteriaceae* in Yunnan Province, China

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SUMMARY: Among 144 carbapenem-resistant *Enterobacteriaceae* strains isolated from 4 hospitals in Yunnan province, 113 were identified as carbapenemase-producing *Enterobacteriaceae* (CPE). *Bla*_{KPC-2} (99/113, 87.6%) was the most common carbapenemase gene and *Klebsiella pneumoniae* (100/113, 88.5%) was the most common species. *Bla*_{NDM-1} (11/113, 9.7%), *bla*_{IMP-4} (10/113, 8.8%), and *bla*_{IMP-1} (1/113, 0.9%) genes were also detected. Extended-spectrum β -lactamase genes were common in CPE, and the SHV- and CTX-M-types were predominant.

Carbapenems have been considered ideal antimicrobial agents to be used against gram-negative bacteria (1–3). However, the emergence and dissemination of carbapenem-resistant strains among *Enterobacteriaceae* have become a serious problem worldwide (4), which has made the empirical choice of appropriate antimicrobial treatment very difficult (5). Considering the importance of *Enterobacteriaceae* as nosocomial bacteria and the fact that infections caused by carbapenemase-producing strains often result in high mortality (6), investigation of the molecular characteristics of CPE is critical.

A total of 144 non-duplicate *Enterobacteriaceae* strains with decreased susceptibility to carbapenems isolated from inpatients at 4 hospitals (including 3 tertiary teaching hospitals and 1 country-class hospital), from 2011 to 2013, were included. Antimicrobial susceptibility testing was performed initially using gram-negative susceptibility cards on the VITEK 2 compact system (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. Isolates that were not susceptible to imipenem (minimum inhibitory concentration $\geq 2 \mu\text{g/mL}$) and/or meropenem (minimum inhibitory concentration $\geq 2 \mu\text{g/mL}$) were collected. These included 63 from the First People's Hospital of Kunming City (Kunming city, 1,100 beds), 65 from the First People's Hospital of Yunnan Province (Kunming city, 2,000 beds), 15 from the First Affiliated Hospital of Kunming Medical University (Kunming city, 2,162 beds), and 1 from Jingdong County people's hospital (Jingdong, 300 beds). The specimens yielding the isolates were sputum [57], blood [34], urine [28], pus [14], drainage [7], wound secretion [2], vaginal swab [1], and

pleural fluid [1].

PCR was performed for carbapenemase genes as well as extended-spectrum β -lactamase (ESBL) and AmpC genes including those of Ambler class A (*bla*_{CTX-M}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{KPC}, *bla*_{IMI}, and *bla*_{GES}), class B (*bla*_{VIM}, *bla*_{IMP}, *bla*_{NDM}, and *bla*_{SPM}), class C (*bla*_{CMY}, *bla*_{ACT-1}, and *bla*_{DHA-1}), and class D (*bla*_{OXA-48}). The primers used in this study were described previously (1,7). Products were sequenced on an ABI PRISM 3730AXL sequencer analyzer and the results were compared to reported sequences from GenBank. A total of 113 CPE strains were detected, including isolates of *K. pneumoniae* [100], *Escherichia coli* [5], *E. cloacae* [3], *C. freundii* [2], *K. oxytoca* [1], *P. mirabilis* [1], and *S. marcescens* [1]. The prevalence rate of CPE among carbapenem-resistant *Enterobacteriaceae* isolates was 78.5% (113/144), which was higher than rates previously reported in other provinces and cities in mainland China (28.5%) (7) and Taiwan (5%) (8). The higher rate of carbapenemase production indicates that these enzymes were the main mechanism of carbapenem-resistance among *Enterobacteriaceae* isolates in Yunnan province. *Bla*_{KPC-2} was the most common type carbapenemase gene, and was present in 87.6% (99/113) of CPE. This is consistent with a previous study in other provinces and cities in mainland China, wherein 63.1% (41/65) of CPE were found to harbor the *bla*_{KPC-2} (7). In addition, 11, 10, and 1 CPE isolate (s) produced NDM-1, IMP-4, and IMP-1, respectively. Interestingly, 8 *K. pneumoniae* strains co-harbored 2 carbapenemase genes (Table 1). *K. pneumoniae* co-harboring 2 carbapenemase genes has been previously identified in China, wherein these genes were found to be co-localized on a plasmid of approximately 300 kb (9). Further research is needed to uncover the mechanism of plasmid-mediated drug resistance in CPE in Yunnan.

Among 144 *Enterobacteriaceae* strains, 31 were carbapenem-resistant but did not harbor a carbapenemase gene suggesting that other resistance mechanisms might be involved. The positive rate from the modified Hodge test of 31 strains was 77.4% (24/31), and thus, we speculated that those strains might harbor other carba-

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Table 1. The antibiogram and molecular characterization of 113 CPE strains in present study

Carbapenemase gene	Species	No. of isolates	IPM				MEM				No. of isolates co-harboring ESBL gene
			Range	MIC ₅₀	MIC ₉₀	Resistant rate (%)	Range	MIC ₅₀	MIC ₉₀	Resistant rate (%)	
<i>bla</i> _{KPC-2}	KPN	87	4– > 64	64	64	100	8– > 64	> 64	> 64	100	84
	ECO	3				100				100	2
	KOX	1				100				100	1
	PMI	1				100				100	1
	SMA	1				100				100	1
<i>bla</i> _{NDM-1}	CFR	2	4–8	8	8	100	4–8	4	8	100	1
	KPN	1				100				100	0
	ECO	1				100				100	1
<i>bla</i> _{IMP}	KPN	4	4–8	8	8	100	1–8	2	8	25	3
	ECL	3				100				67	1
	ECO	1				100				100	0
<i>bla</i> _{KPC} and <i>bla</i> _{NDM-1}	KPN	5	32–64	32	64	100	> 64	> 64	> 64	100	5
<i>bla</i> _{KPC} and <i>bla</i> _{IMP}	KPN	1	64	—	—	100	8	—	—	100	1
<i>bla</i> _{IMP} and <i>bla</i> _{NDM-1}	KPN	2	4	4	4	100	8	8	8	100	1

KPN, *K. pneumoniae*; ECO, *E. coli*; ECL, *E. cloacae*; KOX, *K. oxytoca*; PMI, *P. mirabilis*; SMA, *S. marcescens*; CFR, *C. freundii*; IPM, imipenem; MEM, meropenem; MIC, minimum inhibitory concentration; ESBL, extended-spectrum β -lactamase.

penemase genes beyond those amplified by PCR in this study. In addition, the absence or decreased expression of outer membrane protein could have contributed to the high minimum inhibitory concentration (MIC) values of carbapenems (10).

SHV- and CTX-M-type ESBLs were confirmed in 90.3% (102/113) of CPE isolates in this study. The types of ESBL strains differed according to the hospital from which they were isolated. Strains isolated from First People's Hospital of Kunming City were mainly SHV11 type; CTX-M-15 was the predominant ESBL type in the First People's Hospital of Yunnan Province and Jingdong County People's Hospital SHV12 and CTX-M-15 were common in the First Affiliated Hospital of Kunming Medical University. Taken together, the ESBLs are common in CPE isolates in Yunnan province, and SHV and CTX-M were the predominant types. AmpCs have always been detected with CPE (11), but *bla*_{ACT-1}, *bla*_{CMY}, and *bla*_{DHA-1} were not identified in this study using PCR.

The microdilution susceptibility testing method was used to further determine MICs for clinically important antimicrobial agents (12). MIC results were interpreted as specified by the M100-S23 Clinical and Laboratory Standards Institute (CLSI) document (13), except for those of tigecycline, which were interpreted as defined by the US Food and Drug Administration (susceptible: MIC ≥ 2 mg/L; resistant: MIC ≥ 8 mg/L). All 113 CPE strains were resistant to imipenem, ceftazidime, piperacillin, and piperacillin/tazobactam. Most CPE isolates were resistant to meropenem, but 3 IMP-4-producing CPE strains (2 isolates of *K. pneumoniae* and 1 of *E. cloacae*) were sensitive, and 1 IMP-4-producing *K. pneumoniae* isolate was of intermediate susceptibility. The MIC₅₀ and MIC₉₀ values of imipenem and meropenem for KPC-2-producing strains were higher than those for class B Ambler carbapenemase-producing strains including those producing IMP- and NDM-type β -lactamases in vitro (Table 1). Tigecycline is an available option for treating CPE isolates, and is even being used in pediatric patients (14). Although

none of the CPE isolates were found to be resistant against to this drug in the present study, the efficacy of tigecycline has been questioned when treating serious infections, as higher mortality was observed compared to that with carbapenem treatment (15).

It is noteworthy that 1 KPC-2-producing *K. pneumoniae* strains was detected in Jingdong County People's Hospital in this study; the presence of CPE in a country-class hospital is worrisome. CPE strains are predominantly involved in nosocomial and systemic infections, it is therefore crucial to implement efficient infection control measures to limit the spread of these pathogens.

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Conflict of interest None to declare.

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