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SUPPLEMENT ARTICLE

Phenotypic and Genotypic Characterization of Carbapenem-resistant *Enterobacteriaceae*: Data From a Longitudinal Large-scale CRE Study in China (2012–2016)

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Background. Carbapenem-resistant *Enterobacteriaceae* (CRE) strains are a major threat to global health. The development of effective control measures requires more detailed phenotypic and genotypic characterization of CRE.

Methods. CRE isolates were collected from 65 hospitals in 25 provinces across China between January 1, 2012, and December 31, 2016. The isolates were characterized by antimicrobial susceptibility testing and multilocus sequence typing. Genes encoding carbapenemases, mobilized colistin resistance (*mcr-1*), and β-lactamases were detected by polymerase chain reaction and DNA sequencing.

Results. A total of 1801 independent CRE isolates (1201 *Klebsiella pneumoniae*, 282 *Escherichia coli*, and 179 *Enterobacter cloacae*) were collected during the study period. Overall, 96.9%, 89.7%, 54.5%, 49.9%, and 40% of CRE strains were susceptible to colistin, tigecycline, amikacin, minocycline, and fosfomycin, respectively. Notably, 1091/1201 (91%) *K. pneumoniae*, 225/282 (80%) *E. coli*, and 129/179 (72%) *E. cloacae* harbored carbapenemase gene. *K. pneumoniae* carbapenemase (KPC) was predominant in *K. pneumoniae* (77%), whereas New Delhi metallo-β-lactamase (NDM) was predominant in *E. coli* (75%) and *E. cloacae* (53%). The *mcr-1* gene was detected in 13 NDM-carrying *E. coli* isolates (4.6%). Sequence type (ST)11 and ST167 were predominant among the 100 *K. pneumoniae* and 47 *E. coli* STs, respectively. KPC-ST11, which accounted for 64% of *K. pneumoniae* isolates, had higher levels of resistance than non-ST11 strains to aztreonam, fosfomycin, and amikacin ($P < .001$). The proportions of KPC and NDM enzymes in CRE increased from 2012 to 2016 (54%–59% and 12%–28%, respectively).

Conclusions. The number of CRE strains harboring carbapenemase is increasing. KPC-ST11 *K. pneumoniae*, the predominant strain, shows a reduced susceptibility to most available antibiotics.

Keywords. carbapenem-resistant *Enterobacteriaceae*; molecular epidemiology; KPC-2; NDM; carbapenemases.

Enterobacteriaceae are opportunistic pathogens that cause severe nosocomial infections, including bloodstream and abdominal infections and pneumonia. The emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) poses a global healthcare challenge. Infections caused by these so-called superbugs are associated with high mortality because therapeutic options are limited [1–4]. In recent decades, sporadic CRE events and outbreaks have been reported in many countries and regions, including China [5–7].

There are 2 major carbapenem-resistance mechanisms in *Enterobacteriaceae*: the production of carbapenemase or of extended-spectrum β-lactamase (ESBL) and/or AmpC cephalosporinase (AmpC) in combination with membrane impermeability and active efflux [8–10]. The first bla_{KPC}-positive *Klebsiella pneumoniae* isolate recorded in China was identified in 2004 in Zhejiang Province [11]. Since then, bla_{KPC}-positive *Enterobacteriaceae* have been reported in different regions of China. Our surveillance during 2004–2008 showed that the main resistance mechanism of CRE was the loss or reduced expression of porin proteins, along with ESBL or AmpC production [12]. However, in the last 10 years, the prevalence of CRE strains producing carbapenemase has increased, especially among *K. pneumoniae* and *Escherichia coli* [13, 14]. In the United States and in European countries, *K. pneumoniae* ST258 has contributed significantly to the dissemination of

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bla$_{KPC}$-positive *K. pneumoniae* [5], although sequence type (ST)11 is predominant in China [15].

Currently, there is no comprehensive CRE monitoring network in China for CRE epidemiology and antimicrobial resistance surveillance. A longitudinal large-scale CRE study can be useful for controlling nosocomial infections, as it can provide a basis for the development of new detection methods and treatment measures. In the present study, we investigated the status of the major strains, carbapenemase types, STs, and antimicrobial resistance characteristics of CRE strains in China.

MATERIALS AND METHODS

CRE Network

Our research group established a CRE network to investigate the epidemiology of CRE in China starting from 2014. There were 2 stages to this study: first, from 2012 to 2013, we collected 150 CRE isolates from 16 tertiary hospitals, and second, starting in 2014, we expanded the collection area. The number of participating hospitals increased from 25 in 2014 to 65 in 2016. Peking University People's Hospital was the lead unit in this project and was responsible for the collection, identification, and sorting of isolates.

Bacterial Isolates

From January 1, 2012, to December 31, 2016, we collected 1801 non-repetitive clinical CRE isolates from 65 hospitals in 25 provinces and municipalities across China. During the study period, *Enterobacteriaceae* isolates resistant to any carbapenem (imipenem, meropenem, or ertapenem), as determined by standard methods, were obtained from individual patients at participating hospitals. The provinces and municipalities were distributed throughout Northern China (including Inner Mongolia, Beijing, Shanxi, Hebei, and Tianjin), Eastern China (including Anhui, Jiangsu, Shandong, Fujian, Shanghai, and Zhejiang), Southern China (including Guangdong), Central China (including Hunan, Hubei, and Henan), Northeastern China (including Jilin, Liaoning, and Heilongjiang), Northwestern China (Gansu, Ningxia, Xinjiang, and Shaanxi), and Southwestern China (including Chongqing and Yunnan). All isolates were reidentified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Daltonik, Bremen, Germany) at Peking University People's Hospital and were stored at −80°C for antimicrobial susceptibility testing and investigation of resistance mechanisms.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was evaluated by the agar dilution and microdilution methods at Peking University People's Hospital according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (M07-A9, 2012), and the results were interpreted according to CLSI categories and minimum inhibitory concentration (MIC) breakpoints [16]. The breakpoint of tigecycline for *Enterobacteriaceae* was based on the US Food and Drug Administration standard. The breakpoint of cefoperazone/sulbactam for *Enterobacteriaceae* was referred to cefoperazone in CLSI. The antibiotics cefoxitin, cefotaxime, ceftriaxone, ceftazidime, cefepime, piperacillin/tazobactam, cefoperazone/sulbactam, ertapenem, imipenem, meropenem, amikacin, ciprofloxacin, colistin, fosfomycin, chloramphenicol, and levofloxacin were tested by the agar dilution method. Tigecycline was tested by the broth microdilution method. *Pseudomonas aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as quality control standards for antimicrobial susceptibility testing.

Investigation of Resistance Mechanisms

For all CRE strains, polymerase chain reaction (PCR) was used to detect genes encoding carbapenemases (*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{SIM}$, and *bla*$_{OXA-48}$), ESBLs, and AmpC β-lactamases (*bla*$_{CTX-M}$, *bla*$_{DHA}$, and *bla*$_{CMY}$), as previously described [17–23]. The colistin resistance gene *mcr*-1 was also detected by PCR, as previously described [24]. PCR products were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) and sequenced by Sanger sequencing on an ABI PRISM 3730XL system (Applied Biosystems, Foster City, CA, USA).

Multilocus Sequence Typing (MLST)

MLST of *K. pneumoniae* was performed according to the protocol described on the Pasteur Institute MLST website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). *E. coli* MLST was performed as described on the EnteroBase website (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). *Enterobacter cloacae* MLST was performed as described on the *E. cloacae* MLST databases website (https://pubmlst.org/ecloacae/). The sequences of 7 housekeeping genes were compared with those in the MLST databases.

Statistical Analysis

Data were analyzed using SPSS v.19.0 software (SPSS Inc., Chicago, IL, USA). For categorical data, different groups were compared using the χ^2 test. A P value < .05 was considered statistically significant. Susceptibility data were analyzed using WHONET v.5.6 (http://www.whonet.org/contact.html).

RESULTS

Distribution of Isolates

Of the 1801 CRE isolates, *K. pneumoniae* was the most abundant species (n = 1201), followed by *E. coli* (n = 282), *E. cloacae* (n = 179), *Citrobacter freundii* (n = 44), *Klebsiella oxytoca* (n = 29), *Serratia marcescens* (n = 28), *Enterobacter aerogenes* (n = 24), *Raoultella ornithinolytica* (n = 6), *Citrobacter braakii* (n = 3), *Citrobacter koseri* (n = 3), and *Raoultella planticola* (n = 2). The proportion of *K. pneumoniae* was 78% in 2012, and it increased from 46.8% in 2013 to 70.9% in 2016 (Supplementary Figure S1). The abundance of other species

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changed little over the years. *K. pneumoniae* accounted for the highest proportion of all specimen types, ranging from 44.4% from wounds to 74.4% from the respiratory tract (Supplementary Table S1). Most of the isolates were obtained from the respiratory tract (47.6%, 858/1801), followed by blood (16.9%, 304/1801), urine (16.8%, 303/1801), and abdominal fluid (6.5%, 117/1801).

Antimicrobial Susceptibility Testing Results

Antimicrobial susceptibility findings of the major species are shown in Table 1. The 1801 CRE isolates showed high susceptibility to colistin (96.9%), followed by tigecycline (89.7%), amikacin (54.5%), minocycline (49.9%), fosfomycin (40%), and chloramphenicol (26.9%). The tested strains showed low susceptibility to carbapenem (<12.7%). Fewer than 7.6% of the CRE strains were susceptible to third- or fourth-generation cephalosporins and β-lactam combination agents (eg, cefoperazone/sulbactam and piperacillin/tazobactam). There were clear interspecies variations in susceptibility; for example, *E. coli* was less susceptible to colistin than other species (93.9% vs 97.1%–98.5%). The susceptibility rate of *K. pneumoniae* to amikacin was 42.2%, compared with >74% for *E. coli*, *E. cloacae*, and *C. freundii*. Only 21% of *K. pneumoniae* isolates were susceptible to fosfomycin, compared with >76% of the *E. coli*, *E. cloacae*, and *C. freundii* isolates.

Screening for Carbapenemase and Other Antimicrobial Resistance Genes

Of the 1801 CRE isolates, 1544 (85.7%) were found to produce carbapenemases (Table 2). *K. pneumoniae* was the most abundant carbapenemase-producing species (1091/1201, 90.8%), followed by *C. freundii* (38/44, 86.4%), *E. coli* (225/282, 79.8%), and *E. cloacae* (111/179, 62%). *K. pneumoniae* carbapenemase (KPC)-2 was the most common carbapenemase type in both *K. pneumoniae* (919/1201, 76.5%) and *Serratia marcescens* (14/28, 50%); New Delhi metallo-β-lactamase (NDM)-5 was the most common type in *E. coli* (147/282, 52.1%); and NDM-1 was the most common type in *E. cloacae* (90/179, 50.3%), *C. freundii* (24/44, 54.5%), and *K. oxytoca* (14/29, 48.3%). Only 6/1801 strains were found to express 2 types of carbapenemase (*K. pneumoniae* and *C. freundii*, n = 2; *E. cloacae* and *K. oxytoca*, n = 1). The carbapenemase OXA-48 was rare in China (2/1801, 0.1%), with only 2 *K. pneumoniae* isolates harboring the corresponding gene. However, 740/1201 *K. pneumoniae* isolates were found to harbor genes encoding ESBLs (mainly CTX-M-65 and CTX-M-14), whereas only 14.4% harbored AmpC genes (DHA-1 and ACT-20). Only 39.4% and 13.8% of *E. coli*, and 16.2% and 24.6% of *E. cloacae* isolates carried ESBL and AmpC genes, respectively. The carbapenemase types in CRE differed across regions throughout China (Supplementary Tables S2–S4). The frequency of KPC in *K. pneumoniae* increased from 61.5% in 2012 to 80.1% in 2016, whereas that of NDM in *E. coli* and *E. cloacae* increased from 20.8%–25% in 2013 to 84.1%–52.6% in 2016 (Figure 1). The colistin resistance gene *mcr-1* was detected in 13/282 (4.6%) *E. coli* isolates in this study and coexisted with NDM-5 in one strain. We did not detect the *mcr-1* gene in other CRE species.

Carbapenem MIC Distribution of Different Carbapenemases

The MICs of the 3 carbapenemases identified in the isolates are shown in Figure 2. The distribution of imipenem and meropenem MICs for NDM-positive strains was 8 mg/L (28.5%–28.7%), followed by 4 mg/L (22.1%–23.6%), and 16 mg/L (15.4%–18.6%). Over 96% of the NDM strains had an MIC >2 mg/L for ertapenem. The most frequently observed MIC of meropenem and ertapenem in KPC-positive strains was ≥16 mg/L (86.6%–76.7%). Fewer than 3.5% of the KPC-producing strains were in the carbapenem-susceptible range. The most frequent imipenem and meropenem MIC for IMP-positive strains was 1 mg/L (24.6%–32.3%). Only 1.5% of IMP-producing strains had high imipenem and meropenem MICs (>32 mg/L).

Geographic Distribution of STs and Carbapenemase-producing CRE Strains

A total of 100 *K. pneumoniae* ST types were classified (Table 3). ST11 was the most prevalent ST in China (790/1201, 65.8%), followed by ST17 (35/1201, 2.9%), ST15 (31/1201, 2.6%), and ST48 (22/1201, 1.8%). *K. pneumoniae* ST types were geographically diverse. ST11 was the most prevalent type in Northern (306/408, 75%), Eastern (268/416, 64.4%), Southern (94/135, 69.6%), Central (75/114, 65.8%), Northeastern (15/29, 51.7%), and Southwestern (18/26, 69.2%) China. ST17 (19/73, 26.0%) was the most prevalent ST in Northwestern China, whereas ST11 accounted for 19.2% of *K. pneumoniae* isolates in this area (Figure 3).

There were 47 different *E. coli* STs (Table 3). The most prevalent type was ST167 (69/282, 24.5%), followed by ST410 (31/282, 11.0%), ST617 (13/282, 4.6%), and ST131 (12/282, 4.3%). ST167 was the most prevalent type in Northern (15/67, 22.4%), Eastern (33/105, 31.4%), Southern (5/41, 12.2%), and Northwestern (12/26, 46.2%) China. The most prevalent ST in the Northeast was ST410 (6/13, 46.0%) (Figure 3). There were 52 different *E. cloacae* STs (Table 3); ST74 was the most prevalent type (15/179, 8.4%), followed by ST418 (12/179, 6.7%), ST256 (9/179, 5.0%), and ST754 (6/179, 3.4%).

Relationship Between ST and Carbapenemase Genes

Most *K. pneumoniae* ST11 isolates (96.2%) produced KPC-2 carbapenemase (Table 3), whereas ST17 isolates were more likely to produce NDM carbapenemase (91.4%). ST11-KPC-2 *K. pneumoniae* was the most prevalent carbapenem-resistant *K. pneumoniae* (CRKP) strain in China. Most *E. coli* isolates harbored only NDM; only a few ST167 and ST 131 isolates carried KPC. ST167-NDM isolates accounted for 21.6% of all carbapenem-resistant *E. coli* isolates. Most *E. cloacae* isolates harbored NDM, and all ST418 isolates produced NDM. VIM was found in 4 *E. cloacae* isolates.

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Table 1. Antimicrobial Susceptibility Testing Results of CRE Isolates

	All Strains (n = 1801)				*Klebsiella pneumoniae* (n = 1201)				*Escherichia coli* (n = 282)				*Enterobacter cloacae* (n = 179)				*Citrobacter freundii* (n = 42)			
Antibiotic Name	%R	%S	MIC_{50}	MIC_{90}	%R	%S	MIC_{50}	MIC_{90}	%R	%S	MIC_{50}	MIC_{90}	%R	%S	MIC_{50}	MIC_{90}	%R	%S	MIC_{50}	MIC_{90}
Amikacin	44.8	54.5	8	>256	57.5	42.2	>256	>256	24.4	74.9	2	>256	12.1	86.8	2	>256	14.3	78.6	2	>256
Aztreonam	89	9.6	>256	>256	95.7	3.7	>256	>256	76.6	19.7	256	>256	70.5	28.8	64	>256	71	25.8	32	>256
Cefepime	90.2	4	64	256	93.5	2.3	64	256	94.6	1.8	256	>256	75.9	10.9	32	128	83.3	2.4	64	256
Cefoperazone/Sulbactam	90.7	4.8	>256	>256	94.6	2.8	>256	>256	90.6	3.6	>256	>256	74.7	14.4	256	>256	88.1	4.8	>256	>256
Cefotaxime	98.4	1.2	256	>256	98.5	1	256	>256	99.6	0.4	>256	>256	97.1	2.9	256	>256	100	0	256	>256
Cefotaxime/Clavulanic acid	95.1	4	128	>256	95.1	4.1	128	256	96.4	3.6	>256	>256	95.4	2.9	256	>256	100	0	256	>256
Cefoxitin	96.1	2.2	>256	>256	95.4	2.7	256	>256	97.5	0.7	>256	>256	98.8	0.6	>256	>256	100	0	>256	>256
Ceftazidime	95.2	3.2	256	>256	96.1	2.6	256	>256	97.5	0.7	>256	>256	93.7	5.7	>256	>256	97.6	0	>256	>256
Ceftazidime/Clavulanic acid	91.6	6.8	128	>256	92.6	5.8	64	>256	90.9	7.6	>256	>256	93.6	6.4	>256	>256	95.2	0	>256	>256
Ceftriaxone	98.5	1.3	>256	>256	98.8	1.1	>256	>256	99.6	0.4	>256	>256	97.1	2.3	>256	>256	100	0	>256	>256
Chloramphenicol	64.5	26.9	64	>256	70.3	21.5	64	>256	57.4	36	32	>256	57	34.9	32	>256	38.1	52.4	8	256
Ciprofloxacin	80.6	16.7	64	128	86.3	12	64	128	87.8	11.5	64	128	50.9	41.6	4	128	64.3	33.3	16	64
Ertapenem	92.2	3.8	64	64	94.6	2.8	64	64	92.7	3.4	64	64	82.3	6.5	8	64	89.7	3.4	16	64
Fosfomycin	52.1	40	256	>256	69.6	21	>256	>256	21.3	76.4	2	>256	11.8	81	32	256	17.5	82.5	1	256
Imipenem	80.2	12.7	16	64	86.8	8.6	16	64	69.9	16.1	4	32	56.9	29.3	4	32	69	19	8	32
Levofloxacin	77.7	19.4	32	128	84.3	14	32	128	86.7	12.2	16	64	46	47.7	4	64	54.8	38.1	8	32
Meropenem	80.9	12.5	32	64	87.1	7.7	64	64	75.6	13.6	8	32	56.3	35.1	4	32	69	23.8	8	32
Minocycline	32.5	49.9	8	32	31.1	50.2	4	32	31.4	51.3	4	32	46	45.4	8	128	35.7	50	4	32
Piperacillin/Tazobactam	87.9	7.6	>256	>256	92.8	5.1	>256	>256	88.8	5	>256	>256	67.8	21.8	256	>256	78.6	19	>256	>256
Colistin	2.5	96.9	0.25	0.5	1.4	98.5	0.25	0.5	4	93.9	0.25	0.5	2.9	97.1	0.25	0.5	2.4	97.6	0.25	0.5
Tigecycline	3.5	89.7	1	4	3.1	89.4	1	4	0.7	96.8	0.5	1	10.3	79.9	1	6	0	95.2	0.5	1

Abbreviations: CRE, carbapenem-resistant *Enterobacteriaceae*; $MIC_{50/90}$, 50%/90% minimum inhibitory concentration.

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Table 2. Prevalence of Resistant Genes Harbored by All CRE Strains

Organism	No.	Carbapenemase					Total	ESBL	AmpC	mcr-1
		KPC	NDM	IMP	Other	Two types of Carbapenemase				
Klebsiella pneumoniae	1201	KPC-2 (919, 76.5%); KPC-12 (6, 0.5%); KPC-24 (1, 0.1%)	NDM-1 (112, 9.3%); NDM-5 (21, 1.7%); NDM-7 (2, 0.2%); NDM-9 (1, 0.1%)	IMP-1 (2, 0.2%); IMP-4 (14, 1.2%); IMP-24 (2, 0.2%); IMP-26 (3, 0.2%)	OXA-48 (2, 0.2%); SIM-1 (2, 0.2%); VIM-1 (2, 0.2%);	2[a], 0.2%	1091, 90.8%	740, 61.6%	173, 14.4%	
Escherichia coli	282	KPC-2 (9, 3.2%)	NDM-1 (52, 18.4%); NDM-4 (6, 2.1%); NDM-5 (147, 52.1%); NDM-7 (1, 0.4%); NDM-9 (5, 1.8%)	IMP-4 (5, 1.8%)			225, 79.8%	111, 39.4%	39, 13.8%	13, 4.6%
Enterobacter cloacae	179	KPC-2 (6, 3.4%)	NDM-1 (90, 50.3%); NDM-5 (4, 2.2%)	IMP-1 (3, 1.7%); IMP-4 (18, 10.1%); IMP-26 (3, 1.7%)	VIM-1 (4, 2.2%);	1[b], 0.5%	129, 72.1%	29, 16.2%	44, 24.6%	
Citrobacter freundii	44	KPC-2 (5, 11.4%)	NDM-1 (24, 54.5%)	IMP-4 (7, 15.9%)		2[c], 4.5%	38, 86.4%	16, 36.4%	21, 47.7%	
Klebsiella oxytoca	29	KPC-2 (5, 17.2%)	NDM-1 (14, 48.3%)	IMP-1 (2, 6.9%); IMP-4 (6, 20.7%)		1[d], 3.4%	28, 96.6%	10, 34.5%	3, 10.3%	
Serratia marcescens	28	KPC-2 (14, 50%)					14, 50%	1, 3.6%	1, 3.6%	
Enterobacter aerogenes	24	KPC-2 (1, 4.2%)	NDM-1 (5, 20.8%)				6, 25%	9, 37.5%	5, 20.8%	
Raoultella ornitholytica	6	KPC-2 (2, 33.3%)	NDM-1 (3, 50%)				5, 83.3%	2, 33.3%	2, 33.3%	
Citrobacter braakii	3		NDM-1 (3, 100%)				3, 100%	2, 66.7%		
Citrobacter koseri	3		NDM-1 (3, 100%)				3, 100%			
Raoultella planticola	2		NDM-1 (2, 100%)				2, 100%	2, 100%		
Total	1801	961, 53.4%	495, 27.5%	65, 3.6%	10, 0.6%	6, 0.3%	1544, 85.7%	922, 51.2%	290, 16.1%	13, 0.7%

Abbreviations: AmpC, AmpC cephalosporinase; CRE, carbapenem-resistant *Enterobacteriaceae*; ESBL, extended-spectrum β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; *mcr*-1, colistin resistance gene; VIM, Verona integron-encoded metallo-β-lactamase.

[a]KPC-2 + NDM-1; [b]NDM-5 + IMP-26; [c]KPC-2 + IMP-1; [d]NDM-1 + IMP-26.

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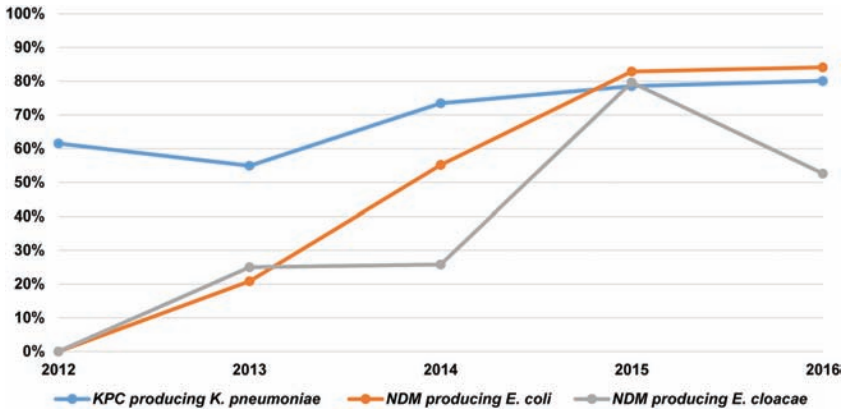

Figure 1. Major carbapenemases found in CRE species by year. Abbreviation: CRE, carbapenem-resistant *Enterobacteriaceae*.

Antimicrobial Susceptibility of *K. pneumoniae* ST11 and *E. coli* ST167

The susceptibility of *K. pneumoniae* ST11 to drugs other than colistin and minocycline was lower than that of non-ST11 isolates (Figure 4). The 2 groups of bacteria differed significantly in terms of susceptibility to aztreonam (0% vs 12.5%, $P < .001$), fosfomycin (3.6% vs 59.5%, $P < .001$), ciprofloxacin (0.4% vs 35.7%, $P < .001$), levofloxacin (0.5% vs 41.6%, $P < .001$), amikacin (0.3% vs 26.9%, $P < .001$), and chloramphenicol (9.9% vs 14.1%, $P < .001$). There were no statistically significant differences between ST167 *E. coli* isolates and non-ST167 *E. coli* isolates with respect to susceptibility to meropenem, chloramphenicol, minocycline, fosfomycin, colistin, and tetracycline (Figure 5). In contrast, ST167 was less susceptible than non-ST167 *E. coli* to amikacin (58.0% vs 80.5%, $P < .001$), aztreonam (9.0% vs 23.8%, $P = .006$), imipenem (8.7% vs 18.6%, $P = .049$), ciprofloxacin (0% vs 15.3%, $P < .001$), and levofloxacin (0% vs 16.2%, $P < .001$).

DISCUSSION

The continual emergence of CRE strains is a major threat to public health worldwide. The China Antimicrobial Resistance Surveillance Report (http://www.carss.cn/), the largest survey of antimicrobial resistance in China, reported that the rate of carbapenem resistance in *K. pneumoniae* increased from 6.4% in 2014 to 8.7% in 2016, whereas in *E. coli*, the rate remained stable at under 2% over this period. The results of our study reflect this trend, revealing that *K. pneumoniae* accounts for the largest percentage of domestic CRE strains and is the fastest growing species. The number of deaths attributable to CRE is not insignificant [25]; patients with CRE infection, especially bloodstream infection, have high mortality rates [26, 27]. In this study, a significant portion of isolates were obtained from blood specimens, suggesting that CRE bloodstream infection is a major problem.

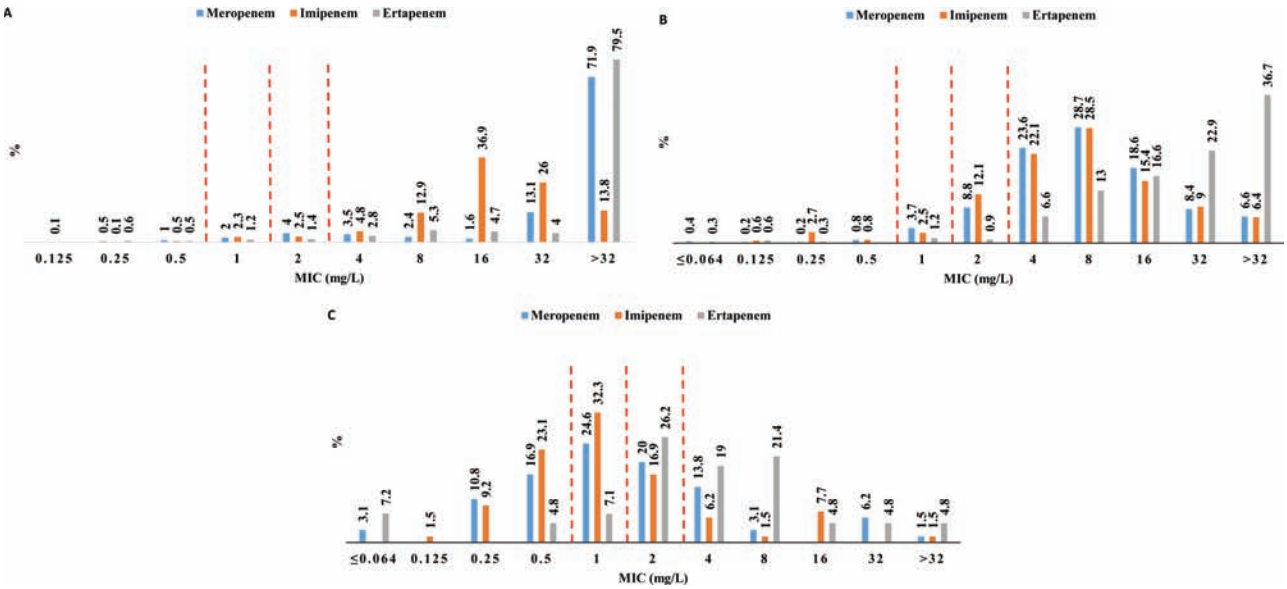

Figure 2. Carbapenem MIC distribution among strains harboring different carbapenemase genes. MIC distribution among (*A*) bla_{KPC}-positive (n = 972), (*B*) bla_{NDM}-positive (n = 499), and (*C*) bla_{IMP}-positive (n = 67) CRE strains. Abbreviations: CRE, carbapenem-resistant *Enterobacteriaceae*; MIC, minimum inhibitory concentration.

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Table 3. Carbapenemase Distribution Determined by CRE MLST

	ST	No.	IMP	KPC	NDM	OXA-48	SIM-1	VIM-1
Klebsiella pneumoniae	ST11	790	1 (0.1)	767 (97.1)	8 (1)			
(n = 1201)	ST17	35		3 (8.6)	32 (91.4)			
	ST15	31		23 (74.2)	1 (3.2)			
	ST48	22		19 (86.4)	1 (4.5)			
	ST37	11			7 (63.6)			
	ST290	9		6 (66.7)	1 (11.1)			
	ST147	7	1 (14.3)	2 (28.6)	3 (42.9)			
	ST1	6			4 (66.7)			
	ST895	6			6 (100)			
	ST23	6		3 (50)	2 (33.3)			
	Other STs	278	19 (6.8)	103 (37.1)	71 (25.5)	2 (0.7)	2 (0.7)	2 (0.7)
Escherichia coli	ST167	69		1 (1.4)	61 (88.4)			
(n = 282)	ST410	31			30 (96.8)			
	ST617	13	1 (7.7)		12 (92.3)			
	ST131	12		3 (25)	5 (41.7)			
	ST405	12			5 (41.7)			
	ST361	10			8 (80)			
	ST46	9			6 (66.7)			
	ST10	7			7 (100)			
	ST156	4			4 (100)			
	ST354	4			3 (75)			
	Other STs	111	4 (3.6)	5 (4.5)	70 (63.1)			
Enterobacter cloacae	ST74	15	1 (6.7)		6 (40)			
(n = 179)	ST418	12			12 (100)			
	ST256	9	2 (22.2)		6 (66.7)			1 (11.1)
	ST175	6			4 (66.7)			2 (33.3)
	ST754	6	1 (16.7)		4 (66.7)			
	ST88	6			5 (83.3)			
	ST111	5	1 (20)		4 (80)			
	ST145	5	1 (20)		4 (80)			
	ST51	5			4 (80)			
	ST66	3		1 (33.3)	2 (66.7)			
	Other STs	107	18 (16.8)	5 (4.7)	43 (40.2)			1 (0.9)

Abbreviations: CRE, carbapenem-resistant *Enterobacteriaceae*; KPC, *Klebsiella* pneumoniae carbapenemase; MLST, multilocus sequence typing; NDM, New Delhi metallo-β-lactamase; ST, sequence type; VIM, Verona integron-encoded metallo-β-lactamase.

Treatment of CRE infections is challenging, and some of the few effective drugs, so far, such as ceftazidime-avibactam and colistin, are not available in many countries, including China. Individualized therapy must be used to treat CRE infections based on *in vitro* antimicrobial susceptibility profiles, molecular type, infection severity, and the patient's health status [28]. Our study describes the antimicrobial susceptibility profiles and molecular epidemiological characteristics of CRE in China, which can inform the choice of treatment. For example, we found that colistin and tetracycline have high activity against CRE in China, which is consistent with surveillance data from the United States [29] and European countries [30]. The susceptibility of different CRE species to fosfomycin varied, with a lower rate in *K. pneumoniae* (21%) than in *E. coli*, *E. cloacae*, and *C. freundii* (>76%). Over 74% of the isolates of the latter three species were susceptible to amikacin. This result provides important data for selecting specific drug and aminoglycoside combinations for the empiric therapy of infections caused by these species.

Carbapenemase-producing CRE strains carry resistance genes on mobile plasmids that can shuttle between resistant and susceptible strains [31, 32]. The dissemination of mobile resistance genes, especially those encoding KPC and NDM, is a major reason for the increase in nosocomial and community infections caused by CRE. CRKP strains harboring KPC are prevalent in the United States, Israel, Romania, Greece, Italy, and some parts of the Mediterranean region [33]. Our data showed that KPC was the main drug resistance factor of CRKP in most regions of China (found in 76% of isolates). Only CKRP from the Northwest produced a high level of NDM. CRKP strains in some European countries, such as France and Turkey, are more affected by OXA-48-like carbapenemases [5, 34]. However, OXA-48 was rare in China, with only 2 isolates from the Eastern region found to be OXA-48 carriers. It was reported that OXA-48-producing *K. pneumoniae* and *E. coli* were isolated from a female patient in Eastern China who had returned from Europe [35].

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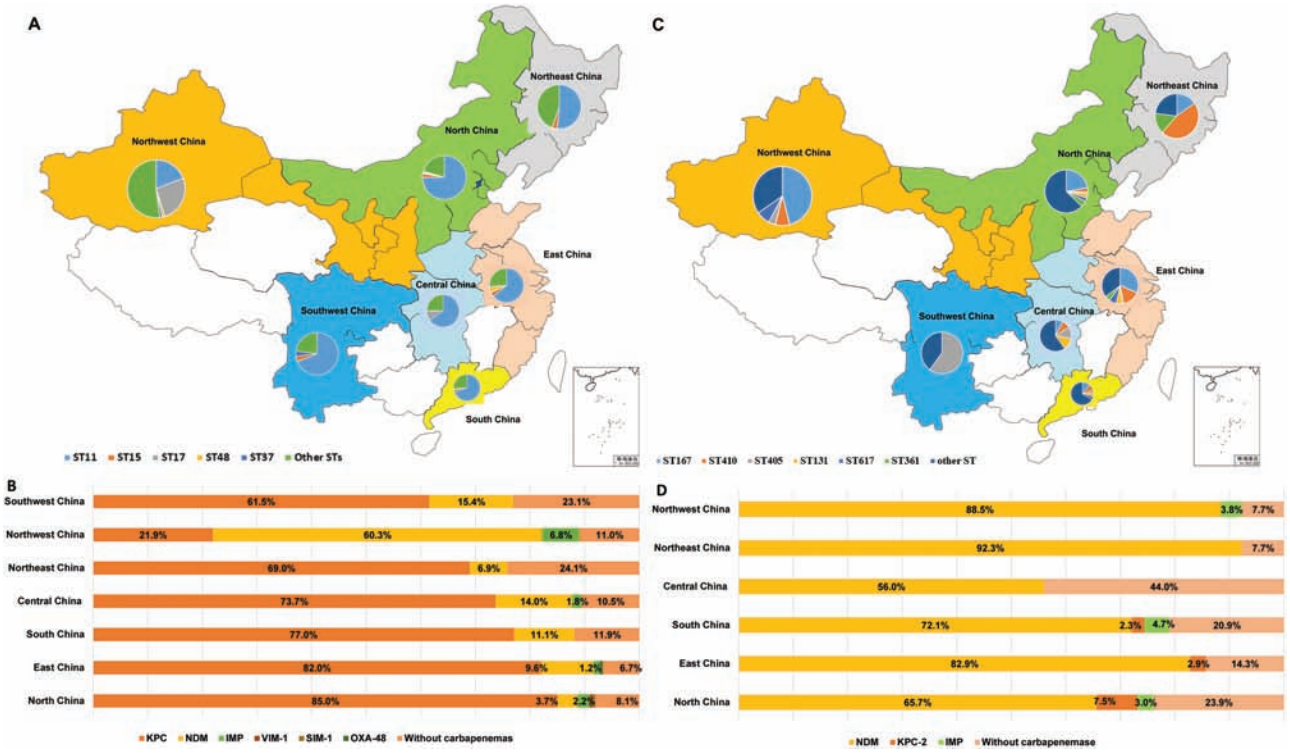

Figure 3. Distribution of STs of *K. pneumoniae* and *E. coli* and carbapenemase production in different regions of China. Distribution of (*A*) *K. pneumoniae* STs and (*B*) carbapenemase-producing *K. pneumoniae* strains (B) in different regions of China. Distribution of (*C*) *E. coli* STs and (*D*) carbapenemase-producing *E. coli* strains in different regions of China. Abbreviation: ST, sequence type.

Although the positive rate of ESBL genes was >60% in CRKP in this study, we were unable to confirm this by phenotypic testing because the ESBL phenotype is no longer sensitive to carbapenemase. Accordingly, the incidence of ESBL-positive *K. pneumoniae* has been declining for years, according to the CHINET survey [36]. Asian countries such as India are a major reservoir of NDM producers. Our study showed that NDM was the main mechanism of carbapenem resistance in *E. coli* and *E. cloacae*. Compared with the results of previous studies in China [12, 13], the prevalence of KPC and NDM carbapenemases in CRE increased between 2012 and 2016, whereas that of IMP decreased. Particular attention should be paid to the

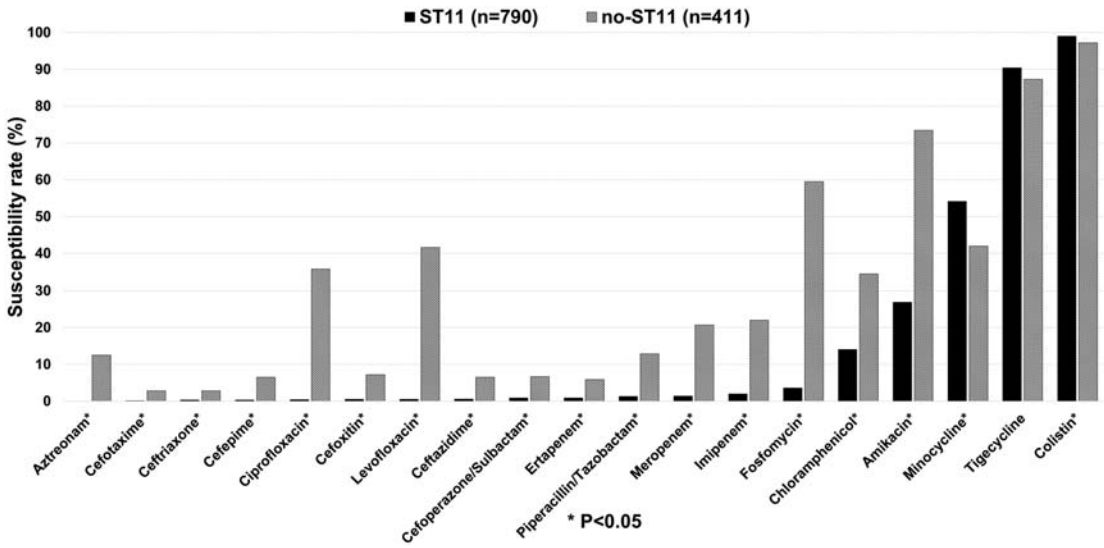

Figure 4. Comparison of susceptibility between ST11 and non-ST11 *K. pneumoniae* isolates.

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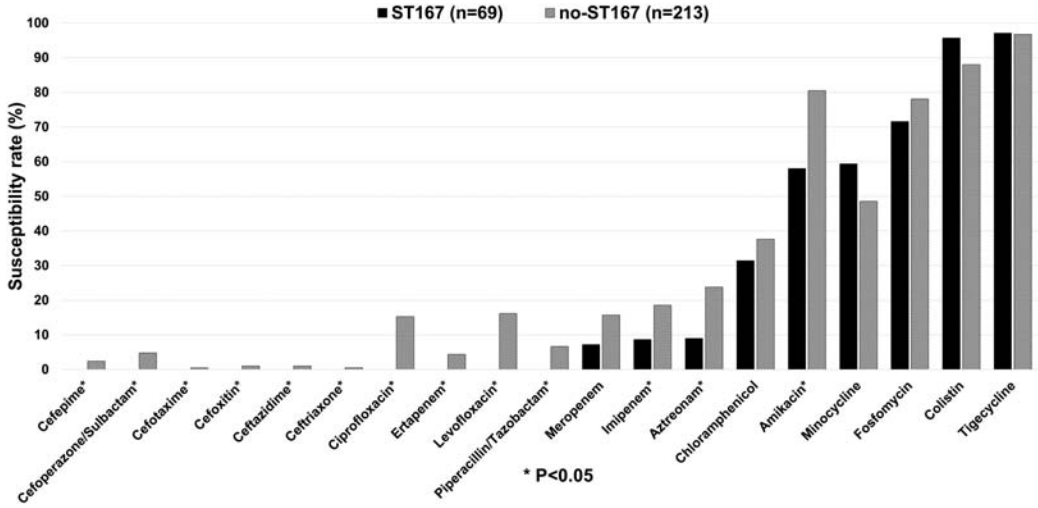

Figure 5. Comparison of susceptibility between ST167 and non-ST167 *E. coli* isolates.

high proportion of NDM-5 coexisting with *mcr*-1 in the same *E. coli* strain [37]. The *mcr-1* gene is particularly abundant in China [38–40] and can be transferred between strains.

The main ST was, and still is, derived from the clonal expansion of *K. pneumoniae* ST258, which has become prevalent in many parts of the world [5] since it was first detected in the United States [41]. However, our data showed that ST11 was the most abundant *K. pneumoniae* ST type in China. Most ST11 CRKP isolates carried KPC-2 carbapenemase. Moreover, ST11 showed a higher resistance than non-ST11 strains. We believe that the most abundant *K. pneumoniae* strain in China is ST11-KPC, which should be the focus of infection control measures and clinical studies. Other STs, such as ST17, are likely to harbor NDM carbapenemase and cause local epidemics. The most common clone of *E. coli* in China was ST167, which also showed higher resistance than non-ST167 strains. This result is in disagreement with an earlier report stating that ST131 was the most prevalent strain [15]. Moreover, *E. coli* ST varied across regions; specifically, ST167 was the most prevalent ST in Northern, Eastern, Southern, and Northwestern China, whereas ST167 was predominant in Northern China.

Carbapenemases differ in terms of hydrolytic activity, with class B β-lactamases exhibiting the highest activity [42, 43]. However, in our study, most IMP-producing strains had imipenem and meropenem MIC values <4 mg/L. Therefore, carbapenems may be clinically effective against IMP-producing strains [44]. The cutoff for the sentinel drug for the detected carbapenemase is controversial. We found that >50% of IMP-producing strains had low imipenem and meropenem MICs (≤1 mg/l). Thus, cutoff values of 0.25 mg/L for meropenem and imipenem and 0.5 mg/L for ertapenem can be used to screen for carbapenemase in *Enterobacteriaceae*.

This study had some limitations. Some hospitals did not preserve all CRE strains, resulting in the loss of some data. Additionally, we did not carry out a detailed analysis of drug-resistant plasmids, which should be examined at later stages by second- or third-generation sequencing.

In conclusion, to our knowledge this is the first longitudinal large-scale CRE surveillance of CRE in China, covering 25 provinces and municipalities. Our major findings were as follows: (1) CRE caused by carbapenemase production is increasing; (2) KPC and NDM are the major carbapenemases produced by CRE in China, and their proportions among carbapenemases are increasing; (3) *K. pneumoniae* ST11 and *E. coli* ST167 are the most abundant CRE strains in China and have a reduced susceptibility to available antibiotics; and (4) the MICs of meropenem and imipenem (0.25 mg/L) and of ertapenem (0.5 mg/L) can serve as cutoff values for screening for carbapenemases in *Enterobacteriaceae*. These results provide a basis for developing more effective measures for controlling the spread of CRE.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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