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Prevalence of Carbapenem-Resistant *Enterobacteriaceae* in Seoul, Korea

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The prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) is increasing globally. However, a few studies have addressed their epidemiology in Seoul, Korea. In this study, we conducted one-year surveillance of CRE among 1,468 clinical isolates of *Enterobacteriaceae* at the hospital in Seoul with molecular characterization of carbapenemase genes. About 85% of CRE-positive samples were isolated from the elderly age group (above 60 years). The most common isolated organisms were *Klebsiella pneumoniae (K. pneumoniae)* (56.5%) and *Escherichia coli (E.coli)* (17.0%). We detected six different Carbapenemase-producing *Enterobacteriaceae* (CPE) of bla_{KPC}, bla_{NDM}, bla_{OXA}, bla_{VIM}, bla_{IMP}, and bla_{GES} alone or in combination with other *bla* genes. Typically, 853 (58.1%) isolates were tested positive for at least one CPE. KPC (*K. pneumoniae* carbapenemase)-2 was the most common CPE type (46.0%) followed by NDM (New Delhi metallo-β-lactamase)-1 (5.9%). KPC-2 was most commonly found in *K. pneumoniae* (494/676 isolates [73.1%]) and *E.coli* (107/676 isolates [15.8%]), whereas NDM-1 was commonly found in *Enterobacter cloacae* complex (20/86 isolates [23.3%]). Detailed information and molecular characteristics of CPE is essential to prevent the spread of these pathogens.

Key Words: Carbapenem-resistant *Enterobacteriaceae* (CRE), Carbapenemase-producing *Enterobacteriaceae* (CPE), *Klebsiella pneumoniae*

No potential conflict of interest relevant to this article was reported.

INTRODUCTION

Carbapenem-resistant *Enterobacteriaceae* (CRE) is an emerging problematic infectious agent, with reports of its prevalence worldwide (1-3). CRE outbreak in hospitals has become a critical issue. Transmission by patients with CRE, as well as carriers of CRE, contribute significantly to in-hospital CRE transmission (4). Carbapenemase-producing *Enterobacteriaceae* (CPE) are an important and increasing threat to global health (5). Infections due to these organisms are associated with significant morbidity and mortality (1).

CPE produce enzymes that fall into three classes according to the Ambler classification: class A β-lactamases (*Klebsiella pneumoniae* carbapenemase [KPC]), class B metallo-β-lactamases (New Delhi metallo-β-lactamase [NDM], imipenemase [IMP], and Verona integron-encoded metallo-β-lactamase [VIM]), and class D β-lactamases (oxacillinase [OXA]-48). The carbapenemase genes in *Enterobacteriaceae* have been shown to be associated with mobile genetic elements such as plasmids or transposons, thereby facilitating infection outbreaks (6).

An understanding of the epidemiology of the emergence of CPE and the changing burden over time is critical to the implementation of control programs and the management of individual patients (7). The purpose of this study is to investigate the prevalence and molecular epidemiology of CPE isolates collected from Seoul area in 2018.

MATERIALS AND METHODS

Bacterial strains

From January 2018 to December 2018, a total of 1,468 CRE clinical isolates were retrospectively collected from 113 hospitals in Seoul (Korea). Isolation sites were blood, urine, stool, sputum, lesion, bile, pus, tracheal aspiration, and etc. Bacterial identification was performed using Bruker Biotyper MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and VITEK 2 (bioMérieux, Marcy l'Etoile, France).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out by broth microdilution using customized Sensititre plates (TREK Diagnostic Systems, Cleveland, OH, USA). CRE were found to be resistant to imipenem, meropenem, doripenem, and ertapenem based on the Clinical and Laboratory Standards Institute (CLSI) guidelines (8).

PCR detection and sequencing of CPE genes

PCR and subsequent sequencing were conducted to detect and identify the main CPE (*bla*$_{KPC}$ and *bla*$_{GES}$ from class A; *bla*$_{IMP}$, *bla*$_{VIM}$, and *bla*$_{NDM}$ from class B; and *bla*$_{OXA}$ from class D)-encoding genes, as previously described (9) (Table 1). Briefly, the template DNAs were prepared by boiling, and PCR amplification was conducted using Quick Taq HS DyeMix (TOYOBO, Japan) with specific primer pairs. The PCR conditions were: 94°C for 5 min, followed by 30 cycles of

Table 1. Primer pairs used for amplification of CPE

Target genes	Sequences (5'→3')	Product size (bp)
IMP	F TGA GCA ATG TAT CTG TAT TC R TTA GTT GCT TGG TTT TGA TG	740
OXA-48	F TTG GTG GCA TCG ATT ATC GG R GAG CAC TTC TTT TGT GAT GGC	743
VIM	F TGG TCT ACA TGA CCG CGT CT R CGA CTG AGC GAT TTG TGT G	766
NDM	F CAA TAT TAT GCA CCC GGT CG R ATC ATG CTG GCC TTG GGG AA	720
KPC	F ATG TCA CTG TAT CGC CGT CT R TTT TCA GAG CCT TAC TGC CC	893
GES	F GCG CTT CAT TCA CGC ACT AT R GCG TAA TCT CTC TCC TGG GC	753
SME	F AAC GGC TTC ATT TTT G R GCT TCC GCA ATA GTT TTA TCA	830
GIM	F TCG ACA CAC CTT GGT CTG AA R AAC TTC CAA CTT TGC CAT GC	477
SIM	F TAC AAG GGA TTC GGC ATC G R TAA TGG CCT GTT CCC ATG TG	570
SPM	F AAA ATC TGG GTA CTC AAA CG R ACA TTA TCC GCT GGA ACA GG	271

denaturation at 94˚C for 30 s, annealing at 56˚C for 20 s and elongation at 72˚C for 30 s; and a final extension at 72˚C for 7 min. PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Analyses of nucleotide sequences were carried out by BIOFACT Co. (Daejeon, Korea). Sequences were compared with available sequences in GenBank using the BLAST program of the National Center for Biotechnology Information (NCBI).

RESULTS

CRE isolates and their resistance characteristics

While stratifying all CRE isolates by age group, it was observed that 407 (27.7%) isolates were from 70-79 years age group, 392 (26.7%) were from 80-89 years age group, 252 (17.2%) were from 60-69 years age group, 138 (9.4%) were from 50-59 years age group, 58 (4.0%) were from 40-49 years age group, 55 (3.7%) were from above 90 years age group, 49 (3.3%) were from 0-9 years age group, 41 (2.8%) were from 30-39 years age group, 20 (1.4%) were from 20-29 years age group, and 6 (0.4%) were from 10-19 years age group (Table 2). For all the analyzed isolates from patients, 806 (54.9%) were from male patients and 589 (40.1%) were from female patients (Table 2). The types of CRE isolates are shown in Table 3. Of 1,468 study isolates, *Klebsiella pneumoniae* (*K. pneumoniae)* was the most common isolated CRE organism in this study (830 isolates, 56.5%) followed by *Escherichia coli* (*E. coli*) (249 isolates, 17%), *Enterobacter cloacae* complex (136 isolates, 9.3%), *Klebsiella aerogenes* (63 isolates, 4.3%), *Proteus mirabilis* (41 isolates, 2.8%), *Citrobacter koseri* (37 isolates, 2.5%), and *Citrobacter freundii* (34 isolates, 2.3%).

Genetic characterization of carbapenemases genes among CRE isolates

The distribution of different carbapenemases among the *Enterobacteriaceae* isolates is summarized in Table 4. Of 1,468 samples tested, 853 (58.1%) were tested positive for at least one CPE. KPC-2 was the most common CPE type (46.0%), followed by NDM-1 (5.9%), NDM-5 (1.8%), NDM-5 co-infected with OXA-181 (0.8%), OXA-181 (0.7%), OXA-232 (0.5%), NDM-4 (0.5%), OXA-48 (0.3%), VIM-2 (0.3%), and NDM-1 with OXA-232 (0.2%). Also, a relatively low detection frequency of IMP-1, KPC-2 with NDM-1, KPC-2 with GES-5, KPC-4, and NDM-13, KPC-4 with NDM-1, OXA-48 with NDM-5, IMP-6, GES-5, and GES-5 with VIM-2 at 0.1% was observed, respectively. KPC-2 was most commonly found in *K. pneumoniae* (494/676 isolates [73.1%]) and *E. coli* (107/676 isolates [15.8%]), whereas NDM-1 was mostly found in *Enterobacter cloacae* complex (20/86 isolates [23.3%]) (Table 5).

Table 2. Distribution of CRE by gender and age group

Age	No. of male (%)	No. of female (%)	No. of unknown (%)	Total (%)
0-9	24 (1.6)	23(1.6)	2(0.1)	49(3.3)
10-19	5 (0.3)	1(0.1)	0(0.0)	6(0.4)
20-29	12(0.8)	6(0.4)	2(0.1)	20(1.4)
30-39	17(1.2)	23(1.6)	1(0.1)	41(2.8)
40-49	41(2.8)	17(1.2)	0(0.0)	58(4.0)
50-59	95(6.5)	39(2.7)	4(0.3)	138(9.4)
60-69	161(11.0)	82(5.6)	9(0.6)	252(17.2)
70-79	247(16.8)	149(10.1)	11(0.7)	407(27.7)
80-89	177(12.1)	208(14.2)	7(0.5)	392(26.7)
>=90	21(1.4)	34(2.3)	0(0.0)	55(3.7)
Unknown	6(0.4)	7(0.5)	37(2.5)	50(3.4)
Total	806(54.9)	589(40.1)	73(5.0)	1,468(100.0)

Table 3. Distribution of species in CRE isolates

Types of isolates	No. of isolates	Percentage (%)
Klebsiella pneumoniae	830	56.5
Escherichia coli	249	17.0
Enterobacter cloacae complex	136	9.3
Klebsiella aerogenes	63	4.3
Proteus mirabilis	41	2.8
Citrobacter koseri	37	2.5
Citrobacter freundii	34	2.3
Serratia marcescens	15	1.0
Klebsiella oxytoca	15	1.0
Providencia rettgeri	14	1.0
Morganella morganii	8	0.5
Proteus vulgaris	8	0.5
Citrobacter braakii	4	0.3
Raoultella ornithinolytica	3	0.2
Providencia stuartii	2	0.1
Citrobacter amalonaticus	1	0.1
Hafnia alvei	1	0.1
Citrobacter youngae	1	0.1
Escherichia hermannii	1	0.1
Kluyvera cryocrescens	1	0.1
Citrobacter farmeri	1	0.1
Kluyvera intermedia	1	0.1
Raoultella planticola	1	0.1
Proteus penneri	1	0.1
Total	1,468	100

Table 4. Distribution of carbapenemase genotypes by CRE isolates (n=1,468)

Types of isolates	No. of isolates	Percentage (%)
KPC-2	676	46.0
NDM-1	86	5.9
NDM-5	26	1.8
NDM-5, OXA-181	12	0.8
OXA-181	11	0.7
OXA-232	8	0.5
NDM-4	8	0.5
OXA-48	5	0.3
VIM-2	4	0.3
NDM-1, OXA-232	3	0.2
IMP-4	2	0.1
NDM-4, OXA-181	2	0.1
IMP-1	1	0.1
KPC-2, NDM-1	1	0.1
KPC-2, GES-5	1	0.1
KPC-4	1	0.1
NDM-13	1	0.1
KPC-4, NDM-1	1	0.1
OXA-48, NDM-5	1	0.1
IMP-6	1	0.1
GES-5	1	0.1
GES-5, VIM-2	1	0.1
NEG*	615	41.9
Total	1,468	100

*carbapenemase gene negative

Table 5. Distributions of genotypes of CRE isolates (n=1,468)

Types of CPE / Types of Enterobacteriaceae	KPC-2	GES-5, VIM-2	NEG	NDM-1	NDM-5	NDM-5, OXA-181	OXA-181	OXA-232	NDM-4	OXA-48	VIM-2	NDM-1, OXA-232	IMP-4	NDM-4, OXA-181	IMP-1	KPC-2, NDM-1	KPC-2, GES-5	KPC-4	NDM-13	KPC-4, NDM-1	OXA-48, NDM-5	IMP-6	GES-5	NEG*
Klebsiella pneumoniae	494		279	18	8	2	4	8	6	1	2	3		2				1		1			1	279
Escherichia coli	107		80	18	18	10	6		2	4					1		1		1		1			80
Enterobacter cloacae complex	5		108	20							2		1											108
klebsiella aerogenes	5		54	2			1									1								54
Proteus mirabilis			39	1																		1		39
Citrobacter koseri	37																							
Citrobacter freundii	9		10	15																				10
Serratia marcescens	7	1	7																					7
Klebsiella oxytoca	10			4									1											
Providencia rettgeri			14																					14
Proteus vulgaris			8																					8
Morganella morganii			7	1																				7
Citrobacter braakii			3	1																				3
Raoultella ornithinolytica				3																				
Providencia stuartii			2																					2
Citrobacter youngae			1																					1
Kluyvera intermedia	1																							
Citrobacter amalonaticus			1																					1
Kluyvera cryocrescens	1																							
Escherichia hermannii				1																				
Raoultella planticola				1																				
Hafnia alvei			1																					1
Proteus penneri			1																					1
Citrobacter farmeri				1																				

*carbapenemase gene negative

DISCUSSION

CRE is of significant concern to public health, both at the community level and for the health-care facilities (10). The worldwide global increase in infections with CRE is of great concern due to the association of infections with the highly virulent bacteria with high morbidity and mortality rates (10). Until now, the carbapenem resistance rate among *Enterobacteriaceae* isolates from Korea has been relatively low and stable (11).

When we considered CRE isolates, the age distribution was skewed toward older patients, accounting for 85% of isolates (above 60 years of age).

Our findings that *K. pneumoniae* was the dominant type (identified in 56.5% of isolated), followed by *E. coli* (only 17.0%) is in agreement with those of previous studies done in community hospitals in Bahrain, Taiwan, and US where *K. pneumoniae* was the most prevalent species (91%) (10, 12, 13). *K. pneumoniae* will play a major role in carbapenem resistance because it has been repeatedly identified to be the most common species among CRE, hereafter called carbapenem-resistant *K. pneumoniae* (CRKP) (14).

It was found that the emergence of CRE has become a formidable public health threat as it had increased four-fold over the past 10 years worldwide (15), particularly among *K. pneumoniae* and *E. coli*, as has been reported in the global antibiotic resistance estimates published by the World Health Organization in 2014 (16). The results of the present study on the leading causes of CRE were similar to those of previous studies that focused on community hospitals. However, the prevalence rate of *E. coli* and *K. pneumoniae* was either on a little lower or higher side than the rate specified in previous reports. This may be due to the difference between countries and the reporting years (10, 17, 18).

Three main carbapenemases are reported worldwide: KPC, NDM, and OXA-48-like (18). KPC the most common transmissible class A genes are mostly found worldwide (19, 20). KPC-2, NDM-1, NDM-5, and OXA-181 are the most common carbapenemases identified in the present study, and the outcome is similar to previous findings describing the detection of KPC, NDM, and OXA-48 worldwide (21, 22). Korea is known for outbreaks of *K. pneumoniae* that produce KPC-2, NDM-1, and OXA-232 (20, 23, 24). After the first KPC producer (KPC-2 in *K. pneumoniae*) was identified in 1996 in the eastern United States (25), reports of KPC-2 in the New York, NY, area began to appear in 2004; KPC-expressing *K. pneumoniae* is currently an alarming problem (26-28). This report is especially disturbing because New York demonstrated large outbreaks of extended-spectrum β-lactamase (ESBL)-producing *Klebsiella* (29, 30) for which carbapenems were considered to be one of the few treatment options (16). Since numerous studies on KPC-2 genes have been carried out worldwide (31, 32), we need to carefully monitor CPE and perform in-depth research on CPE using surveillance systems.

Previous studies indicated increasing numbers of *Enterobacteriaceae* as frequent carriers of genes encoding two of the most concerning subclasses of carbapenemases: KPC, which has become endemic in parts of America, Southern Europe, Israel, and China; and the New Delhi Metallo-β-lactamase (NDM), which has become endemic in Northern Europe and the Asia Pacific region, most remarkably in the UK and India (33-35). Mechanisms of carbapenem nonsusceptibility can be divided broadly into carbapenemase production CPE and a combination of β -lactamase (ESBL and AmpC) production with porin loss, and efflux pump overexpression (non-carbapenemase-producing carbapenem nonsusceptible *Enterobacteriaceae* [NCPCRE]) (36). These mechanisms generally appear paired among themselves or with carbapenemase-production (1). All three alternative mechanisms aim to block the penetration of the antibiotics within the bacterial cell (37). The data from a case-control study comparing patients with NCPCRE (cases) and patients with CPE (controls) reported in the United States indicate 843 unique patients with first-episode CRE, including 387 (45.9%) NCPCRE and 456 (54.1%) CPE (38). Orsi and colleagues (39) demonstrated that compared to *Klebsiella pneumoniae* carbapenemase (KPC)-producing CRE, NCPCRE were associated with prior antibiotic exposure, demonstrating that patient-level risk factors may differ according to mechanisms of resistance. Another study performed in Taiwan from 2010 to 2012 presented the detection of Carbapenemase in 5.0% of the CRE isolates but the prevalence and carbapenemase differed by species (40).

Although KPC is the most common carbapenemase worldwide, NDM has also been shown to be important sporadic outbreaks around the globe (41, 42). The prevalence of NDM was in agreement with a study on Global distribution (43). The analysis of a total of 1,468 isolates of *Enterobacteriaceae* for the presence of CPE demonstrated NDM as the second gene (9.6%, 141/1,468) and mostly NDM-1 (64.5%, 91/141) in *Enterobacteriaceae*. CPE distribution likely appears to vary geographically; for example, high rates in Greece (VIM and KPC), Romania, Poland and Denmark (NDM), and in the Indian subcontinent (NDM, KPC, and OXA-181) (44, 45). MBL-producing *Enterobacteriaceae* isolates have also been reported in several Latin American countries and Europe (45). In a multinational survey spanning 2012-2014, VIM-producing CPE were recovered in Mexico and NDM-1-producing CPE in Venezuela (45).

CRE continues to evolve, posing an increasing threat to patients of all ages (1). Early identification of carbapenemase producers in clinical infections, at the carriage state, or both, is therefore mandatory to prevent the development of hospital-based outbreaks (44). Consequently, it is hypothesized that constant surveillance and regular education are important for the successful mitigation of health-care-associated infection and to decrease the incidence rate of CRE. Molecular characterization of CPE is essential for epidemiological surveillance to monitor the insurgence of the CPE pandemic.

In conclusion, this study revealed the overall prevalence of CRE in Seoul, Korea and highlights the need for continued studies as a way to control the emergence of new CPE varieties as a basis for further epidemiological surveillance

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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