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Genotypic Distribution and Antimicrobial Susceptibilities of Carbapenemase-Producing *Enterobacteriaceae* Isolated From Rectal and Clinical Samples in Korean University Hospitals Between 2016 and 2019

Seri Jeong, M.D.[1], Nuri Lee, M.D.[1], Min-Jeong Park, M.D.[1], Kibum Jeon, M.D.[2], Han-Sung Kim, M.D.[3], Hyun Soo Kim, M.D.[4], Jae-Seok Kim, M.D.[5], and Wonkeun Song, M.D., Ph.D.[1]

[1]Department of Laboratory Medicine, Kangnam Sacred Heart Hospital, Hallym University College of Medicine, Seoul, Korea; [2]Department of Laboratory Medicine, Hangang Sacred Heart Hospital, Hallym University College of Medicine, Seoul, Korea; [3]Department of Laboratory Medicine, Hallym University Sacred Heart Hospital, Hallym University College of Medicine, Anyang, Korea; [4]Department of Laboratory Medicine, Dongtan Sacred Heart Hospital, Hallym University College of Medicine, Hwaseong, Korea; [5]Department of Laboratory Medicine, Kangdong Sacred Heart Hospital, Seoul, Korea

Background: The emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) represents a major clinical problem. Recently, the occurrence of CPE has increased globally, but epidemiological patterns vary across region. We report the trends in the genotypic distribution and antimicrobial susceptibility of CPE isolated from rectal and clinical samples during a four-year period.

Methods: Between January 2016 and December 2019, 1,254 nonduplicated CPE isolates were obtained from four university hospitals in Korea. Carbapenemase genotypes were determined by multiplex real-time PCR. Antimicrobial susceptibility was profiled using the Vitek 2 system (bioMérieux, Hazelwood, MO, USA) or MicroScan Walkaway-96 system (Siemens West Sacramento, CA, USA). The proportions of carbapenemase genotypes and nonsusceptibility were analyzed using Pearson's chi-square test.

Results: Among the 1,254 CPE isolates, 486 (38.8%), 371 (29.6%), 357 (28.5%), 8 (0.6%), 8 (0.6%), and 24 (1.9%) were *Klebsiella pneumoniae* carbapenemase (KPC), oxacillinase (OXA)-48-like, New Delhi metallo-β-lactamase (NDM), imipenemase (IMP), Verona integron-encoded metallo-β-lactamase (VIM), and multiple producers, respectively. The predominant species was *K. pneumoniae* (72.6%), followed by *Escherichia coli* (6.5%). More than 90% of the isolates harboring *KPC, NDM*, and *OXA-48-like* were nonsusceptible to cephalosporins, aztreonam, and carbapenems.

Conclusions: The impact of CPE is primarily due to KPC-, NDM-, and OXA-48-like-producing *K. pneumoniae* isolates. Isolates carrying these carbapenemase are mostly multidrug-resistant. Control strategies based on these genotypic distributions and antimicrobial susceptibilities of CPE isolates are required.

Key Words: Carbapenemase-producing *Enterobacteriaceae*, *Klebsiella pneumoniae*, *Klebsiella pneumoniae* carbapenemase, New Delhi metallo-β-lactamase, oxacillinase-48-like

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Corresponding author:
Wonkeun Song, M.D., Ph.D.
Department of Laboratory Medicine, Kangnam Sacred Heart Hospital, Hallym University College of Medicine, 1 Shingil-ro, Youngdeungpo-gu, Seoul 07441, Korea
Tel: +82-2-829-5259
Fax: +82-2-847-2403
E-mail: swonkeun@hallym.or.kr

INTRODUCTION

The emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) is a major clinical concern because these bacteria are nonsusceptible to various classes of antimicrobials, which can lead to therapeutic failure and worse outcomes [1]. CPE mainly harbor three major classes of β-lactamases 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) [2]. In CPE that cause infection outbreaks, the carbapenemase genes are located on mobile genetic elements, such as plasmids or transposons [3, 4].

The occurrence of CPE has increased globally, but their epidemiological patterns vary across region. KPC-producing *K. pneumoniae* was first isolated in North Carolina, USA, in 1996 [5]. Numerous outbreaks related to these strains have been reported in the USA, South America, Greece, Italy, Israel, and China [1]. NDM producers were identified in early 2008 from an Indian patient hospitalized previously in New Delhi, India; since then, they have been detected in most countries related with India [6]. Recently, isolates from the Balkan states and the Middle East have served as reservoirs of NDM producers [7]. The first OXA-48-producing *K. pneumoniae* strain was isolated in Istanbul, Turkey, in 2001 [8]. Various strains, including OXA-48 strains, have caused several outbreaks in Mediterranean and Southern Europe, the Indian subcontinent, and Northern Africa [2, 7].

Multicenter studies on the prevalence and molecular epidemiology or antimicrobial susceptibilities of CPE have been conducted in Korea [9, 10]. Reporting the recent status of genotypic distribution of CPE in relation to antimicrobial susceptibilities based on a multicenter study is necessary to increase the awareness about and control of these isolates. We report the genotypic distribution and antimicrobial susceptibilities of CPE isolated from rectal and clinical samples in four Korean university hospitals during a 4-year period.

MATERIALS AND METHODS

Study design and CPE isolate identification

Between January 2016 and December 2019, CPE isolates were prospectively collected from four university hospitals in a metropolitan area of Korea (two hospitals, Kangnam Sacred Heart Hospital with 572 beds [A] and Hangang Sacred Heart Hospital with 158 beds [B] in Seoul, and two, Hallym University Sacred Heart Hospital with 834 beds [C] and Dongtan Sacred Heart Hospital with 660 beds [D] in Gyeonggi-do). In total, 1,254 non-duplicated CPE isolates harboring carbapenemase genes were collected (N=308, hospital A; N=55, hospital B; N=856, hospital C; N=35, hospital D). The isolates had been obtained from rectal samples taken for epidemiological and infection control purposes and clinical samples taken during the work-up of patients with suspected infections. The following clinical samples were provided: sputum (N=158, 12.6%), urine (N=130, 10.3%), blood (N=58, 4.6%), body fluid (N=36, 2.9%), wound or pus (N=22, 1.8%), and swabs (N=10, 0.8%). The isolates from patients in the intensive care unit (ICU) were more than a half of the included CPE isolates (N=792, 63.2%). Bacteria were identified using a Vitek 2 system (bioMérieux, Hazelwood, MO, USA) at hospitals B and D, amatrix-assisted laser desorption ionization-time-of-flight mass spectrometry on a Vitek-MS instrument (bioMérieux) at hospital A, and a MicroScan Walkaway-96 system (Siemens, West Sacramento, CA, USA) at hospital C. After collecting carbapenem-nonsusceptible *Enterobacteriaceae* isolates (based on a minimal inhibitory concentration [MIC]>0.5 μg/mL for ertapenem or MIC>1 μg/mL for imipenem or meropenem) from the four hospitals, the modified Hodge test (MHT) and carbapenemase inhibition test (CIT) were used for CPE detection at hospital A [11]. Clinical information, such as hospital of origin, sampling date, isolation site, department, and hospitalization ward, was collected by reviewing the medical records. The study protocol was approved by the Institutional Review Board of Kangnam Sacred Heart Hospital, Seoul (HKS 2020-03-020), which waived the need for informed consent. The data used in this study have been deposited in Harvard Dataverse and are accessible through https://doi.org/10.7910/DVN/QR9DR6.

Genotypic detection of carbapenemase genes

PANA Real Typer CRE kits (PANAGENE Inc., Daejeon, Korea) utilizing peptide nucleic acid-mediated multiplex real-time PCR were used to type all CPE isolates that tested carbapenemase-positive using MHT and CIT. The primers and probes, which were designed to target six carbapenemase genes (bla_{KPC}, bla_{GES}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and $bla_{OXA\text{-}48\text{-}like}$) and an internal control, were provided by PANAGENE Inc. The sequences have been previously published [12]. The multiplex real-time PCR assay was conducted in a 96-well plate on a CFX-96 Real-Time PCR detection system (Bio-Rad Laboratories Inc., Hercules, CA, USA) using the PANA Real Typer CRE kit per the manufacturer's instructions. Reaction mixtures contained 5 μL of genomic DNA, 1

μL of Taq DNA polymerase, and 19 μL of the primer/probe and multiplex real-time PCR master mixtures. Thermal cycles were as follows: 50°C for 2 minutes and 95°C for 15 minutes; 45 cycles of 95°C for 15 seconds, 58°C for 45 seconds, and 72°C for 15 seconds; 95°C for 5 minutes; 35°C for 5 minutes; and a melting-curve step (35°C to 80°C in 0.5°C increments for 5 seconds). Fluorescence was acquired on all four channels (Blue/FAM for bla_{KPC} and bla_{IMP}, Green/HEX for bla_{GES} and bla_{NDM}, Yellow/ROX for bla_{VIM} and bla_{OXA-48}, and Violet/Cy5 for the internal control). After each run, the threshold cycle (Ct) was measured based on the signal strength, at which the fluorescence exceeded the threshold. Rectal and clinical samples with a Ct

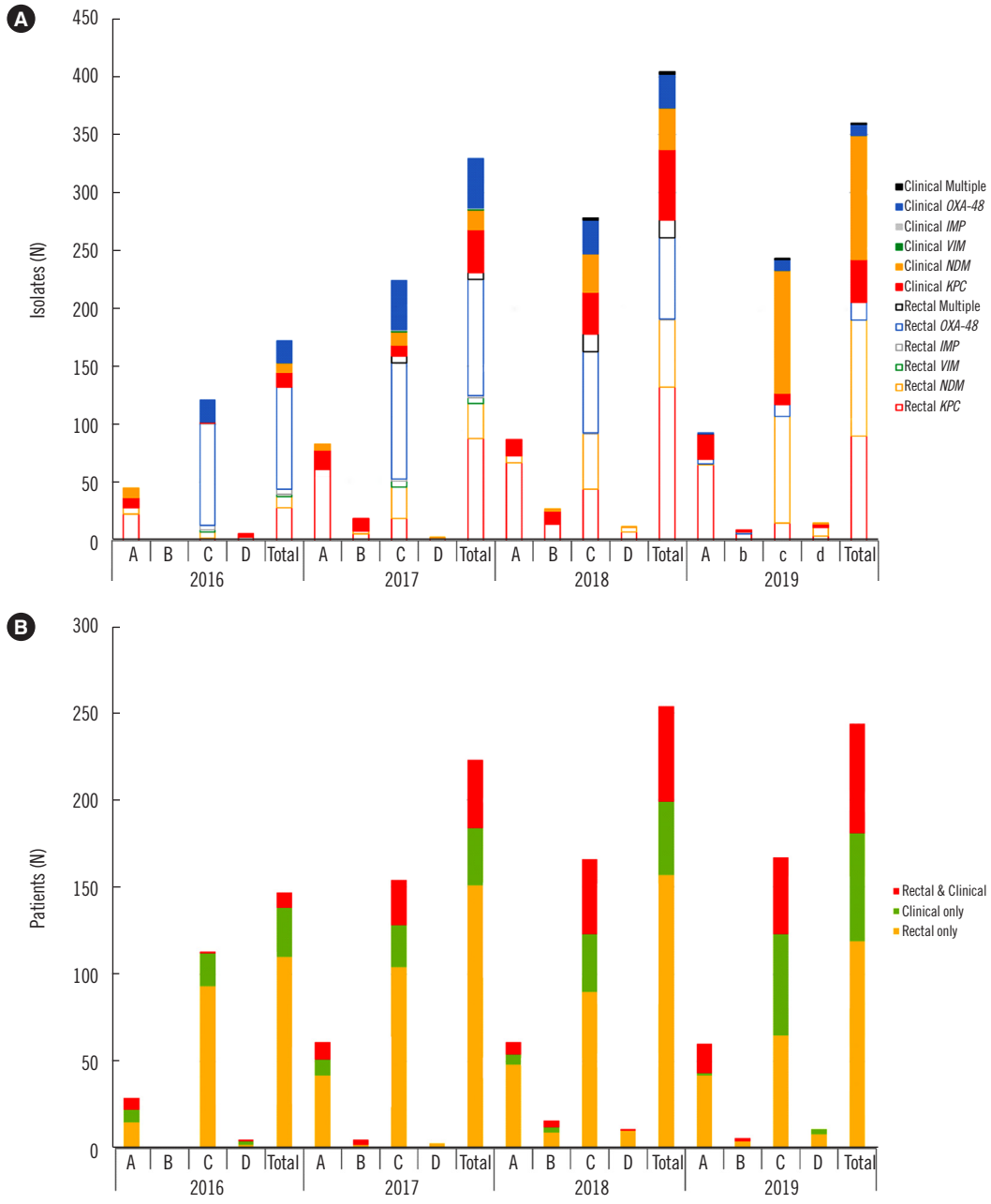

Fig. 1. Distributions of genotypes and patients according to the hospital. (A) Carbapenemase genotypes and (B) patients with carbapenemase-producing *Enterobacteriaceae* isolates according to year and sample type.
Abbreviations: A, Kangnam Sacred Heart Hospital; B, Hangang Sacred Heart Hospital; C, Hallym University Sacred Heart Hospital; D, Dongtan Sacred Heart Hospital; KPC, *K. pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA-48, oxacillinase-48; IMP, imipenemase; VIM, Verona integron-encoded metallo-β-lactamase.

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value <35 were regarded positive. RNase-free water was used as a negative control in each run.

Antimicrobial susceptibility testing

The MICs of the isolates were determined using the Vitek 2 system at hospitals A, B, and D and the MicroScan Walkaway-96 system at hospital C. Nonsusceptibility rates were calculated by dividing the number of isolates showing nonsusceptibility by the total number of isolates. The analyzed antimicrobial agents were cefotaxime, ceftazidime, cefepime, aztreonam, ertapenem, imipenem (or meropenem), amikacin, gentamicin, ciprofloxacin (or levofloxacin), trimethoprim-sulfamethoxazole, and tigecycline. MIC breakpoints were applied according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (M100S) [13]. For tigecycline, MIC results were interpreted according to the European Committee for Antimicrobial Susceptibility Testing criteria [14]. Intermediate susceptibility was determined according to the criteria set in the CLSI guidelines and per the European Committee for Antimicrobial Susceptibility Testing [13, 14].

Statistical analysis

Statistical analyses were performed using PASW version 18.0 (SPSS Inc., Chicago, IL, USA), Analyse-it Method Evaluation Edition software (version 2.26; Analyse-it Software Ltd., Leeds, UK), and R statistical software (version 3.6.3; R Foundation for Statistical Computing, Vienna, Austria). Categorical variables were compared using Pearson's chi-square test or Fisher's exact test. All tests were two-sided, and differences with $P<0.05$ were considered significant.

RESULTS

Distribution of CPE isolates

The CPE isolates comprised 910 *K. pneumoniae* (72.6%), 81 *Escherichia coli* (6.5%), 79 *Enterobacter cloacae* (6.3%), 47 *Citrobacter freundii* (3.7%), 45 *Klebsiella oxytoca* (3.6%), 28 *Serratia marcescens* (2.2%), 20 *Klebsiella aerogenes* (1.6%), 13 *Citrobacter koseri* (1.0%), 12 *Providencia rettgeri* (1.0%), 3 *Enterobacter hormaechei* (0.2%), 3 *Enterobacter kobei* (0.2%), 3 *Pantoea agglomerans* (0.2%), 2 *Citrobacter braakii* (0.2%), 2

Table 1. Distribution of the most prevalent carbapenemase genotypes according to carbapenemase-producing *Enterobacteriaceae* species

	KPC			NDM			OXA-48-like		
	Rectal	Clinical	Subtotal	Rectal	Clinical	Subtotal	Rectal	Clinical	Subtotal
Klebsiella pneumoniae	239 (49.2)	115 (23.7)	354 (72.8)	114 (31.9)	124 (34.7)	238 (66.7)	217 (58.5)	93 (25.1)	310 (83.6)
Escherichia coli	29 (6.0)	8 (1.6)	37 (7.6)	16 (4.5)	5 (1.4)	21 (5.9)	18 (4.9)	4 (1.1)	22 (5.9)
Enterobacter cloacae	16 (3.3)	4 (0.8)	20 (4.1)	26 (7.3)	5 (1.4)	31 (8.7)	4 (1.1)	1 (0.3)	5 (1.3)
Citrobacter freundii	3 (0.6)	2 (0.4)	5 (1.0)	27 (7.6)	3 (0.8)	30 (8.4)	10 (2.7)	0 (0.0)	10 (2.7)
Klebsiella oxytoca	11 (2.3)	5 (1.0)	16 (3.3)	5 (1.4)	3 (0.8)	8 (2.2)	17 (4.6)	1 (0.3)	18 (4.9)
Serratia marcescens	10 (2.1)	9 (1.9)	19 (3.9)	1 (0.3)	8 (2.2)	9 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)
Klebsiella aerogenes	12 (2.5)	3 (0.6)	15 (3.1)	2 (0.6)	0 (0.0)	2 (0.6)	2 (0.5)	1 (0.3)	3 (0.8)
Citrobacter koseri	11 (2.3)	2 (0.4)	13 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Providencia rettgeri	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	12 (3.4)	12 (3.4)	0 (0.0)	0 (0.0)	0 (0.0)
Enterobacter hormaechei	1 (0.2)	1 (0.2)	2 (0.4)	1 (0.3)	0 (0)	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
Enterobacter kobei	2 (0.4)	0 (0.0)	2 (0.4)	1 (0.3)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
Pantoea agglomerans	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.6)	0 (0.0)	2 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)
Citrobacter braakii	1 (0.2)	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.3)
Enterobacter asubriae	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Kluyvera ascorbate	0 (0.0)	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.3)
Raoutella planticola	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.6)	0 (0.0)	2 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)
Klebsiella ozaenae	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.3)
Klebsiella variicola	1 (0.2)	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	336 (69.1)	150 (30.9)	486 (100.0)	197 (55.2)	160 (44.8)	357 (100)	271 (73.0)	100 (27.0)	371 (100)

Data are expressed as number (%).
Abbreviations: KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA-48, oxacillinase-48.

Table 2. Distribution of carbapenemase-producing *Enterobacteriaceae*

	Hospital A			Hospital B			Hospital C			Hospital D		
	Rectal	Clinical	Subtotal	Rectal	Clinical	Subtotal	Rectal	Clinical	Subtotal	Rectal	Clinical	Subtotal
Klebsiella pneumoniae	133 (21.6)	42 (6.8)	175 (28.5)	19 (17.3)	18 (16.4)	37 (33.6)	72 (4.4)	49 (3.0)	121 (7.4)	15 (21.4)	6 (8.6)	21 (30.0)
Escherichia coli	22 (3.6)	3 (0.5)	25 (4.1)	3 (2.7)	2 (1.8)	5 (4.5)	4 (0.2)	3 (0.2)	7 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
Enterobacter cloacae	16 (2.6)	4 (0.7)	20 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Serratia marcescens	10 (1.6)	9 (1.5)	19 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Klebsiella oxytoca	8 (1.3)	0 (0.0)	8 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.1)	5 (0.3)	7 (0.4)	1 (1.4)	0 (0.0)	1 (1.4)
Klebsiella aerogenes	8 (1.3)	1 (0.2)	9 (1.5)	3 (2.7)	2 (1.8)	5 (4.5)	1 (0.1)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Citrobacter koseri	10 (1.6)	2 (0.3)	12 (2.0)	1 (0.9)	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Citrobacter freundii	3 (0.5)	0 (0.0)	3 (0.5)	0 (0.0)	2 (1.8)	2 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Enterobacter hormaechei	1 (0.2)	1 (0.2)	2 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Enterobacter kobei	2 (0.3)	0 (0.0)	2 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Citrobacter braakii	1 (0.2)	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Klebsiella variicola	1 (0.2)	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
KPC	215 (35.0)	63 (10.2)	278 (45.2)	26 (23.6)	24 (21.8)	50 (45.5)	79 (4.8)	57 (3.5)	136 (8.3)	16 (22.9)	6 (8.6)	22 (31.4)
Klebsiella pneumoniae	4 (0.7)	0 (0.0)	4 (0.7)	2 (1.8)	0 (0)	2 (1.8)	106 (6.5)	124 (7.6)	230 (14.1)	1 (1.4)	0 (0.0)	2 (2.9)
Enterobacter cloacae	2 (0.3)	0 (0.0)	2 (0.3)	0 (0.0)	2 (1.8)	2 (1.8)	24 (1.5)	3 (0.2)	27 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)
Citrobacter freundii	0 (0.0)	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	26 (1.6)	2 (0.1)	28 (1.7)	1 (1.4)	0 (0.0)	1 (1.4)
Escherichia coli	5 (0.8)	0 (0.0)	5 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	5 (0.3)	3 (0.2)	8 (0.5)	6 (8.6)	2 (2.9)	8 (11.4)
Providencia rettgeri	0 (0.0)	11 (1.8)	11 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Serratia marcescens	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	8 (0.5)	9 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)
Klebsiella oxytoca	1 (0.2)	1 (0.2)	2 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.2)	2 (0.1)	5 (0.3)	1 (1.4)	0 (0.0)	1 (1.4)
Klebsiella aerogenes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.1)	0 (0.0)	2 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Pantoea agglomerans	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.1)	0 (0.0)	2 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Raoutella planticola	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.1)	0 (0.0)	2 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Enterobacter hormaechei	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)	0 (0.0)	1 (1.4)
Enterobacter kobei	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
NDM	12 (2.0)	13 (2.1)	25 (4.1)	2 (1.8)	2 (1.8)	4 (3.6)	172 (10.5)	143 (8.8)	315 (19.3)	11 (15.7)	2 (2.9)	13 (18.6)
Klebsiella pneumoniae	2 (0.3)	0 (0.0)	2 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	215 (13.2)	93 (5.7)	308 (18.9)	0 (0.0)	0 (0.0)	0 (0.0)
Escherichia coli	1 (0.2)	1 (0.2)	2 (0.3)	1 (0.9)	0 (0.0)	1 (0.9)	16 (1.0)	3 (0.2)	19 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)
Klebsiella oxytoca	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	17 (1.0)	1 (0.1)	18 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)
Citrobacter freundii	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (0.6)	0 (0.0)	10 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)
Enterobacter cloacae	1 (0.2)	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.2)	1 (0.1)	4 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
Klebsiella aerogenes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.1)	1 (0.1)	3 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
Citrobacter braakii	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Kluyvera ascorbate	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Klebsiella ozaenae	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
OXA-48-like	4 (0.7)	1 (0.2)	5 (0.8)	1 (0.9)	0 (0.0)	1 (0.9)	266 (16.3)	99 (6.1)	365 (22.4)	0 (0.0)	0 (0.0)	0 (0.0)
Total	462 (75.1)	153 (24.9)	615 (100.0)	58 (52.7)	52 (47.3)	110 (100)	1,034 (63.4)	598 (36.6)	1,632 (100.0)	53 (75.7)	16 (22.9)	70 (100.0)

Data are expressed as number (%).

Abbreviations: KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA-48, oxacillinase-48; Hospitals A, Kangnam Sacred Heart Hospital; B, Hangang Sacred Heart Hospital; C, Hallym University Sacred Heart Hospital; D, Dongtan Sacred Heart Hospital.

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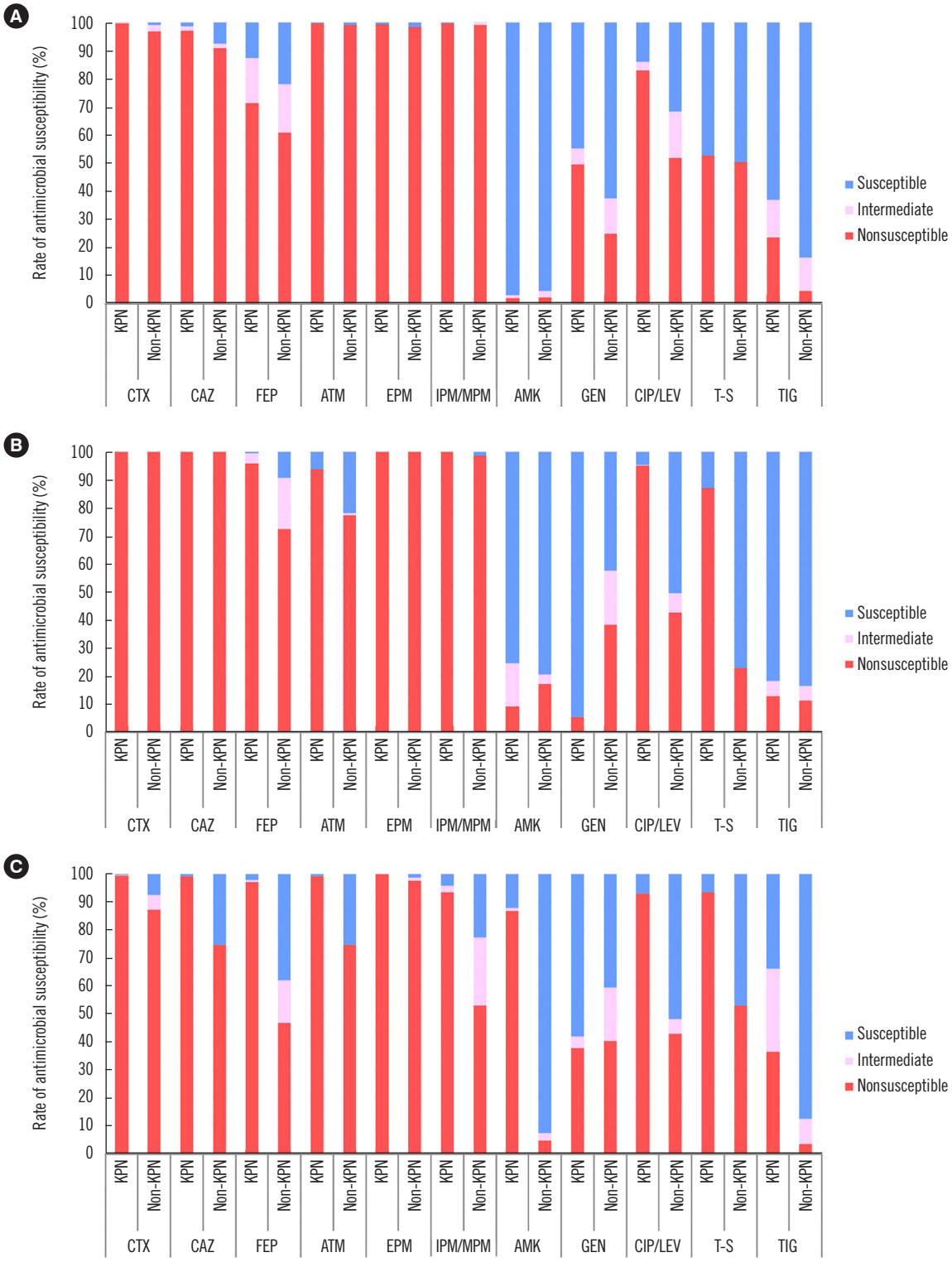

Fig. 2. Antimicrobial susceptibility profiles of *Enterobacteriaceae* isolates producing (A) KPC, (B) NDM, and (C) OXA-48-like.
Abbreviations: CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; EPM, ertapenem; IMP/MPM, imipenem (or meropenem); AMK, amikacin; GEN, gentamicin; CIP/LEV, ciprofloxacin (or levofloxacin); T-S, trimethoprim-sulfamethoxazole; TIG, tigecycline; KPN, *K. pneumoniae*; KPC, *K. pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA-48, oxacillinase-48.

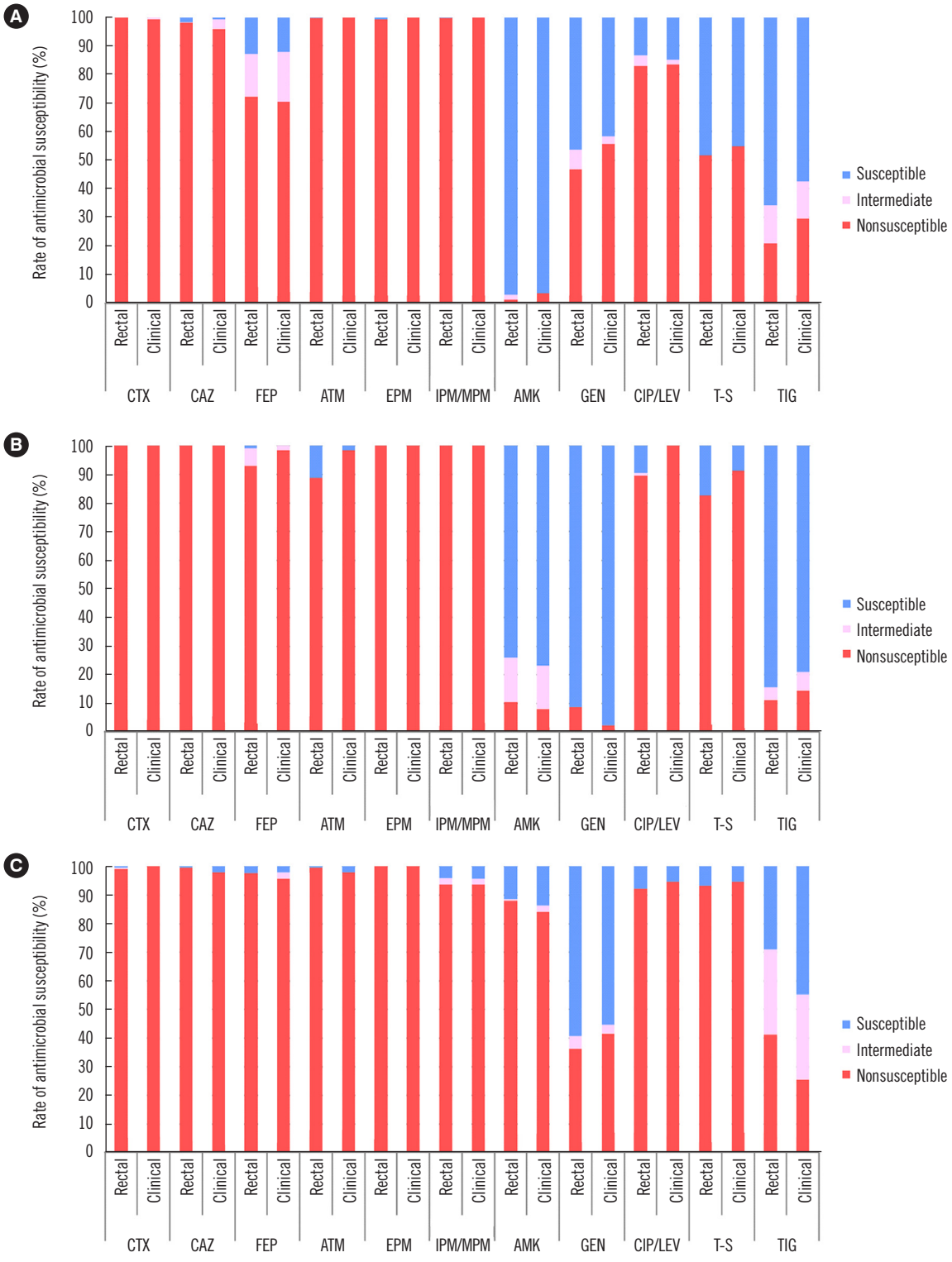

Fig. 3. Antimicrobial susceptibility profiles of *K. pneumoniae* isolates producing (A) KPC, (B) NDM, and (C) OXA-48-like by sample types.
Abbreviations: CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; EPM, ertapenem; IMP/MPM, imipenem (or meropenem); AMK, amikacin; GEN, gentamicin; CIP/LEV, ciprofloxacin (or levofloxacin); T-S, trimethoprim-sulfamethoxazole; TIG, tigecycline; KPC, *K. pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA-48, oxacillinase-48.

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Enterobacter asubriae (0.2%), 2 *Kluyvera ascorbate* (0.2%), 2 *Raoutella planticola* (0.2%), 1 *Klebsiella ozaenae* (0.1%), and 1 *Klebsiella variicola* (0.1%) isolates.

The distribution of carbapenemase genes identified among the CPE isolates is illustrated in Fig. 1A. Among the 1,254 CPE isolates, 486 (38.8%), 371 (29.6%), 357 (28.5%), 8 (0.6%), 8 (0.6%), and 24 (1.9%) were KPC, OXA-48-like, NDM, IMP, VIM, and multiple producers harboring more than two types of genes, respectively. Twenty-four CPE (1.9%) isolates harbored more than two carbapenemase genes, and most of these isolates (N=19, 1.5%) contained both *OXA-48*-like and *NDM*. The samples comprised rectal (N=829, 66.9%) and clinical (N=415, 33.1%) samples. The number of CPE isolates from clinical samples alone (P=0.018) and from rectal plus clinical samples (P<0.001) increased yearly (Fig. 1B).

The distribution of carbapenemase genotypes according to species is presented in Table 1. The most prevalent species among the KPC, NDM, and OXA-48-like producers was *K. pneumoniae* (72.8% of KPC, 66.7% of NDM, and 83.6% of OXA-48-like producers), followed by *E. coli* isolates (7.6% of KPC, 5.9% for each of NDM and OXA-48-like producers). *E. cloacae* (8.7%) and *C. freundii* (8.4%) isolates were more frequently NDM producers than KPC or OXA-48-like producers. The distribution of CPE isolates according to the four hospitals is shown in Table 2. In hospital C, the proportion of *NDM* (19.3%) and *OXA-48*-like (22.4%) was larger than that of *KPC* (8.3%), unlike in the other hospitals (P<0.001), reflecting local differences.

Antimicrobial susceptibility profile

More than 90% of *KPC*-harboring *Enterobacteriaceae* isolates were nonsusceptible to cefotaxime, ceftazidime, aztreonam, ertapenem, imipenem, and meropenem (Fig. 2A). The isolates harboring *NDM* showed similar nonsusceptibility patterns; however, more than 90% of *K. pneumoniae* isolates (95.8% of NDM vs. 71.5% of KPC producers) were nonsusceptible to cefepime (Fig. 2B). As for *Enterobacteriaceae* harboring *OXA-48*-like, *K. pneumoniae* isolates were highly nonsusceptible (nonsusceptibility rate >90%) to cefotaxime, ceftazidime, cefepime, aztreonam, ertapenem, imipenem, meropenem, ciprofloxacin, levofloxacin, and trimethoprim-sulfamethoxazole when compared with non-*K. pneumoniae* isolates (Fig. 2C). All *K. pneumoniae* isolates harboring *KPC, NDM*, and *OXA-48*-like genes showed higher tigecycline nonsusceptibility rates (12.9%–36.5%) than non-*K. pneumoniae* isolates (3.8%–11.3%) (P<0.001).

Regarding *K. pneumoniae*, representing most of the CPE isolates, more than 90% of the isolates with *KPC, NDM*, and *OXA-48*-like were nonsusceptible to cefotaxime, ceftazidime, cefepime, aztreonam, ertapenem, imipenem, and meropenem. *KPC*-harboring *K. pneumoniae* isolates and *NDM*-harboring *K. pneumoniae* from rectal samples showed lesser nonsusceptibility rates (<90%) to cefepime and aztreonam, respectively (Fig. 3). *K. pneumoniae* harboring *OXA-48*-like in both rectal and clinical samples presented more than 90% of nonsusceptibility to ciprofloxacin, levofloxacin, and trimethoprim-sulfamethoxazole.

DISCUSSION

Increasing trends in CPE prevalence and spread have been identified worldwide [7, 15]. Concordant with previous studies, we mainly identified KPC (38.8%), OXA-48-like (29.6%), and NDM (28.5%) producers [9, 16]. The predominantly isolated species (*K. pneumoniae* and *E. coli*) were also similar to those reported in other studies [9, 16].

In the USA and Europe, *K. pneumoniae* is the main pathogen harboring *KPC*, and the first case of infection with a KPC-producing extremely nonsusceptible *K. pneumoniae* isolate in Korea was reported in 2010 [17]. The first outbreak of KPC-producing *K. pneumoniae* in Korea in 2013 was likely due to clonal spread [18].

The Indian subcontinent is recognized as an NDM endemic zone, and the associated species are mostly *K. pneumoniae* and *E. coli*. NDM-producing *K. pneumoniae* isolates producing nearly identical *Xba*I pulsed-field gel electrophoresis patterns were first detected in Korea in 2010 [19]. Nosocomial transmission of genetically related NDM-producing *E. coli* from four elderly patients was reported in a Korean hospital in 2011 [20]. In this study, *E. cloacae* and *C. freundii* were frequently detected NDM producers in rectal samples. These species were also more commonly detected among NDM rather than KPC and OXA-48-like producers in Canada and major strains in Korea in a study conducted in 2015 [9, 21].

OXA-48-like producers are often detected in Northern Africa and the Mediterranean area and is increasingly being reported in *Enterobacteriaceae*, such as *K. pneumoniae* and *E. coli* [7, 16]. Korea is known for outbreaks of *K. pneumoniae* and *E. coli* that produce OXA-232, which was identified as an *OXA-48* variant differing in a few amino acids [22, 23]. Clonal and horizontal spread of these *OXA-232*-harboring species has caused persistent outbreaks in Korea [9, 22].

Although the proportions of IMP (0.6%) and VIM (0.6%) producers among CPE were relatively low in this study, strains with these genes cause sporadic outbreaks mainly in Southeast Asia

and the Americas, respectively [24]. In Korea, an outbreak of IMP-producing carbapenem-nonsusceptible *K. pneumoniae* strains from four ICU patients was reported in 2017 [25]. After the first identification of VIM in 1999, it has spread mainly in *Pseudomonas aeruginosa*, with several outbreaks [26, 27].

The predominant CPE genotypes in Korea showed hospital-specific differences. In particular, hospital C had more NDM and OXA-48-like producers than KPC compared with the other hospitals. Thus, the control of nosocomial spread of CPE may require different measures depending on the local circumstances.

KPC-producing *Enterobacteriaceae* isolates were highly nonsusceptible to cefotaxime, ceftazidime, aztreonam, ertapenem, imipenem, and meropenem. Treatment options for infections caused by KPC producers remain limited because these strains are mostly multidrug-nonsusceptible [7]. Among cephalosporins, nonsusceptibility rates for cefepime (71.5% of *K. pneumoniae* isolates and 60.9% of non-*K. pneumoniae* isolates) were lower than those for cefotaxime and ceftazidime (>90% of both *K. pneumoniae* and non-*K. pneumoniae* isolates). Cefepime is a fourth-generation cephalosporin proven to be an extended-spectrum antimicrobial against gram-negative pathogens. Compared with other cephalosporins, cefepime exhibits high affinity for KPC, which has led to high susceptibility rates in previous studies [28 29]. The use of cefepime in combination with amoxicillin-clavulanate has been recommended for the treatment of KPC-producing *K. pneumoniae* infections [30].

In line with our results, NDM-producing *Enterobacteriaceae* were nonsusceptible to nearly all antimicrobials, except tigecycline [2, 31]. Typically, metallo-β-lactamases, such as NDM, weakly hydrolyze aztreonam [32]. However, the NDM-producing *K. pneumoniae* isolates exhibited a high nonsusceptibility rate (>90%) to aztreonam in clinical samples. The isolates likely had extended-spectrum β-lactamase (ESBL) or permeability defects simultaneously [31]. Plasmids containing *NDM* are diverse and harbor numerous nonsusceptible genes related to carbapenemases, such as OXA-48, VIM types, ESBLs, AmpCs, and sulfamethoxazole-nonsusceptible genes, and thus frequently provide multidrug or extensive drug nonsusceptibility [7, 31].

In general, OXA-48-like producers effectively hydrolyze penicillins, whereas they weakly hydrolyze carbapenems and cephalosporins [23]. Non-*K. pneumoniae* isolates harboring *OXA-48-like* in our study showed similar trends. However, OXA-48-like-producing *K. pneumoniae* isolates exhibited high nonsusceptibility (>90%) to all cephalosporins, aztreonam, carbapenems, fluoroquinolones, and even trimethoprim-sulfamethoxazole. When combining ESBL or permeability defects, OXA-48 producers showed a high level of nonsusceptibility to broad-spectrum cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems [23]. In a previous study in Korea, all OXA-232-producing *K. pneumoniae* isolates co-produced ESBL, such as CTX-M-15 [9]. Therefore, only tigecycline remains effective against these OXA-48-producing *K. pneumoniae* isolates [7, 9, 23]. The dissemination of the *OXA-48*-like gene to various *Enterobacteriaceae* has been worrisome, because it is unusually located on a small and self-transferable plasmid [23, 33].

This study has some limitations. We only evaluated carbapenemases, not ESBLs and AmpCs. However, most CPE isolated in Korea also harbor an ESBL [9]. Differences in the commercial platforms utilized for the identification and antimicrobial susceptibility testing in the different hospitals might have affected the results. In addition, hospital size likely influenced the distribution results.

In summary, the impact of CPE in Korea is primarily due to KPC-, NDM-, and OXA-48-like-producing *K. pneumoniae* isolates. *Enterobacteriaceae* isolates harboring these carbapenemase genes were mostly multidrug-nonsusceptible or extensively drug-nonsusceptible. Moreover, a stepwise and significant increase in CPE was noted in recent years. These genetic trends have affected the antimicrobial susceptibility patterns. These worsening antimicrobial nonsusceptibility trends should be reflected in precise and aggressive control strategies to combat CPE.

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AUTHOR CONTRIBUTIONS

Song W designed the study; Jeong S and Lee N analyzed the data and wrote the manuscript; and Kim HS, Kim HS, Park MJ, and Kim JS edited the manuscript. All authors reviewed and approved the manuscript.

CONFLICTS OF INTEREST

None declared.

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ORCID

Seri Jeong https://orcid.org/0000-0002-4199-7033
Kibum Jeon https://orcid.org/0000-0002-1333-5366
Nuri Lee https://orcid.org/0000-0001-7727-8279
Han-Sung Kim https://orcid.org/0000-0002-5481-5390
Hyun Soo Kim https://orcid.org/0000-0002-7026-6715
Min-Jeong Park https://orcid.org/0000-0002-2775-2128
Jae-Seok Kim https://orcid.org/0000-0001-6025-0341
Wonkeun Song https://orcid.org/0000-0001-5056-9033

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