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Epidemiological Study of KPC-2 Producing *Klebsiella pneumoniae* Isolated in Daejeon During a 4-Year Period

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Abstract

The emergence and dissemination of carbapenemase-producing *Enterobacteriaceae* (CPE), particularly the *Klebsiella pneumoniae* carbapenemase-2 (KPC-2)-producing *Klebsiella pneumoniae*, has been rapidly increasing worldwide and is becoming a serious public health threat. Since the epidemiology and characteristics of these KPC-2-producing *K. pneumoniae* vary according to the region and period under consideration, this study investigated the prevalence of carbapenemases and the epidemiological relationship of 78 carbapenem-resistant *K. pneumoniae* (CRKP) isolated from tertiary hospitals in Daejeon, from March 2017 to December 2020. The antimicrobial susceptibility tests were identified using the disk-diffusion method. PCR and DNA sequencing were used to determine the carbapenemase genes. In addition, molecular epidemiology was performed by multilocus sequence typing (MLST). Among the 78 CRKP isolates, 35 isolates (44.9%) were carbapenemase-producing *K. pneumoniae* (CPKP) and the major carbapenemase type was KPC-2 (30 isolates, 85.7%). The New Delhi metallo-enzyme-1 (NDM-1) and NDM-5 were identified in 4 isolates (11.4%) and 1 isolate (2.9%), respectively. MLST analysis showed 10 sequence types (STs) and the most prevalent ST was ST307 (51.4%, 18/35). All the ST307 isolates were KPC-2-producing *K. pneumoniae* and were multidrug-resistant (MDR). In addition, ST307 has gradually emerged during a four-year period. These findings indicate that continuous monitoring and proper infection control are needed to prevent the spread of KPC-2-producing *K. pneumoniae* ST307.

Keywords

Beta-lactamase KPC-2
Carbapenemase
Klebsiella pneumoniae

1. Introduction

Following the increasing spread of *Enterobacteriaceae* that produce extended-spectrum β-lactamase (ESBL), such as TEM, SHV, and CTX-M, which can degrade β-lactam antibiotics, carbapenems, which have excellent broad-spectrum therapeutic effects, have been used as the primary antibiotics for ESBL-producing *Enterobacteriaceae* [1]. Recently, with the increased use of carbapenems, carbapenem-resistant *Enterobacteriaceae* (CRE) has been rapidly increasing worldwide, becoming a serious problem [2,3]. These CREs can be classified into carbapenemase-nonproducing *Enterobacteriaceae* (CNPE) and carbapenemase-producing *Enterobacteriaceae* (CPE) [4]. Inactivation of antibiotics due to carbapenemase production is a major mechanism for acquiring carbapenem resistance, and there are concerns about the spread of carbapenem resistance through homologous or heterologous transfer by plasmid and transposon [5].

The first reported CPE was in 1993 producing non-metalloenzyme carbapenemase (NMC), and various CPEs have been identified ever since with an increasing occurrence frequency [2]. In the case of South Korea, looking at the number of CPE isolates reported to the Korean Centers for Disease Control and Prevention (KCDC), there has been a sharp increase each year, with 565 cases in 2015, 1,453 cases in 2016, and 2,657 cases in 2017 [6].

Furthermore, the species of CPE isolates reported since 2012 have mostly been *Klebsiella pneumoniae*, and since 2015, the predominant carbapenemase enzyme in CPE isolates reported has been *Klebsiella pneumoniae* carbapenemase (KPC), with KPC-2 being the most frequently identified subtype. Previous studies in South Korea also reported that KPC-2-producing *K. pneumoniae* had the highest proportion among CPE [6-8]. Recent studies in Europe reported an increase in colistin resistance among KPC-producing *K. pneumoniae* [9,10], and domestic research in South Korea has also raised concerns as most CPE, including KPC-producing *K. pneumoniae*, have been reported as multidrug-resistant [11,12].

To continuously monitor and manage the rapid spread and increasing antibiotic resistance of these CPEs, various epidemiological studies tailored to the characteristics of the period, region, and healthcare facilities are necessary. Therefore, this study aims to analyze carbapenemase genes and investigate antibiotic resistance and epidemiological relationships for carbapenem-resistant *K. pneumoniae* (CRKP) isolated from three tertiary hospitals in Daejeon over a four-year period.

2. Materials and methods

2.1. Collection and identification of strains

This study targeted a total of 78 strains of CRKP isolated from three tertiary hospitals in Daejeon from March 2017 to December 2020. Among them, strains repeatedly isolated from the same patient were excluded from the collection. Isolates obtained from clinical specimens were identified using the VITEK® 2 system (bioMérieux, Marcy-l'Étoile, France), and CRKP strains were selected based on their resistance to ertapenem, imipenem, and meropenem.

2.2. Antibiotic susceptibility testing

Antibiotic susceptibility testing for amikacin, gentamicin, ertapenem, imipenem, meropenem, ceftazidime, cefotaxime, cefepime, ciprofloxacin, trimethoprim/sulfamethoxazole, chloramphenicol, tigecycline (bioMérieux) was performed using the disk diffusion method on Mueller-Hinton agar medium (Difco, Cockeysville, MD, USA) following the Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. For quality control, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were tested simultaneously to ensure that the results fell within the acceptable range.

2.3. Detection of carbapenemase

To determine the presence of carbapenemase in the 78 CRKP strains, PCR was performed using previously

used primers (**Table 1**) [14]. First, the target strains were inoculated into brain heart infusion broth (Difco) and cultured at 37°C for 24 hours. Genomic DNA was then extracted using the Genomic DNA Prep Kit (Solgent, Daejeon, Korea). A reaction mixture was prepared, consisting of 5 μL of DNA extraction buffer, 2.5 μL of 10× Taq buffer, 0.5 μL of 10 mM dNTP mix, 10 pmol of each primer, 0.7 U Taq DNA polymerase (Solgent), and distilled water, with a total volume of 25 μL. The PCR process involved an initial denaturation at 94°C for 10 min, followed by 36 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 40 s, extension at 72°C for 50 s, and a final extension at 72°C for 5 min. The PCR products were electrophoresed on a 1% agarose gel and stained with ethidium bromide for 30 minutes, and the bands were visualized. The amplicons were then purified using a PCR purification kit (Solgent) and subjected to DNA sequencing using the BigDye™ Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA, USA) and an ABI PRISM® 3730XL DNA Analyzer (PE Applied Biosystems). The obtained nucleotide sequences for each strain were compared to *Escherichia coli* ATCC 25922 using the BLAST program provided by the NCBI.

2.4. Multilocus sequence typing analysis

Multilocus sequence typing (MLST) analysis was conducted following the method described on the *Klebsiella pneumoniae* MLST database website

Table 1. Oligonucleotide primers used in the current study

Genes	Sequence (5' – 3')	Annealing temperature (°C)	Product size (bp)
Carbapenemase gene primers [14]			
bla_{IMP}	F: GGA ATA GAG TGG CTT AAY TCT C R: GGT TTA AYA AAA CAA CCA CC	52	232
bla_{VIM}	F: GAT GGT GTT TGG TCG CAT A R: CGA ATG CGC AGC ACC AG	52	390
bla_{OXA-48}	F: GCG TGG TTA AGG ATG AAC AC R: CAT CAA GTT CAA CCC AAC CG	52	438
bla_{NDM}	F: GGT TTG GCG ATC TGG TTT TC R: CGG AAT GGC TCA TCA CGA TC	52	621
bla_{KPC}	F: CGT CTA GTT CTG CTG TCT TG R: CTT GTC ATC CTT GTT AGG CG	52	798
MLST gene primers [15]			
*rpo*B	F: GGC GAA ATG GCW GAG AAC CA R: GAG TCT TCG AAG TTG TAA CC	50	501
*gap*A	F: TGA AAT ATG ACT CCA CTC ACG G R: CTT CAG AAG CGG CTT TGA TGG CTT	60	450
mdh	F: CCC AAC TCG CTT CAG GTT CAG R: CCG TTT TTC CCC AGC AGC AG	50	477
pgi	F: GAG AAA AAC CTG CCT GTA CTG CTG GC R: CGC GCC ACG CTT TAT AGC GGT TAA T	50	432
*pho*E	F: ACC TAC CGC AAC ACC GAC TTC TTC GG R: TGA TCA GAA CTG GTA GGT GAT	50	420
*inf*B	F: CTC GCT GCT GGA CTA TAT TCG R: CGC TTT CAG CTC AAG AAC TTC	50	318
*ton*B	F: CTT TAT ACC TCG GTA CAT CAG GTT R: ATT CGC CGG CTG RGC RGA GAG	45	414

Abbreviation: MLST, multilocus sequence typing.

(https://bigsdb.pasteur.fr/klebsiella/). Similar to the carbapenemase detection, a reaction mixture was prepared, consisting of 5 μL of DNA extract, 2.5 μL of 10× Taq buffer, 0.5 μL of 10 mM dNTP mix, 10 pmol of each primer, 0.7 U Taq DNA polymerase (Solgent), and distilled water, with a total volume of 25 μL. Seven housekeeping genes (*rpo*B, *gap*A, *mdh*, *pgi*, *pho*E, *inf*B, *ton*B) were amplified using the Gene Amp PCR System 9600 (Perkin-Elmer, Norwalk, CT, USA). The PCR process involved an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 95°C for 20 s, annealing at 50°C (60°C or 45°C) for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min (**Table 1**) [15]. The PCR products were electrophoresed on a 1% agarose gel and stained with ethidium bromide for 30 min, and the bands were visualized. The amplicons were purified using a PCR purification kit (Solgent) and subjected to DNA sequencing using the BigDye™ Terminator Cycle Sequencing Kit (PE Applied Biosystems) and an ABI PRISM® 3730XL DNA analyzer (PE Applied Biosystems). The obtained nucleotide sequences for each of the seven housekeeping genes were submitted to the MLST database to determine allelic numbers and sequence types (ST).

3. Result

3.1. Confirmation and analysis of carbapenemase

As a result of PCR and nucleotide sequencing analysis to detect carbapenemase genes, out of a total of 78 CRKP strains, 35 strains (44.9%) were confirmed as carbapenemase-producing *K. pneumoniae* (CPKP). Among these, 30 strains (85.7%) were identified as KPC-2, 4 strains (11.4%) as NDM-1, and 1 strain (2.9%) as NDM-5. When looking at the annual frequency of CPKP among the 35 strains, it showed a gradual increase over the four years, with 14.3% (5 strains) in 2017, 20.0% (7 strains) in 2018, 28.6% (10 strains) in 2019, and 37.1% (13 strains) in 2020.

3.2. MLST analysis of CPKP

After conducting MLST on the 35 CPKP strains, a total of 10 sequence types (STs) were identified (**Table 2**). The most common type among these was ST307, which was confirmed in 18 strains (51.4%). Following this, ST789 was identified in 4 strains (11.4%), ST48 and ST147 in 3 strains each (8.6%), and ST11 in 2 strains (5.7%). The remaining five STs (ST25, ST337, ST395, ST714, and ST1944) were identified in one strain each (2.9%).

When examining the types of STs identified annually, in 2017 and 2018, there were four different STs each, with three STs (ST307, ST789, and ST11) being the same in both years (**Table 3**). In 2019, two STs were distributed, and in 2020, five STs were identified. Over the four years from 2017 to 2020, the ST type that was consistently identified was ST307, with the highest proportion in 2018 at 42.9% (3 out of 7 strains), 70.0% in 2019 (7 out of 10 strains), and 53.8% in 2020 (7 out of 13 strains), except for 2017.

Based on the types of clinical samples, the results of the 35 CPKP strains showed that 21 strains (60.0%) were most commonly isolated from feces (including rectal swabs), and 7 STs were identified (**Table 4**). Among these, ST307 was confirmed in 15 strains (71.4%), followed by the remaining 6 STs (ST48, ST147, ST337, ST714, ST781, and ST1944) each being identified in one isolate (4.8%). Next, 6 strains (17.1%) of CPKP were isolated from urine, with 4 STs were identified. ST789 was confirmed in 3 strains (50.0%), while ST11, ST147, and ST307 were each identified in 1 strain (16.7%). Among the five strains (14.3%) isolated from sputum, four STs were confirmed, with ST48 being confirmed in two strains (40.0%), and ST11, ST25, and ST147 being each identified in one strain (20.0%). The two strains (5.7%) isolated from bile were both identified as ST307, and the one strain (2.9%) isolated from blood was identified as ST395.

Analyzing the types of STs based on carbapenemase production, out of the 30 strains confirmed with KPC-

Table 2. MLST analysis of 35 carbapenemase-producing *Klebsiella pneumoniae*

ST	Allelic profile							No. of isolates (%)
	*rpo*B	*gap*A	*mdh*	*pgi*	*pho*E	*inf*B	*ton*B	
307	1	4	2	52	1	1	7	18 (51.4)
789	1	25	1	1	20	10	22	4 (11.4)
48	1	2	2	2	7	5	10	3 (8.6)
147	4	3	6	1	7	4	38	3 (8.6)
11	1	3	1	1	1	3	4	2 (5.7)
25	4	2	1	1	10	1	13	1 (2.9)
337	1	2	11	1	1	1	13	1 (2.9)
395	1	3	2	4	1	1	4	1 (2.9)
714	4	2	2	2	6	3	160	1 (2.9)
1994	115	2	1	6	9	1	182	1 (2.9)

Abbreviation: ST, sequence type.

Table 3. Prevalence of ST based on year in 35 CPKP isolates collected during the four-year period

Years	No. of isolates (%)	ST (*n*)
2017	5 (14.3)	789 (2), 337 (1), 307 (1), 11 (1)
2018	7 (20.0)	307 (3), 789 (2), 395 (1), 11 (1)
2019	10 (28.6)	307 (7), 48 (3)
2020	13 (37.1)	307 (7), 147 (3), 1944 (1), 714 (1), 25 (1)

Abbreviation: ST, sequence type.

Table 4. Prevalence of ST based on specimen in 35 CPKP isolates

Specimens	No. of isolates (%)	ST (*n*)
Stool	21 (60.0)	307 (15), 48 (1), 147 (1), 337 (1), 714 (1), 789 (1), 1944 (1)
Urine	6 (17.1)	789 (3), 11 (1), 147 (1), 307 (1)
Sputum	5 (14.3)	48 (2), 11 (1), 25 (1), 147 (1)
Bile	2 (5.7)	307 (2)
Blood	1 (2.9)	395 (1)

Abbreviation: ST, sequence type.

Table 5. Prevalence of ST based on carbapenemase in 35 CPKP isolates

Carbapenemase	No. of isolates (%)	ST (*n*)
KPC-2	30 (85.7)	307 (18), 789 (4), 48 (3), 11 (2), 25 (1), 337 (1), 395 (1)
NDM-1	4 (11.4)	147 (3), 714 (1)
NDM-5	1 (2.9)	1944 (1)

Abbreviation: ST, sequence type.

2, 7 STs were identified, with ST307 having the highest distribution in 18 strains (60.0%) (**Table 5**). Following these, ST789 was confirmed in 4 strains (13.3%), ST48 in 3 strains (10.0%), ST11 in 2 strains (6.7%), and ST25, ST337, and ST395 each in 1 strain (3.3%). For the 4 strains confirmed with NDM-1, 2 STs were identified, with ST147 having the highest proportion in 3 strains (75.0%), and ST714 being confirmed in 1 strain (25.0%). The one strain confirmed with NDM-5 was distributed within ST1944.

Table 6. Antibiotic susceptibility pattern of 35 CPKP isolates according to ST

ST	*n*	AMK			GEN			ETP			IPM			MPM			CAZ			CTX			FEP			CIP			TMP/SMX			CHL			TGC		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
307	18	17	0	1	4	1	13	0	0	18	0	0	18	0	0	18	0	0	18	0	0	18	0	0	18	0	0	18	0	0	18	3	7	8	6	5	7
789	4	4	0	0	4	0	0	0	0	4	0	0	4	0	0	4	0	0	4	0	0	4	0	0	4	0	0	4	0	0	4	0	1	3	2	2	0
48	3	3	0	0	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	3	0	0
147	3	3	0	0	3	0	0	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	1	0	2	2	1	0
11	2	2	0	0	1	0	1	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	1	0	1	1	0	1	1	0	1	1	1	0
25	1	1	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1
337	1	1	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0
395	1	1	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0
714	1	1	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0
1944	1	1	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	0	1	1	0	0
Total	35	34	0	1	16	1	18	0	0	35	0	0	35	0	0	35	0	0	35	0	0	35	0	2	33	2	0	33	5	0	30	6	8	21	17	10	8

Abbreviation: ST, sequence type; AMK, amikacin; GEN, gentamicin; ETP, ertapenem; IPM, imipenem; MEM, meropenem; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; CIP, ciprofloxacin; TMP/SMX, trimethoprim/sulfamethoxazole; CHL, chloramphenicol; TGC, tigecycline; S, susceptible; I, intermediate resistant; R, resistant.

3.3. Antibiotic susceptibility patterns

After conducting antibiotic susceptibility testing on the 35 CPKP strains, all 35 strains (100%) showed resistance to ertapenem, imipenem, meropenem, ceftazidime, and cefotaxime, while 94.3% (33 strains) showed high resistance to cefepime and ciprofloxacin (**Table 6**). In sequence, 85.7% (30 strains) showed resistance to trimethoprim/sulfamethoxazole, 60.0% (21 strains) to chloramphenicol, and 51.4% (18 strains) to gentamicin, with tigecycline showing a relatively low resistance of 22.9% (8 strains) and amikacin showing very low resistance of 2.9% (1 strain).

Among the 18 strains of ST307, 8 out of 12 antibiotics showed complete resistance (ertapenem, imipenem, meropenem, ceftazidime, cefotaxime, cefepime, ciprofloxacin, trimethoprim/sulfamethoxazole). ST307 showed resistance to amikacin in 1 strain, and it showed resistance to tigecycline in 87.5% (7 strains) out of the 8 strains. It was also identified that 72.2% (13 strains) out of the 18 strains were resistant to gentamicin, and 38.1% (8 strains) out of the 21 strains were resistant to chloramphenicol.

4. Discussion

As the occurrence and frequency of CPE continue to rise worldwide, there are growing concerns about patient treatment and infection control. In South Korea, CPE, including CRKP, is one of the major multidrug-resistant bacteria involved in nosocomial infections and the spread of antimicrobial resistance in the community. It exhibits a persistent increasing trend, necessitating active surveillance and strict infection measures.

In this study, CPKP was identified in 44.9% (35/78) of CRKP strains isolated from tertiary hospitals in Daejeon over the past 4 years. Among the CPKP, KPC-2-producing *K. pneumoniae* was the most prevalent, accounting for 85.7% (30/35). These results are consistent with previous studies by Ahn *et al*. (2016) and Hong *et al*. (2020), which reported KPC-producing

K. pneumoniae as the most common type of CPE [11,12]. KPC-producing CPE has been identified since the late 1990s and has been associated with a significant increase in mortality rates in some regions since its spread in Europe in the mid-2000s [9,10,16]. In South Korea, KPC has been the most commonly identified CPE, with KPC-2 being the most frequently reported subtype since its first identification in *K. pneumoniae* in 2010 [7,17,18].

Additionally, NDM-1-producing *K. pneumoniae* accounted for 11.4% (4/35), and NDM-5-producing *K. pneumoniae* accounted for 2.9% (1/35). KPC-producing *K. pneumoniae* is reported predominantly in countries such as Brazil, Argentina, Poland, Germany, France, Spain, and China, while NDM-producing *K. pneumoniae* is highly predominant in countries such as Canada, Greece, Belgium, Sweden, Norway, India, Pakistan, and China [2,19].

Molecular epidemiological analysis using MLST revealed that ST307 was the most common type, accounting for 51.4% (18/35) of the CPKP strains. ST307 has been consistently distributed from 2017 to 2020 and was confirmed as KPC-2-producing *K. pneumoniae*, showing multidrug resistance. ST307 is recognized as one of the globally dominant clones, reported in Italy, South Korea, the United States, Mexico, and China, and associated with high resistance to fluoroquinolones, third-generation cephalosporins, and carbapenems [8,20-23]. Recent studies have demonstrated an increasing prevalence of KPC-producing *K. pneumoniae*, mainly with ST11 and ST307, since its first identification in South Korea in 2014 [8].

The second most common ST type was ST789, representing 11.4% (4/35) of the CPKP strains, all confirmed as KPC-2-producing *K. pneumoniae*. However, a study from China in 2021 reported ST789 in NDM-5-producing *K. pneumoniae* associated with neonatal infections and categorized it as a new high-risk ST type [24]. Thirdly, ST48 and ST147 accounted for 8.6% each. Notably, 3 ST48 strains were distributed in 2019, and all strains were KPC-2-producing *K. pneumoniae*. In contrast, 3 ST147 strains were distributed in 2020, and all strains were NDM-1-producing *K. pneumoniae*. ST49 was initially reported as the predominant clone in South Korea and Thailand and was linked to concerns about potential high-risk clones due to some exhibiting resistance to tigecycline [25]. ST147 has been reported in recent studies in Poland, Iran, Pakistan, and the United States as NDM-1-producing *K. pneumoniae* [26-28].

ST11, representing 5.7%, was the fourth most common type among the CPKP strains. ST11 is a single locus variant of the globally prevalent ST258, with major clones reported throughout Europe, Asia, and Latin America [5,29]. A study by Andrade *et al*. from Brazil in 2014 reported concerns about the global clonal spread of ST11, as many virulence factors involved in colonization, biofilm formation, phagocytosis defense, and multidrug resistance were identified in ST11 [30]. Other identified ST25, ST337, ST395, ST714, and ST1944 are also likely to develop into high-risk major clones in Korea in the future, and follow-up studies are required.

In summary, this study identified a persistently increasing prevalence of multidrug-resistant ST307 KPC-2-producing *K. pneumoniae* in the Daejeon region over the past four years, reflecting the global dominance of this clone. To prevent the spread of CPE, continued surveillance and effective infection control measures are crucial.

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Disclosure statement

The author declares no conflict of interest.

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