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Molecular Characterization of Carbapenem-resistant, Colistin-resistant *Klebsiella pneumoniae* Isolates from a Tertiary Hospital in Jeonbuk, Korea

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Klebsiella pneumoniae resistant to both carbapenem and colistin has become a major clinical challenge. Although such resistant isolates are rare, they have been sporadically reported from many areas of the world. To investigate the prevalence and resistant mechanisms to carbapenem-resistant, colistin-resistant *K. pneumoniae* among Korean patients, we selected these resistant *K. pneumoniae* from clinical isolates collected over a period of five years at a tertiary hospital in Jeonbuk, Korea. The minimum inhibitory concentration of a variety of antibiotics against the resistant isolates was determined by the macrodilution method or the E-test. PCR analysis was used to determine sequence types (STs) and identify the genes involved in resistance to carbapenem and colistin. In 338 *K. pneumoniae* clinical isolates, two exhibited both carbapenem and colistin resistance. The ST of these belonged to ST11 and ST258, the most prevalent STs with *K. pneumoniae* carbapenemases, but the isolates did not carry any prevalent carbapenem-hydrolyzing β-lactamase genes. The carbapenem resistance was mediated by loss of porins OmpK35 and/or OmpK36 associated with DHA-1 AmpC β-lactamase. The colistin resistance was caused by amino acid changes in PmrB, PmrC, PmrE, PmrK, and MgrB, including novel amino acid changes in PmrE and PmrK. We first reported novel carbapenem-resistant and colistin-resistant *K. pneumoniae* ST11 and ST258 with mutations within *ompK35*, *ompK36*, *mgrB*, and *pmr* genes in Korea. The results suggest that non-carbapenemase-producing carbapenem-resistant, colistin-resistant *K. pneumoniae* might be prevalent in Korea, and that better measures are necessary to control the spread of the resistant pathogen.

Key Words: *K. pneumoniae*, Carbapenem, Colistin, Porin, *pmr* genes

INTRODUCTION

Klebsiella pneumoniae, a gram-negative bacterium, is an important human opportunistic pathogen that can cause serious hospital- and community-acquired diseases, including septicemia, pneumonia, and urinary tract infections (1). The emergence and dissemination of multidrug-resistant (MDR) and extremely drug resistant (XDR)

K. pneumoniae are of great health concern in many countries (1). In particular, carbapenem-resistant *K. pneumoniae* (CRKP) was recognized as a serious threat worldwide because the isolates often were co-resistant to most antibiotics, such as penicillins, cephalosporins, fluoroquinolones, and aminoglycosides (2). The most prevalent mechanism of resistance to carbapenems has been associated with production of carbapenem-hydrolyzing β-lactamases, including *K. pneumoniae* carbapenemases (KPCs) (Ambler class A), metallo-β-lactamases (MBLs) (Ambler class B), and OXA-48-like β-lactamases (Ambler class D) (2, 3). Carbapenem resistance has been conferred primarily by carbapenemase production mediated by KPCs (4). Worldwide, the predominant KPC-producing *K. pneumoniae* isolates are of sequence types (ST) 11 and 258; ST11 prevails mainly in East Asia, especially in China, and ST258 is most common in North America, Latin America, and several Europe countries (3, 5). In addition, some studies described how non-carbapenemase-producing CRKP (nCP-CRKP) can occur through impaired permeability via loss of outer membrane porins, OmpK35 and OmpK36, linked with AmpC-type β-lactamases (6, 7).

Infection with CRKP has limited therapeutic options, but one of the available antimicrobials is colistin, a last resort antibiotic that acts by disrupting the anionic lipopolysaccharide (LPS) component of the gram-negative outer membrane. The most common mechanism for colistin resistance in *K. pneumoniae* is mediated by modification of the LPS through cationic substitution (8). The LPS modification depends on a large panel of regulatory genes and operons (8, 9). Regulatory genes include those encoding proteins involved in the PmrA/PmrB system, the PhoP/PhoQ system, and a negative regulator (MgrB) of the PhoP/PhoQ system; the *pmrC* gene, *pmrE* gene, and *pmrHFIJKLM* operon are associated with LPS modification. Recently, plasmid-mediated colistin-resistant *mcr* genes were identified in *Escherichia coli* and *K. pneumoniae* (8). More worryingly, colistin-resistant KPC-producing *K. pneumoniae* has been reported sporadically from Korea, Greece, North America, and other European countries in the past few years (8, 10). Therefore, the emergence and spread of colistin resistance in CRKP have increased the importance of long-term surveillance and an approach to gain insight into the exact mechanisms responsible for the resistance.

Although a number of studies has reported the prevalence and mechanism of *K. pneumoniae* resistance to carbapenem or colistin, *K. pneumoniae* that is resistant to both antibiotics has not been well demonstrated despite the increase in colistin-resistant CRKP. In this study, we screened carbapenem-resistant, colistin-resistant *K. pneumoniae* in clinical isolates collected from a Korean tertiary hospital to investigate the prevalence and mechanisms of carbapenem-resistant, colistin-resistant *K. pneumoniae* in Korea. Two isolates with both carbapenem and colistin resistance were identified and analyzed for STs and the genes associated with resistance to these antibiotics. Although the isolates did not carry any prevalent carbapenem-hydrolyzing β-lactamase genes, such as KPCs and OXA-48-like β-lactamases, we identified mutations within the *ompK35, ompK36*, *mgrB*, and *pmr* genes that have not been observed in carbapenem-resistant, colistin-resistant *K. pneumoniae* ST11 and ST258 in Korea.

MATERIALS AND METHODS

Bacterial isolates and antimicrobial susceptibility

From 2008 to 2010 and from 2015 to 2018, 338 clinical isolates of *K. pneumoniae* were obtained from a tertiary hospital in Jeonbuk, Korea (Jeonbuk National University Hospital Culture Collection for Pathogens). The primary identification and antimicrobial susceptibility test were performed with a VITEK-2 system (bioMérieux, Marcy l'Etoile, France). The minimum inhibitory concentration (MIC) of antibiotics was determined by the macrodilution method using 2 ml of cation-adjusted Muller-Hinton broth in a standard glass test tube according to Clinical and Laboratory Standards Institute guidelines (8, 11). The MIC also was measured using the E-test carried out on a Muller-Hinton agar plate according to the manufacturer's instructions (bioMérieux, Marcy l'Etoile, France; Liofilchem Inc., MA, USA). As a control, *K. pneumoniae* ATCC 13883 purchased from the American Type Culture Collection (ATCC, VA, USA) was used.

Multilocus sequence typing (MLST)

MLST of *K. pneumoniae* isolates was performed with seven housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpoB,* and *tonB*), according to a previously described protocol (12). Allele sequences and ST were assigned according to Institute Pasteur's MLST scheme for *K. pneumoniae*.

Analysis of antimicrobial resistance-related genes by polymerase chain reaction (PCR) amplification and sequencing

Whole-bacterial DNA was prepared from bacterial cells lysed by heating at 95°C for 10 min. The colistin resistance-associated genes (*pmrA, pmrB, pmrC, pmrD, pmrE, pmrK, phoP, phoQ,* and *mgrB*) were amplified using specific primers (Table 1) (13-15). PCR amplification detection for plasmid-mediated *mcr* genes was performed using previously described primers (16, 17). The presence of extended-spectrum β-lactamase and carbapenem-hydrolyzing β-lactamase genes, such as *bla*CTX-M, *bla*SHV, *bla*TEM, *bla*KPCs, and *bla*OXA-48, was screened as previously described (18-20). PCR analysis for the *ompK35* and *ompK36* genes, *K. pneumoniae* porin genes associated with carbapenem resistance, was carried using the following primers: *ompK35* forward, 5′- GCCGCCCGCACTCCACGCTG-3′; *ompK35* reverse, 5′-CAGAAAGTTATGGAGCCACG-3′; *ompK36* forward, 5′-GTATCATATTCTTGTTGGATTA-3′; and *ompK36* reverse, 5′-GAAAGTCATATCCATTAATCG-3′. PCR analysis was used with previously described primers to identify the genes encoding AmpC-type β-lactamases from the following six families (21): MOX (MOX-1, MOX-2, CMY-1, and CMY-8 to CMY-11), CIT (LAT-1 to LAT-4, CMY-2 to CMY-7, and BIL-1), DHA (DHA-1 and DHA-2), ACC, EBC (MIR-1T and ACT-1), and FOX (FOX-1 to FOX-5b). The amplified PCR products were confirmed by agarose gel electrophoresis, purified from the agarose gel, and sequenced. The nucleotide and deduced protein sequences were analyzed at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST) program.

Table 1. Primers for PCR amplification of the *pmr*, *mgrB*, *phoP*, and *phoQ* genes

Gene	Primer direction	Primer sequence	Reference
pmrA	Forward	CATTTCCGCGCACTGTCTGC	14
	Reverse	CAGCTTTCAGTTGCAAACAG	
pmrB	Forward	ACCTACGCGAAAAGATTGGC	14
	Reverse	GATGAGGATAGCGCCCATGC	
pmrC (eptA)	Forward	ATGTCGTTATTGACTCTGCGTC	this study
	Reverse	tcaaccagcctctctacatgg	
pmrD	Forward	TAGATGCTTCATGACGCTCTCTC	12
	Reverse	gtgttatttgccggcgtttgtc	
pmrE	Forward	ATGAAAATTACTATTTCCGGTAC	this study
	Reverse	ctaatcattaccaaacagatcg	
pmrK	Forward	cctcaggcaaagtggttc	this study
	Reverse	gccgtccgcgacgggacg	
mgrB	Forward	CAGCCAGCGATGCCAGATTT	13
	Reverse	CCTGGCGTGATTTTGACACGA	
phoP	Forward	GAGCTTCAGACTACTATCGA	14
	Reverse	GGGAAGATATGCCGCAACAG	
phoQ	Forward	ATACCCACAGGACGTCATCA	14
	Reverse	CAGGTGTCTGACAGGGATTA	

RESULTS AND DISCUSSION

Isolation of non-carbapenemase-producing carbapenem-resistant *K. pneumoniae* (nCP-CRKP) with colistin resistance

CRKP has been frequently identified as multidrug-resistant (MDR), including the potential development of resistance to colistin, a last-line antibiotic, and is associated with high rates of morbidity and mortality worldwide (22). To investigate the prevalence and the detailed mechanisms of MDR *K. pneumoniae* with carbapenem and colitis resistance, we assessed carbapenem and colistin resistance from the *K. pneumoniae* isolates collected by a Korean tertiary hospital, Jeonbuk National University Hospital Culture Collection for Pathogens. Out of 338 *K. pneumoniae* isolates, two (0.6%) were identified as both carbapenem and colistin resistance. Interestingly, a high rate of colistin resistance in CRKP was observed as previously reported (23, 24). Two (22%) of nine CRKP possessed colistin resistance (Table 2); the colistin MIC was ≥16 μg/ml using the broth culture macrodilution method, but control *K. pneumoniae* ATCC13883 was not resistant to colistin. The two colistin-resistant isolates, LTH36 and LTH51, were resistant to all tested β-lactam antibiotics, including carbapenems (ertapenem, imipenem, and meropenem) and broad spectrum β-lactams (ceftazidime, cefotaxime, ciprofloxacin, and cefpirome) (Table 2). Intriguingly, the MIC (≥32 μg/ml) for imipenem was higher in the two isolates than those of most CRKP within our health system (imipenem MIC range, 1 to 4 μg/ml) (25). In addition, the isolates were resistant to aztreonam and aminoglycoside antibiotics (amikacin and gentamicin) (Table 2). However, one isolate, LTH51, remained susceptible only to tigecycline, as previously reported in other colistin-resistant *K. pneumoniae*, although the other isolate, LTH36, was not resistant to tigecycline and trimethoprim/sulfamethoxazole (Table 2) (22, 26).

Since the isolates exhibited resistant phenotypes to a variety of β-lactam antibiotics, including carbapenems, we investigated the presence of extend-spectrum-β-lactamase (ESBL) and carbapenem-hydrolyzing β-lactamase genes using PCR analysis and nucleotide sequencing. For ESBL genes, bla_{CTX-M}, bla_{SHV}, and bla_{TEM} genes were analyzed. The LTH36 isolate carried the bla_{SHV-31} and $bla_{TEM-116}$ genes, and the LTH51 isolate revealed the presence of the $bla_{CTX-M-3}$, bla_{SHV-11}, and bla_{TEM-1} genes. For detection of genes associated with carbapenem resistance, we evaluated the most prevalent carbapenem-hydrolyzing β-lactamase genes, bla_{KPCs} and bla_{OXA-48}. However, the bla_{KPC} and bla_{OXA-48} genes were not identified in the two isolates. Since carbapenem resistance can occur with various types of carbapenemases, we screened for other carbapenem- hydrolyzing β-lactamase genes with previously described primers to amplify the following genes (18): $bla_{OXA-48-like}$ including OXA-199 and OXA-370, bla_{VIM}-type, bla_{NDM-1}, bla_{IMP} variants except IMP-3, 16, 27, 31, 34, and 35, $bla_{OXA-23-like}$, $bla_{SME-1\ to\ 5}$, bla_{IMI}-type, bla_{IMP-27}, $bla_{NDM-2\ to\ 10\ and\ 12}$, $bla_{IMP-3\ and\ 34}$, $bla_{GES-1\ to\ 22\ and\ 24}$, bla_{GIM-1}, $bla_{OXA-2-,\ 10-,\ 51-,\ and\ 58-like}$, bla_{DIM-1}, and bla_{CMY-2}. Unexpectedly, the tested genes involved in carbapenem resistance were not detected in the two *K. pneumoniae* isolates, LTH36 and LTH51.

To investigate diversity, distribution, and relationship of our bla_{KPC}- and bla_{OXA-48}-negative CRKP isolates with global isolates, we performed MLST. Interestingly, the STs of our colistin-resistant CRKP isolates LTH36 and LTH51 were ST11 and ST258,

Table 2. Antimicrobial susceptibility profiles of carbapenem-resistant, colistin-resistant *K. pneumoniae* isolates

Isolate	MIC (μg/ml)												
	ETP[a]	IPM[b]	MEM[a]	CST[b]	AMK[a]	GEN[a]	TGC[b]	AZT[b]	CAZ[b]	CTX[b]	CIP[a]	CPR[a]	STX[a]
ATCC13883	‹0.25	1	‹0.25	0.5	1	‹0.25	0.5	‹0.25	0.25	‹0.25	‹0.25	‹0.25	‹0.25
LTH36	≥32	≥32	8	16	≥256	≥256	1	128	≥256	≥256	≥32	8	0.25
LTH51	≥32	≥32	24	32	≥256	≥256	1	≥256	≥256	≥256	≥32	≥256	≥32

Minimum inhibitory concentration (MIC) was measured by [a]quantitative E-test or [b]broth macrodilution assay. *K. pneumoniae* ATCC 13883 was used as a control strain. Antibiotic resistance was considered as an MIC of ›2 μg/ml. ETP, ertapenem; IPM, imipenem; MEM, meropenem; CST, colistin; AMK, amikacin; GEN, gentamicin; TGC, tigecycline; AZT, aztreonam; CAZ, ceftazidime; CTX, cefotaxime; CIP, ciprofloxacin; CPR, cefpirome; STX, trimethoprim/sulfamethoxazole.

respectively, which are the most global STs of KPC-producing CRKP (3, 5, 27). Although the CRKP isolates with ST11 and ST258 were previously isolated in Korea, they carried KPC and showed a different susceptibility to colistin: the ST11 exhibited a colistin-resistant phenotype, but the ST258 did not (10, 28, 29). Taken together, we first isolated *K. pneumoniae* ST11 and ST258 in Korea possessing both carbapenem and colistin resistance despite the absence of previously known carbapenem-hydrolyzing β-lactamase genes.

Characterization of the carbapenem resistance in the nCP-CRKP isolates with colistin-resistance

Because our two isolates were resistant to carbapenems without producing the most prevalent carbapenemases, we investigated an alternative mechanism for carbapenem resistance, which could result from mutation(s) in outer membrane major porins, OmpK35 and OmpK36, in association with AmpC-type β-lactamases. The deficiency of porin as a selective barrier would result in carbapenem resistance through reducing the permeability of porin; in addition, AmpC β-lactamases without carbapenemase activity could be involved in trapping carbapenems, which prevent the antibiotics from accessing their targets (6).

To test this possibility, we analyzed the sequences of porin genes, *ompK35* and *ompK36*, and the presence of *bla*$_{AmpC}$ genes from six families: ACC, CIT, DHA, EBC, FOX, and MOX. In the sequence analysis of *ompK35* and *ompK36* compared with those of a control *K. pneumoniae* ATCC13883 (*ompK35* and *ompk36*, GenBank accession no. CP040993.1), our LTH36 and LTH51 isolates revealed the lack of one or both of the two major porins. The LTH36 isolate revealed 1-bp deletion at position 54 and 1-bp insertion at position 419 of the *ompK35* and *ompk36* genes, respectively, which created amino acid changes and premature terminations after Gly-18 and Ser-139 in OmpK35 and OpmK36, respectively. In the LTH51 isolate, *ompk36* sequencing reveled an ochre nonsense mutation after the start codon that could lead to the loss of OpmK36 porin, although there was wild type of the *ompK35* gene. Furthermore, PCR and sequencing analysis of genes encoding AmpC β-lactamases showed that all of the nCP-CRKP isolates with colistin resistance harbored the DHA-1 *ampC* β-lactamase gene. These results suggest that the carbapenem resistance of the nCP-CRKP isolates LTH36 and LTH51 could be associated with loss of the major porins OmpK35 and/or OpmK36 and involved in DHA-1 AmpC β-lactamase production.

Characterization of the colistin resistance in the nCP-CRKP isolates with colistin resistance

To investigate the molecular mechanisms related to colistin resistance in the nCP-CRKP isolates with colistin resistance, we evaluated genetic mutations in the PmrA/PmrB and PhoP/PhoQ two-component regulatory systems, the PmrD connector of the two-component systems, the MgrB negative regulator of the PhoP/PhoQ, and LPS-modifying enzymes including PmrC, PmrE, and PmrK. Sequence analysis revealed amino acid variations in PmrB, PmrC, PmrE, PmrK, and MgrB compared with the colistin-susceptible *K. pneumoniae* reference strain, MGH78578 (Table 3). The same amino acid changes that were previously reported as contributing to colistin resistance were observed in PmrB (T246A and R256G) and PmrC (C27F, I138V, and Q319R) from the two isolates (8, 30, 31). Furthermore, we identified novel amino acid substitutions in PmrE (V17I, A33S, H68Q, T105A, A165E, D172N, A274G, N354D/A, E371D, and T373A) and PmrK (M114L, V117I, R372K, and G462R) from the LTH36 or LTH51 isolates, although more studies are required to prove a connection between the mutations and colistin resistance. Interestingly, although the two *K. pneumoniae* isolates belonged to different STs, there were the amino acid changes at the same position in PmrB, PmrC, PmrE, and PmrK: PmrB, T246A, and R256G; PmrC, C27F, I138V, and Q319R; PmrE, N354D/A and T373A; PmrK, M114L, V117I, and R372K. In the *mgrB* gene analysis of the two isolates, insertion sequences (ISs) were found in all isolates and caused insertional inactivation of the MgrB protein by missense and frameshift mutations, which resulted in amino acid residue and terminational changes after Leu-17 and Cys-39 of MgrB from the LTH36 and LTH51 isolates, respectively. However, PmrA, PmrD, PhoP, and PhoQ of the isolates were identical to the wild-type proteins of the reference strain *K. pneumoniae* MGH78578, and plasmid-mediated

Table 3. Sequence types and genetic mutations in carbapenem-resistant, colistin-resistant *K. pneumoniae* isolates

Isolate	ST	PmrB	PmrC	PmrE	PmrK	MgrB
LTH36	11	T246A, R256G	C27F, I138V, Q319R	V17I, A33S, H68Q, T105A, A165E, D172N, A274G, N354D, E371D, T373A	M114L, V117I, R372K, G462R ,	Frameshift (IS)
LTH51	258	T246A, R256G	C27F, I138V, Q319R	N354A, T373A	M114L, V117I, R372K	Frameshift (IS)

The insertion sequences (ISs) were inserted after nucleotide position 51 and 117 of *mgrB* (GenBank accession number KF852760.1) from the LTH36 and LTH51, respectively, and were identical to the followed regions: *mgrB* IS of LTH36, accession no. CP066855.1, region 2271513 to 2272285; *mgrB* IS of LTH51, accession no. MW650888.1, region 93977 to 94744. The reference GenBank accession number for *pmrA*, *pmrB*, *pmrC* (*eptA*), *pmrD*, *pmrE*, *pmrK*, *phoP*, and *phoQ* gene is CP000647.1

colistin resistance genes, *mcr-1* to *mcr-9*, were not detected in the isolates. These results suggest that the amino acid changes in PmrB, PmrC, PmrE, PmrK, and MgrB of the colistin-resistant CRKP isolates might be associated with colistin resistance.

In conclusion, we detected a high rate (22%, 2 of 9) of colistin resistance among CRKP although two (0.6%) *K. pneumoniae* isolates with carbapenem and colistin resistance were found in 338 clinical isolates collected by a Korean tertiary hospital. The two isolates belonged to ST11 and ST258, the most prevalent STs of KPC-producing CRKP, but these carbapenem resistances were related to truncating mutations in the major porin *ompK35* and/or *ompK36* genes associated with DHA-1 AmpC β-lactamase. In colistin resistance, these isolates carried novel and previously reported mutations in the *pmrB*, *pmrC*, *pmrE*, *pmrK*, and *mgrB* genes that could confer colistin resistance (Table 3). Taken together, we identified and characterized the detail molecular mechanisms of carbapenem and colistin resistance in *K. pneumoniae* ST11 and ST258 isolate that have not been reported in Korea. These results suggest that colistin-resistant nCP-CRKP with novel mutations emerged in Korea and could spread throughout the country. In addition, this study showed the importance of judicious use of antibiotics with proper susceptibility analysis as well as continuous surveillance and strict infection control measures on colistin-resistant CRKP to prevent the resistant bacteria from becoming prevalent in the hospital setting.

ABBREVIATION

KPC, *K. pneumoniae* carbapenemase; CRKP, carbapenem-resistant *K. pneumoniae*; nCP-CRKP, non-carbapenemase-producing carbapenem-resistant *K. pneumoniae;* MDR, multidrug-resistant; MIC, minimum inhibitory concentration; MLST, multilocus sequence typing; ST, sequence type

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CONFLICT OF INTEREST

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

REFERENCES

1) Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiol Rev* 2017;41:252-75.

2) Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbapenemase-producing *Klebsiella pneumoniae*: Epidemiology, genetic context, treatment options, and detection methods. *Front Microbiol* 2016;7:895.

3) Pitout JD, Nordmann P, Poirel L. Carbapenemase-Producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. *Antimicrob Agents Chemother* 2015;59:5873-84.

4) Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, et al. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect Dis* 2013;13:785-96.

5) Qi Y, Wei Z, Ji S, Du X, Shen P, Yu Y. ST11, the dominant clone of KPC-producing *Klebsiella pneumoniae* in China. *J Antimicrob Chemother* 2011;66:307-12.

6) Hamzaoui Z, Ocampo-Sosa A, Fernandez Martinez M, Landolsi S, Ferjani S, Maamar E, et al. Role of association of OmpK35 and OmpK36 alteration and bla_{ESBL} and/or bla_{AmpC} genes in conferring carbapenem resistance among non-carbapenemase-producing *Klebsiella pneumoniae*. *Int J Antimicrob Agents* 2018;52:898-905.

7) Leavitt A, Chmelnitsky I, Colodner R, Ofek I, Carmeli Y, Navon-Venezia S. Ertapenem resistance among extended-spectrum-beta-lactamase-producing *Klebsiella pneumoniae* isolates. *J Clin Microbiol* 2009;47:969-74.

8) Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. *Clin Microbiol Rev* 2017;30:557-96.

9) Groisman EA. The pleiotropic two-component regulatory system PhoP-PhoQ. *J Bacteriol* 2001;183:1835-42.

10) Rhee JY, Park YK, Shin JY, Choi JY, Lee MY, Peck KR, et al. KPC-producing extreme drug-resistant *Klebsiella pneumoniae* isolate from a patient with diabetes mellitus and chronic renal failure on hemodialysis in South Korea. *Antimicrob Agents Chemother* 2010;54:2278-9.

11) Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 24th informational supplement. CLSI document M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA, 2014.

12) Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol* 2005;43:4178-82.

13) Cheng HY, Chen YF, Peng HL. Molecular characterization of the PhoPQ-PmrD-PmrAB mediated pathway regulating polymyxin B resistance in *Klebsiella pneumoniae* CG43. *J Biomed Sci* 2010;17:60.

14) Choi MJ, Kim S, Ko KS. Pathways Regulating the *pbgP* operon and colistin resistance in *Klebsiella pneumoniae* strains. *J Microbiol Biotechnol* 2016;26:1620-8.

15) Jayol A, Poirel L, Brink A, Villegas MV, Yilmaz M, Nordmann P. Resistance to colistin associated with a single amino acid change in protein PmrB among *Klebsiella pneumoniae* isolates of worldwide origin. *Antimicrob Agents Chemother* 2014;58:4762-6.

16) Kieffer N, Royer G, Decousser JW, Bourrel AS, Palmieri M, Ortiz De La Rosa JM, et al. *mcr-9*, an inducible gene encoding an acquired phosphoethanolamine transferase in *Escherichia coli*, and it's origin. *Antimicrob Agents Chemother* 2019;63:e00965-19.

17) Wang X, Wang Y, Zhou Y, Li J, Yin W, Wang S, et al. Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. *Emerg Microbes Infect* 2018;7:122.

18) Mlynarcik P, Roderova M, Kolar M. Primer evaluation for PCR and its application for detection of carbapenemases in *Enterobacteriaceae*. *Jundishapur J Microbiol* 2016;9:e29314.

19) Schechner V, Straus-Robinson K, Schwartz D, Pfeffer I, Tarabeia J, Moskovich R, et al. Evaluation of PCR-based testing for surveillance of KPC-producing carbapenem-resistant members of the *Enterobacteriaceae* family. *J Clin Microbiol* 2009;47:3261-5.

20) Tofteland S, Haldorsen B, Dahl KH, Simonsen GS, Steinbakk M, Walsh TR, et al. Effects of phenotype and genotype on methods for detection of extended-spectrum-beta-lactamase-producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Norway. *J Clin Microbiol* 2007;45:199-205.

21) Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol* 2002;40:2153-62.

22) Bogdanovich T, Adams-Haduch JM, Tian GB, Nguyen MH, Kwak EJ, Muto CA, et al. Colistin-resistant, *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* belonging to the international epidemic clone ST258. *Clin Infect Dis* 2011;53:373-6.

23) Rojas LJ, Salim M, Cober E, Richter SS, Perez F, Salata RA, et al. Colistin resistance in carbapenem-resistant *Klebsiella pneumoniae*: Laboratory detection and impact on mortality. *Clin Infect Dis* 2017;64:711-8.

24) van Duin D, Doi Y. Outbreak of colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae*: Are we at the end of the road? *J Clin Microbiol* 2015;53:3116-7.

25) Marchaim D, Chopra T, Pogue JM, Perez F, Hujer AM, Rudin S, et al. Outbreak of colistin-resistant, carbapenem-resistant *Klebsiella pneumoniae* in metropolitan Detroit, Michigan. *Antimicrob Agents Chemother* 2011;55:593-9.

26) Tóth A, Damjanova I, Puskás E, Jánvári L, Farkas M, Dobák A, et al. Emergence of a colistin-resistant KPC-2-producing *Klebsiella pneumoniae* ST258 clone in Hungary. *Eur J Clin Microbiol Infect Dis* 2010;29:765-9.

27) Ko KS. Antibiotic-resistant clones in Gram-negative pathogens: presence of global clones in Korea. *J Microbiol* 2019;57:195-202.

28) Livermore DM. Current epidemiology and growing resistance of gram-negative pathogens. *Korean J Intern Med* 2012;27:128-42.

29) Yoo JS, Kim HM, Yoo JI, Yang JW, Kim HS, Chung GT, et al. Detection of clonal KPC-2-producing *Klebsiella pneumoniae* ST258 in Korea during nationwide surveillance in 2011. *J Med Microbiol* 2013;62:1338-42.

30) Cheong HS, Kim SY, Seo J, Wi YM, Peck KR, Ko KS. Colistin resistance and extensive genetic variations in PmrAB and PhoPQ in *Klebsiella Pneumoniae* isolates from South Korea. *Curr Microbiol* 2020;77:2307-11.

31) Jaidane N, Bonnin RA, Mansour W, Girlich D, Creton E, Cotellon G, et al. Genomic insights into colistin-resistant *Klebsiella pneumoniae* from a Tunisian Teaching Hospital. *Antimicrob Agents Chemother* 2018;62:e01601-17.