원저
Lab Med Online
Vol. 8, No. 3: 99-106, July 2018
https://doi.org/10.3343/lmo.2018.8.3.99

임상미생물학 LMO

최근 임상에서 분리된 다제 내성 *Enterobacter cloacae*의 생물학적 유전학적 특성

Biological and Genetic Characteristics of Clinically Isolated *Enterobacter cloacae* with Multidrug Resistance

김재중[1] · 구선회[2]
Jae-Jung Kim, M.T.[1], Sun Hoe Koo, M.D.[2]

가톨릭대학교 대전성모병원 진단검사의학과[1], 충남대학교병원 진단검사의학과[2]
Department of Laboratory Medicine[1], St. Mary's Hospital, Daejeon Catholic University, Daejeon; Department of Laboratory Medicine[2], Chungnam National University Hospital, Daejeon, Korea

Background: From January 2014 to December 2015, 69 clones of *Enterobacter cloacae* showing multidrug resistance to six classes of antimicrobial agents were collected from two medical centers in Korea.
Methods: Minimum inhibitory concentrations were determined using the E-test method, and 17 genes were detected using polymerase chain reaction (PCR). The epidemiological relatedness of the strains was identified using repetitive element sequence-based PCR and multilocus sequence typing.
Results: The 69 *E. cloacae* clones produced extended spectrum β lactamase (ESBL) and AmpC and showed multidrug resistance to cefotaxime, ceftazidime, and aztreonam. We identified the following sequence types: ST56 of type VI for ESBL *SHV* (N=12, 17.4%); ST53, ST114, ST113, and ST550 of types I, IV, VI, and VII, respectively, for *CTX-M* (N=11, 15.9%); and ST668 of type III for the carbapenemase *NDM* gene (N=1, 1.5%). The AmpC *DHA* gene (N=2, 2.89%) was confirmed as ST134, although its type was not identified, whereas *EBC* (*MIR/ACT*; N=18, 26.1%) was identified as ST53, ST24, ST41, ST114, ST442, ST446, ST484, and ST550 of types V, I, III, IV, VII, and VI, respectively. The formed subclasses were $bla_{CTX\text{-}M\text{-}3}$ and $bla_{CTX\text{-}M\text{-}22}$ by *CTX-M-1*, $bla_{CTX\text{-}M\text{-}9}$ and $bla_{CTX\text{-}M\text{-}125}$ by *CTX-M-9*, $bla_{DHA\text{-}1}$ by *DHA*, and $bla_{MIR\text{-}7}$ and $bla_{ACT\text{-}15,17,18,25,27,28}$ by *EBC* (*MIR/ACT*).
Conclusions: There were no epidemiological relationships between the gene products and the occurrence of resistance among the strains.

Key Words: *Enterobacter cloacae*, Extended spectrum β lactamase, AmpC, Repetitive element sequence-based polymerase chain reaction, Multilocus sequence typing, Multidrug resistance

INTRODUCTION

Enterobacter cloacae is a gram-negative bacterium that can cause pathogenic infections in patients in the intensive care unit. This bacterium is widely distributed in human environments, including water, soil, and feces, and can cause respiratory, surgical, and urinary tract infections, as well as sepsis and other diseases in newborns [1-4]. Moreover, this strain is commonly detected in nosocomial infections of the urinary tract, wounds, and blood [5].

Multidrug-resistant *E. cloacae* has been detected in clinical specimens and is associated with the high production of extended spectrum β lactamase (ESBL) and AmpC [6]. Although various resistant strains capable of producing ESBL and AmpC have been identified in recent hospital-acquired infections in Korea, few studies have investigated multidrug-resistant *E. cloacae* isolates producing ESBL and AmpC.

In this study, we investigated the biological and genetic characteristics of multidrug-resistant *E. cloacae* collected from two general hospitals located in the central region of Korea.

Corresponding author: Sun Hoe Koo
Department of Laboratory Medicine, Chungnam National University Hospital, 282 Munhwa-ro, Joong-gu, Daejeon 35015, Korea
Tel: +82-42-280-7798, Fax: +82-42-257-5365, E-mail: shkoo@cnu.ac.kr

Received: November 22, 2017
Revision received: November 27, 2017
Accepted: November 27, 2017

This article is available from http://www.labmedonline.org
© 2018, Laboratory Medicine Online
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

MATERIALS AND METHODS

1. *Enterobacter cloacae* collection

From January 2014 to December 2015, a total of 69 *E. cloacae* clones that were resistant to gentamicin, ertapenem, cefotaxime, ceftazidime, cefepime, ciprofloxacin, aztreonam, and cotrimoxazole (TMP/SMX) were collected without overlap from two medical centers in the central region of Korea using a Vitek 2 Compact 60 instrument (SYSMEX BioMerieux Co., Ltd., Lyon, France) and a Microscan WalkAway 96SI instrument (Siemens Healthcare Diagnostic. Inc., West Sacramento, CA, USA).

2. Antimicrobial susceptibility test

Using Mueller-Hinton agar (ASAN, Daejeon, Korea), the minimum inhibitory concentrations (MICs) were determined by Epsilometer tests (E-tests) based on the Clinical Laboratory Standards Institute guideline [33]. *Escherichia coli* ATCC 25922 was tested at the same time as a control group, and eight types of antibiotic discs were used: gentamicin (BioMerieux SA), ertapenem, cefotaxime, ceftazidime, cefepime, ciprofloxacin, aztreonam, and cotrimoxazole (TMP/SMX).

3. DNA extraction

Single colonies of each *E. cloacae* strain were cultured in sterilized MacConkey agar medium at 37˚C for 18–20 hours, and DNA was extracted using a GeneAll Ribospin extraction kit (GeneAll Biotechnology, Daejeon, Korea) following the manufacturer's instructions. After storage at 20˚C, isolated genomic DNA was used as a DNA template for the amplification of ESBL and AmpC resistance genes and for repetitive element sequence-based polymerase chain reaction (REP-PCR) and multilocus sequence typing (MLST) analysis.

4. Detection of *ESBL* and *AmpC* by PCR

Using an ABI 3730XL system (Applied Biosystems, Foster City, CA, USA), we detected the expression of *CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, TEM, SHV, NDM, IMP, OXA, IMI, VIM, FOX, EBC, CIT, ACC, DHA,* and *MOX* [24-29]. PCR was carried out in a final volume of 25 μL containing 0.5 μL SP Taq (Cosmogenetech, Daejeon, Korea), 2.5 μL of 10× SP Taq buffer, 2.0 μL dNTPs, 5.0 μL Tuning Buffer, 1.0 μL DNA template, 1 μL of each forward and reverse primer (5 μmol), and 12 μL distilled water. ESBL was amplified under the following conditions: pre-denaturation at 95˚C for 5 minutes; 35 cycles of denaturation at 95˚C for 1 minute, annealing at 55˚C for 30 seconds, and extension at 72˚C for 1 minute; and a final extension at 72˚C for 7 minutes. For AmpC, the PCR conditions were as follows: pre-denaturation at 94˚C for 3 minutes; 35 cycles of denaturation at 94˚C for 30 seconds, annealing at 64˚C for 30 seconds, and extension at 72˚C for 1 minute; and a final extension at 72˚C for 7 minutes. The PCR products were verified by electrophoresis on 2% agarose gels containing ethidium bromide and then purified using a PCR purification kit (Cosmogenetech). From the purified DNA, *CTX-M-1, CTX-M-2,* and

Table 1. Sequences of primers for the amplification of the β-lactamase and AmpC genes of *Enterobacter cloacae*

Primer		Primer sequence (5′–3′)	Size	Ref
CTXM-1	F	CCGTCACGCTGTTGTTAGG	693	26
	R	GACGATTTTAGCCGCCGAC		
CTXM-2	F	CGGTGCTTAAACAGAGCGAG	684	
	R	CCATGAATAAGCAGCTGATTGCCC		
CTXM-8	F	ACGCTCAACACCGCGATC	695	
	R	CGTGGGTTCTCGGGGATAA		
CTXM-9	F	GATTGACCGTATTGGGAGTTT	683	
	R	CGGCTGGGTAAAATAGGTCA		
TEM-1	F	ATGAGTATTCAACATTTCCGT	997	27
	R	TTACCAATGCTTAATCAGTGA		
SHV-12	F	CCGGGTTATTCTTATTTGTCGCT	936	
	R	TAGCGTTGCCAGTGCTCG		
NDM-1	F	GCCCAATATTATGCACCCGG	738	28
	R	CTCATCACGATCATGCTGGC		
IMP-1	F	AAGGCGTTTATGTTCATACTTCG	605	
	R	TTTAACCGCCTGCTCTAATGTAA		
OXA-48	F	GATTATCGGAATGCCTGCGG	845	
	R	CTACAAGCGCATCGAGCATCA		
IMI-1	F	AGAGTTCYATTCACCCATCACA	803	29
	R	TCTCCAATCGACCGCATGAA		
VIM-1	F	TGGGCCATTCAGCCAGATC	749	31
	R	TGGGCCATTCAGCCAGATC		
FOX	F	AACATGGGGTATCAGGGAGATG	190	32
	R	CAAAGCGCGTAACCGGATTGG		
EBC	F	TCGGTAAAGCCGATGTTGCGG	302	
	R	CTTCCACTGCGGCTGCCAGTT		
CIT	F	TGGCCAGAACTGACGGCAAA	462	
	R	TTTCTCCTGAACGTGGCTGGC		
ACC	F	AACAGCCTCAGCAGCCGGTTA	346	
	R	TTCGCCGCAATCATCCCTAGC		
DHA	F	AACTTTCACAGG TGTGCTGGGT	405	
	R	CCGTACGCATACTGGCTTTGC		
MOX	F	GCTGCTCAAGGAGCACAGGAT	520	
	R	CACATTGACATAGGTGTGGTGC		

Abbreviations: F, forward; R, reverse.

https://doi.org/10.3343/lmo.2018.8.3.99

CTX-M-9 were sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with sequencing primers and then confirmed by Basic Local Alignment Search Tool (BLAST) [7] software (Table 1).

5. REP-PCR and MLST analysis to examine epidemiological relationships

The epidemiological relationships of the strains were investigated by REP-PCR and MLST. For REP-PCR, the conditions were as follows: pre-denaturation at 94℃ for 15 minutes; 40 cycles of denaturation at 94℃ for 30 seconds, annealing at 60℃ for 1.5 minutes, and extension at 72℃ for 1.5 minutes; and a final extension at 72℃ for 10 minutes. The PCR products were electrophoresed on 2% agarose gels with ethidium bromide and then compared by fingerprints with the naked eye; if at least three bands were different, they were categorized into different types. For MLST, the conditions were as follows: pre-denaturation at 95℃ for 5 minutes; 35 cycles of denaturation at 95℃ for 1 minute, annealing at 55℃ for 30 seconds, and extension at 72℃ for 1 minute; and a final extension at 72℃ for 7 minutes, followed by electrophoresis on 2% agarose gels with ethidium bromide. DNA sequences were analyzed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Finally, the sequencing results of seven housekeeping genes were compared against the MLST database [8], and the allele number and sequence types (STs) were determined (Table 2).

Table 2. Primers for the multilocus sequence typing analysis of *Enterobacter cloacae*

Primer			Sequence (5′–3′)	Position in the target gene	Ref
A	dnaA	F	AYAACCCGCTGTTCCTBTATGGCGGCAC	500–527*	33
		R	KGCCAGCGCCATCGCCATCTGACGCGG	1222–1248*	
	fusA	F	TCGCGTTCGTTAACAAAATGGACCGTAT	413–440*	
		R	TCGCCAGACGGCCCAGAGCCAGACCCAT	1291–1318	
	gyrB	F	TCGACGAAGCGCTCGCGGGTCACTGTAA	143–170	
		R	GCAGAACCGCCCGCGGAGTCCCCTTCCA	1268–1295	
	leuS	F	GATCARCTSCCGGTKATCCTGCCGGAAG	1342–1369*	
		R	ATAGCCGCAATTGCGGTATTGAAGGTCT	2159–2186*	
	pyrG	F	AYCCBGAYGTBATTGCRCAYMAGGCGAT	56–83*	
		R	GCRCGRATYTCVCCCTSHTCGTCCCAGC	563–590*	
	rplB	F	GTAAACCGACATCTCCGGGTCGTCGCCA	17–44*	
		R	ACCTTTGGTCTGAACGCCCCACGGAGTT	735–762*	
	rpoB	F	CCGAACCGTTCCGCGAACATCGCGCTGG	252–280*	
		R	CCAGCAGATCCAGGCTCAGCTCCATGTT	973–1000*	
S	gyrB	F	AAAACCGGTACYATGGTGCGTTTCTGG	484–510*	
		R	GCAGAACCGCCCGCGGAGTCCCCTTCC	1269–1295*	
	fusA	R	ATCTCTTCACGYTTGTTAGCGTGCATCT	1094–1121*	

*These primers were used for sequencing respective amplicons.
Abbreviations: A, amplification; F, forward; R, reverse; S, sequencing.

6. Statistical analysis

Chi-square or Fisher's exact tests were performed to determine resistance according to the occurrence of resistant genes. A *P* value of less than 0.05 was considered statistically significant. SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

RESULTS AND DISCUSSION

E. cloacae clones collected from two medical centers in the central region of Korea exhibited multidrug resistance because of the production of ESBL (N=19, 27.5%) and AmpC (N=20, 29.0%). In general, the amounts of ESBL detected from urine and sputum specimens were higher than those reported by Park et al. [9] and Courdon et al. [10], and more AmpC was generated than what was previously reported by Souna et al. [11]. The resistance rates were higher than the 27.6% for cefotaxime, 21.8% for ceftazidime, and 21.1% for aztreonam reported by the Centers for Disease Control and Prevention of Korea (KARMS) in 2014. The collected *E. cloacae* clones also showed various levels of resistance against gentamicin, ertapenem, cefepime, ciprofloxacin, and cotrimoxazole.

The most common mechanism through which *Enterobacteria* acquires resistance involves the production of AmpC and ESBL. Similar to *E. coli*, *E. cloacae* also obtains resistance through AmpC β-lactamase [12], and resistance against third-generation cephalosporins is caused by ESBL [13]. In this study, *EBC* (*MIR/ACT*; N=18, 26.1%), *SHV* (N=12, 17.4%), and *CTX-M* (N=11, 15.9%) were produced, and the carbapenemases *OXA* (N=1, 1.5%), *NDM* (N=1, 1.5%), and *DHA* (N=1, 1.5%) were generated in some strains (Table 3). These results are similar to those of a report by Harada et al. [14] showing that levels of *SHV* and *CTX-M* had increased globally. The results of this study were also consistent with a report in 2007 showing that the amounts produced were highest for *CTX-M* in the intestinal bacteria *E. coli* and for *SHV-12* in *K. pneumoniae* found in Daejeon city [15]. There were no correlations among the occurrence of genes, resistance due to mismatch, and occurrence of resistance (*P*=0.653, 0.759, and 0.419, respectively). The produced subclasses were $bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-125}}$ for *CTX-M-1*, and $bla_{\text{MIR-7}}$ and $bla_{\text{ACT-15,17,18,25,27,28}}$ for *DHA*. These results differ from those in *E. coli*, which carries *CTX-M-14* and *CTX-M-15* genes

Table 3. Characteristics of multidrug-resistant *Enterobacter cloacae* isolates based on repetitive element sequence-based polymerase chain reaction and multilocus sequence typing

Isolate	Specimen source	REP-PCR	MLST (STs)	β-lactamase	AmpC	GM	ETP	CT	TZ	PM	CL	AT	TS
K1	W	II	668	NDM-1	.	0.5	0.25	>32	16	0.25	0.064	12	0.064
K2	S	I	24	.	DHA, EBC	0.5	0.25	>32	16	0.25	0.064	12	0.064
K3	W	IX	134	.	DHA	1.5	0.064	1	4	0.047	0.38	0.5	0.5
K4	O	V	41	.	EBC	>256	1	>48	>32	24	1.5	128	>32
K5	U	.	280	.	.	0.5	3	>32	>256	1	0.064	128	0.125
K6	U	.	.	.	.	0.5	0.25	>32	32	0.05	0.047	24	0.064
K7	S	.	78	.	.	0.38	0.75	>32	>256	2	0.047	128	0.125
K8	B	I	.	.	.	0.5	0.032	>32	3	16	>32	16	>32
K9	U	VII	.	.	.	0.5	0.19	>32	>256	1.5	0.032	24	0.064
K10	O	VII	279	.	.	0.5	0.125	>32	128	1.5	0.032	16	0.064
K11	O	I	45	SHV-12	.	0.05	0.75	>32	>256	2	0.38	64	0.5
K12	U	VI	41	.	EBC	2	0.25	>32	128	2	1.5	>256	>256
K13	S	VII	53	.	EBC	0.5	0.38	>32	>256	1	0.47	128	0.064
K14	BF	I	.	.	.	0.38	0.5	>32	>256	0.075	0.5	64	0.32
K15	U	.	190	.	.	0.75	0.008	0.19	0.5	0.064	0.064	0.25	0.064
K17	O	VII	245	.	.	>256	0.032	>32	2	8	0.047	8	0.38
K18	U	V	78	.	.	1	0.19	1	1	0.75	1.5	0.38	0.25
K19	U	V	422	.	EBC	>256	1	>32	32	48	>32	64	0.25
K20	B	VI	133	CTX-M-9	.	0.25	0.19	>32	48	0.38	0.023	24	0.064
K21	N.S	.	148	.	.	6	0.38	>32	1.5	3	1	2	>32
K22	Ctip	IX	244	.	.	0.38	0.25	>32	128	1.5	0.047	24	0.094
K23	W	I	.	.	.	0.5	0.25	>32	>256	3	0.064	128	0.047
K24	S	I	53	CTX-M-9	EBC	0.5	0.19	>32	>256	2	0.32	24	0.064
K25	S	I	53	.	EBC	4	0.5	>32	256	3	0.75	64	>32
K26	O	V	484	.	EBC	0.38	0.25	>32	256	1	0.75	96	0.047
K27	S	.	584	.	.	2.5	0.38	>32	64	0.5	0.047	24	0.094
K28	U	VII	550	CTX-M-9	EBC	0.25	0.5	>32	>256	1	0.064	96	0.064
K29	W	.	.	.	.	6	1.5	>32	>256	6	4	128	>32
K30	U	I	144	.	.	1	0.19	>32	>256	1.5	0.032	24	0.064
K31	B	I	604	.	.	1	1.5	>32	>256	16	0.19	256	0.5
K32	S	IX	78	.	.	0.5	0.25	>32	>256	2	0.032	32	0.094
K33	W	III	.	.	.	0.38	0.047	>32	3	16	24	16	>32
K34	U	V	466	.	EBC	0.75	0.023	0.5	1	0.38	3	0.25	1.5
K35	U	VIII	477	.	.	0.75	0.5	>32	16	0.125	0.16	8	0.094
K36	U	VIII	477	.	.	0.75	0.125	>32	256	0.75	0.047	16	0.125
K37	S	III	148	.	.	0.5	0.064	>32	>256	0.5	0.047	24	0.094
K38	U	IV	114	.	.	0.38	0.38	>32	256	4	0.064	48	0.064
K39	S	IV	114	SHV-12	EBC	0.38	0.19	>32	>256	3	0.047	48	0.064
K40	S	IV	114	.	EBC	0.38	0.25	>32	256	4	0.047	48	0.064
K41	BF	.	.	CTX-M-1	EBC	0.5	0.38	>32	>256	4	0.047	48	0.094
K42	S	IV	114	.	EBC	0.5	0.19	>32	64	0.75	0.064	32	0.064
K43	U	I	51	CTX-M-9, SHV-12	.	0.5	0.25	>32	>256	3	0.047	48	0.094
K44	U	IV	114	.	.	6	0.5	>32	256	3	0.19	48	0.064
K45	S	X	.	CTX-M-9, SHV-12	.	0.5	0.25	>32	>256	3	0.047	48	0.094
K46	BF	VI	125	.	.	0.5	0.125	>32	64	0.5	0.047	32	0.064
K47	U	IV	114	SHV-12	EBC	0.38	0.38	>32	48	0.38	0.032	32	0.047

(*Continued to the next page*)

https://doi.org/10.3343/lmo.2018.8.3.99

Table 3. Continued

Isolate	Specimen source	REP-PCR	MLST (STs)	β-lactamase	AmpC	GM	ETP	CT	TZ	PM	CL	AT	TS
K48	U	II	.	CTX-M-9, SHV-12	EBC	0.38	0.38	>32	>256	3	0.032	48	0.094
K49	U	II	175	CTX-M-9, SHV-12	.	0.5	0.32	0.125	0.19	0.064	0.016	0.047	0.094
K50	S	VI	56	SHV-12	.	0.5	0.032	0.19	0.38	0.047	0.016	0.064	0.094
K51	S	II	782	.	.	0.5	0.023	6	16	0.5	0.125	32	>32
K52	PF	IV	114	CTX-M-9	EBC	3	0.25	>32	>256	16	0.75	48	0.094
K53	U	II	.	.	.	0.5	0.25	>32	256	3	0.032	32	0.094
K54	U	VI	.	.	.	0.5	0.047	0.19	0.25	0.047	0.016	0.047	0.125
K55	S	X	.	.	.	0.5	0.008	0.064	0.19	0.047	0.006	0.047	0.064
K56	S	II	.	.	.	0.5	0.38	>32	128	0.5	0.064	32	0.125
K57	BW	II	51	.	.	0.5	0.064	0.75	0.25	0.064	0.023	0.064	0.19
K58	S	XI	350	CTX-M-9, SHV-12	.	0.5	0.125	>32	64	2	0.032	24	0.064
K59	U	II	175	CTX-M-9, SHV-12	.	0.38	0.094	>32	48	1.5	0.016	24	0.094
K60	S	VI	56	SHV-12	.	6	0.19	24	16	0.1	0.19	16	>32
K61	S	III	114	.	.	6	0.064	>32	32	0.5	0.19	32	>32
K62	O	VIII	477	SHV-12	.	0.5	0.25	>32	>256	3	0.32	48	0.094
K63	U	III	148	.	.	0.5	0.25	>32	256	0.5	0.032	24	0.064
K64	U	V	133	.	.	0.25	0.75	>32	>256	3	0.064	64	0.064
K65	BF	III	24	OXA-48	EBC	128	0.38	>32	32	1	0.023	24	>32
K66	U	III	114	.	.	0.38	0.004	0.125	0.125	0.032	0.032	0.047	0.047
K67	S	III	53	.	EBC	12	0.75	>32	64	32	12	128	4
K68	U	III	114	.	.	0.38	0.19	>32	64	0.75	0.5	48	0.047
K69	U	V	61	.	.	48	0.19	>32	24	24	>32	48	48
K70	U	I	329	.	.	0.75	0.19	>32	96	1.5	0.032	24	24

Abbreviations: ATM, aztreonam; B, blood; BF, bile fluid; BW, bronchial wash; CIP, ciprofloxacin; CTX, cefotaxime; Ctip, catheter tip; ETP, ertapenem; GM, gentamicin; NS, nasal swab; O, other; PF, pleural fluid; FEP, cefepime; S, sputum; TS, cotrimoxazole; CAZ, ceftazidime; U, urine; W, wound; ".", Not detected.

with high frequency, and from those in *K. pneumoniae*, in which the *CTX-M-14* and *CTX-M-3* genes are more prevalent. Furthurmore, *bla*$_{CTX-M-3}$ was isolated from plasmids of *E. coli* and *K. pneumoniae* by Liu et al. [16] in 2007 and was also reported by Kim et al. [17] in Korea in 2008. Unlike reports showing that *bla*$_{CTX-M-3}$-producing strains were repeatedly found in the same hospital [18, 19], this gene was detected in only one strain, and we concluded that the resistance by *bla*$_{CTX-M-3}$ had not yet spread. Despite the first report of *bla*$_{CTX-M-22}$ from *E. coli* and *K. pneumoniae* in China in 2002 [20], this gene has not been reported in Korea until now. Moreover, the carbapenemase gene *NDM* was found in four cases in Korea in 2011 [21], and *OXA* was found among intestinal bacteria in Korea [22]; thus, resistance genes in plasmids were thought to have been transferred horizontally from bacteria such as *E. coli* and *K. pneumoniae*. The *DHA* and *EBC* (*MIR/ACT*) genes, which have been shown to cause inductive resistance to AmpR [23, 24], were also shown to cause inductive resistance in this study, although there were no epidemiological relationships among strains, as types and clone classes were different (Fig. 1, Table 3).

As previously reported by Girlich et al. [25], ST114, which was frequently identified, was highly correlated with *EBC* (*MIR/ACT*) AmpC, along with ST53 and ST41. There were also correlations between ST175 and *CTXM-9*, ST175 and *SHV* ESBL, and ST477 and *SHV* ESBL. However, as no resistance genes were detected, a correlation was not identified for ST78.

In summary, multidrug-resistant *E. cloacae* clones collected from two medical centers in the central region of Korea exhibited no epidemiological relationships and showed multidrug resistance through the generation of ESBL and AmpC.

요 약

배경: 2014년 1월부터 2015년 12월까지 중부지방 2곳의 종합병원에서 6개 계열 8종 항균제에 다제 내성을 나타내는 *Enterobacter*

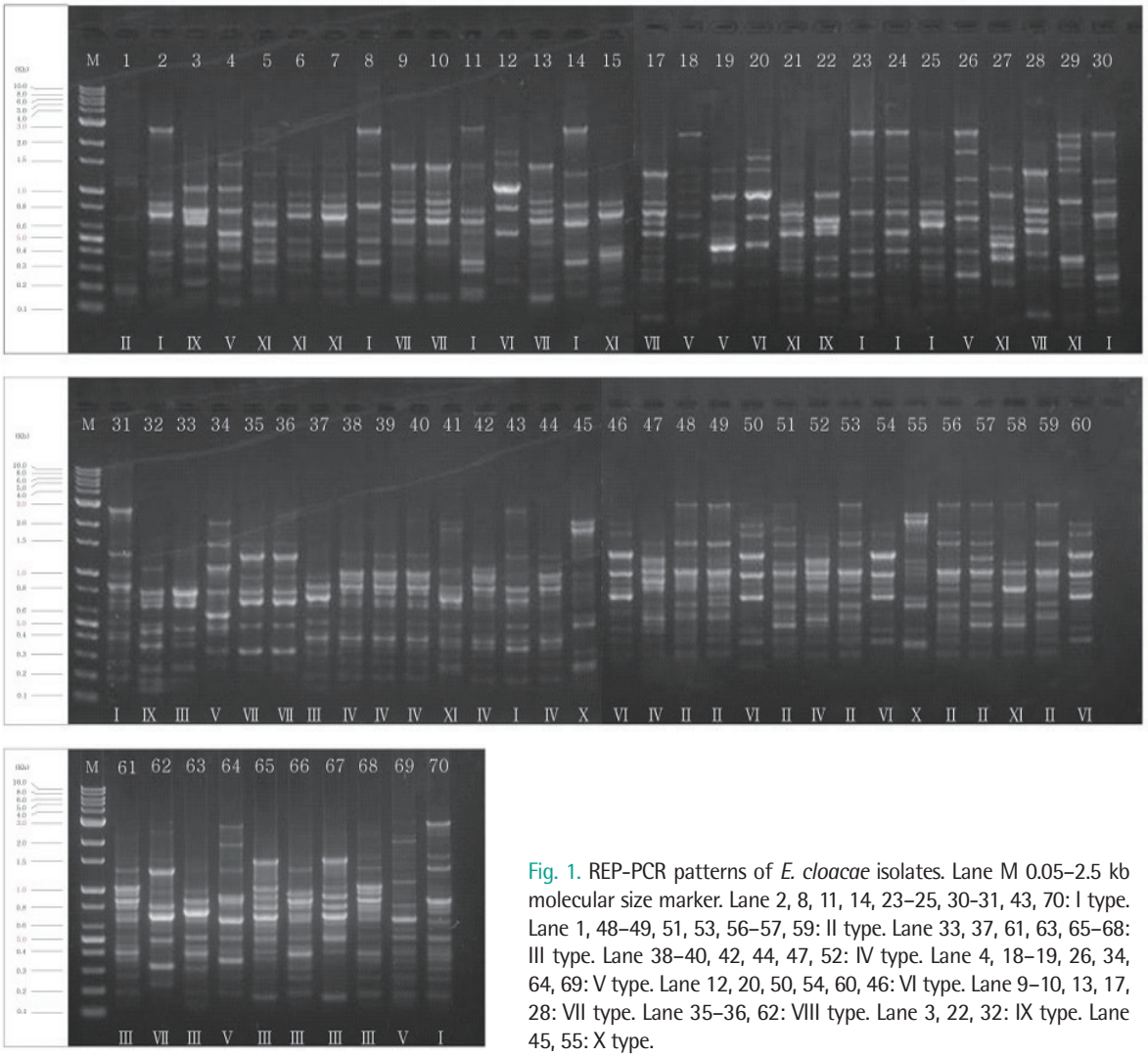

Fig. 1. REP-PCR patterns of *E. cloacae* isolates. Lane M 0.05–2.5 kb molecular size marker. Lane 2, 8, 11, 14, 23–25, 30–31, 43, 70: I type. Lane 1, 48–49, 51, 53, 56–57, 59: II type. Lane 33, 37, 61, 63, 65–68: III type. Lane 38–40, 42, 44, 47, 52: IV type. Lane 4, 18–19, 26, 34, 64, 69: V type. Lane 12, 20, 50, 54, 60, 46: VI type. Lane 9–10, 13, 17, 28: VII type. Lane 35–36, 62: VIII type. Lane 3, 22, 32: IX type. Lane 45, 55: X type.

cloacae 69주를 수집하였다.

방법: E-test법으로 최소억제농도(MIC)를 구하였고, *TEM, SHV, CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, NDM, OXA, IMI, IMP, VIM, FOX, EBC, CIT, ACC, DHA, MOX* 등 17개 유전자를 PCR 증폭 후 검출하였다. 균주 간 역학적 연관성은 REP-PCR과 multilocus sequence typing (MLST)법으로 확인하였다.

결과: *E. cloacae* 69주는 ESBL과 AmpC를 생성하며 cefotaxime, ceftazidime, aztreonam에 다제 내성을 나타냈다. ESBL *SHV* (N=12, 17.4%)는 VI type의 ST56로, *CTX-M* (N=11, 15.9%)은 I, IV, VI, VII type의 ST53, ST114, ST133, ST550로, carbapenemase *NDM* (N=1, 1.5%)은 II type의 ST24, OXA (N=1, 1.5%)는 III type의 ST668로 확인되었다. AmpC *DHA* (N=2, 2.89%)는 type이 동정되지 않은 ST134, *EBC* (*MIR/ACT*) (N=18, 26.1%)는 V, I, III, IV, VII, VI type의 ST53, ST24, ST41, ST114, ST422, ST466, ST484, ST550로 확인되었다. *CTX-M-1*은 $bla_{CTX-M-3}$, $bla_{CTX-M-22}$, *CTX-M-9*은 $bla_{CTX-M-9}$, $bla_{CTX-M-125}$, *DHA*는 bla_{DHA-1}, *EBC* (*MIR/ACT*)는 bla_{MIR-7}, $bla_{ACT-15,17,18,25,27,28}$ 아형을 생성하였다.

결론: 유전자 생성과 내성발생 균주 간 역학적 연관성은 없었다.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

ACKNOWLEDGMENTS

This work was supported by a research fund from Chungnam National University (2016-1635-01).

 https://doi.org/10.3343/lmo.2018.8.3.99

REFERENCES

1. Sanders WE Jr and Sanders CC. *Enterobacter* spp.: pathogens poised to flourish at the turn of the century. Clin Microbiol Rev 1997;10:220-41.
2. Dalben M, Varkulja G, Basso M, Krebs VL, Gibelli MA, van der Heijden I, et al. Investigation of an outbreak of *Enterobacter cloacae* in a neonatal unit and review of the literature. J Hosp Infect 2008;70:7-14.
3. Fernandez A, Pereira MJ, Suarez JM, Poza M, Trevino M, Villalón P, et al. Emergence in Spain of a multidrug-resistant *Enterobacter cloacae* clinical isolate producing SFO-1 extended-spectrum beta-lactamase. J Clin Microbiol 2011;49:822-8.
4. Hamada Y, Watanabe K, Tatsuya T, Mezaki K, Takeuchi S, Shimizu T, et al. Three cases of IMP-type metallo-β-lactamase-producing *Enterobacter cloacae* bloodstream infection in Japan. J Infect Chemother 2013;19:956-8.
5. Schaberg DR, Culver DH, Gaynes RP. Major trends in the microbial etiology of nosocomial infection. Am J Med 1991;91:S72-S75.
6. Sirot D, Sirot J, Labia R, Morand A, Courvalin P, Darfeuille-Michaud A, et al. Transferable resistance to third-generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: identification of CTX-1, a novel β-lactamase. J Antimicrob Chemother 1987;20:323-34.
7. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. Available from http://www.ncbi.nlm.nih.gov/BLAST. Accessed.
8. Jolley K. *Enterobacter cloacae* MLST Databases. Available from http://pubmlst.org/ecloacae/. Accessed.
9. Park YJ, Park SY, Oh EJ, Park JJ, Lee KY, Woo GJ, et al. Occurrence of extended-spectrum β-lactamases among chromosomal AmpC-producing *Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens* in Korea and investigation of screening criteria. Diagn Microbiol Infect Dis 2005;51:265-9.
10. Coudron PE, Moland ES, Sanders CC. Occurrence and detection of extended-spectrum β-lactamases in members of the family *Enterobacteriaceae* at a veterans medical center: seek and you may find. J Clin Microbiol 1997;35:2593-7.
11. Souna D, Amir AS, Bekhoucha SN, Berrazeg M, Drissi M. Molecular typing and characterization of TEM, SHV, CTX-M, and CMY-2 β-lactamases in *Enterobacter cloacae* strains isolated in patients and their hospital environment in the west of Algeria. Med Mal Infect 2014;44:146-52.
12. Livermore DM. β-lactamases in laboratory and clinical resistance. Clin Microbiol Rev 1995;8:557-84.
13. Jeong SH. Extended-spectrum beta-lactams-resistant gram-negative bacilli. Asian Conf Clin Pathol 2000;6:S61-S62.
14. Harada S, Ishii Y, Yamaguchi K. Extended-spectrum beta-lactamases: implications for the clinical laboratory and therapy. Korean J Lab Med 2008;28:401-12.
15. Ko CS, Sung JY, Koo SH, Kwon GC, Shin SY, Park JW. Prevalence of extended-spectrum beta-lactamase in *Escherichia coli* and *Klebsiella pneumoniae* from Daejeon. Korean J Lab Med 2007;27:344-50.
16. Liu, SY, Su LH, Yeh YL, Chu C, Lai JC, Chiu CH. Characterization of plasmids encoding CTX-M-3 extened-spectrum β-lactamase from *Enterobacteriaceae* isolated at a university hospital in Taiwan. Int J Antimicrob Agents 2007;29:440-5.
17. Kim CK, Yum JH, Yong D, Jeong SH, Lee K, Chong Y. Detection of CTX-M-type extended-spectrum β-lactamase in clinical isolates of chromosomal AmpC beta-lactamase-producing *Enterobacteriaceae* from Korea and their molecular characteristics. Korean J Clin Microbiol 2008;11:90-7.
18. Baraniak A, Sadowy E, Hryniewicz W, Gniadkowski M. Two different extended-spectrum β-lactamase (ESBLs) in one of the first ESBL-producing *Salmonella* isolates in Poland. J Clin Microbiol 2002;40:1095-7.
19. Baraniak A, Fiett J, Sulikowska A, Hryniewicz W, Gniadkowski M. Countrywide spread of CTX-M-3 extended-spectrum β-lactamaseproducing microorganisms of the family *Enterobacteriaceae* in Poland. Antimicrob Agents Chemother 2002;46:151-9.
20. Yu Y, Ji S, Chen Y, Zhou W, Wei Z, Li L, et al. Resistance of strains producing extended-spectrum β-lactamases and genotype distribution in China. J Infect 2007;54:53-7.
21. Author XX. First cases of NDM-1 (New Delhi Metallo-beta-lactamase)-producing carbapenem resistant *Enterobacteriaceae* in Korea. Available from http://cdc.go.kr/CDC/cms/content/mobile/52/12552_view.html. Accessed.
22. Jeong SH, Lee KM, Lee J, Bae IK, Kim JS, Kim HS, et al. Clonal and horizontal spread of the $bla_{OXA-232}$ gene among *Enterobacteriaceae* in a Korean hospital. Diagn Microbiol Infect Dis 2015;82:70-2.
23. Barnaud G, Arlet G, Danglot C, Philippon A. Cloning and sequencing of the gene encoding the AmpC β-lactamase of *Morganella morganii*. FEMS Microbiol Lett 1997;148:15-20.
24. Poirel L, Guibert M, Girlich D, Naas T, Nordmann P. Cloning, sequence analyses, expression, and distribution of ampC-ampR from *Morganella morganii* clinical isolates. Antimicrob Agents Chemother 1999;43:769-76.
25. Girlich D, Poirel L, Nordmann P. Clonal distribution of multidrug-resis-

tant *Enterobacter cloacae*. Diagn Microbiol Infect Dis 2015;81:264-8.
26. Ryoo NH, Kim EC, Hong SG, Park YJ, Lee K, Bae IK, et al. Dissemination of SHV-12 and CTX-M-type extended-spectrum β-lactamases among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* and emergence of GES-3 in Korea. J Antimicrob Chemother 2005;56:698-702.
27. Chang FY, Siu LK, Fung CP, Huang MH, Ho M. Diversity of SHV and TEM β-lactamases in *Klebsiella pneumoniae*: gene evolution in Northern Taiwan and two novel β-lactamases, SHV-25 and SHV-26. Antimicrob Agents Chemother 2001;45:2407-13.
28. Bae IK, Kang HK, Jang IH, Lee W, Kim K, Kim JO. Detection of carbapenemase in clinical *Enterobactereriaceae* isolates using the VITEK AST-N202 card. Infect Chemother 2015;47:167-74.
29. Huang L, Wang X, Feng Y, Xie Y, Xie L, Zong Z. First identification of an IMI-1 carbapenemase-producing colistin-resistant *Enterobacter cloacae* in China. Ann Clin Microbiol Antimicrob 2015;14:51.
30. Tato M, Coque TM, Ruíz-Garbajosa P, Pintado V, Cobo J, Sader HS. Complex clonal and plasmid epidemiology in the first outbreak of *Enterobacteriaceae* infection involving VIM-1 metallo-β-lactamase in Spain: toward endemicity? Clin Infect Dis 2007;45:1171-8.
31. Perez-Perez FJ and Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 2002;40:2153-62.
32. Miyoshi-Akiyama T, Hayakawa K, Ohmagari N, Shimojima M, Kirikae T. Multilocus sequence typing (MLST) for characterization of *Enterobacter cloacae*. PLoS One 2013;8:e66358.
33. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; Document M100-S28. Wayne, PA: Clinical and Laboratory Standards Institute, 2018.

https://doi.org/10.3343/lmo.2018.8.3.99