Mechanisms of ertapenem resistance in Enterobacteriaceae isolates in a tertiary university hospital

Hae-Sun Chung,[1] Dongeun Yong,[2] Miae Lee[1]

[1]Department of Laboratory Medicine, Ewha Womans University School of Medicine, Seoul, Korea
[2]Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul, Korea

Correspondence to
Professor Miae Lee, Department of Laboratory Medicine, Ewha Womans University School of Medicine, Seoul 07985, Korea; miae@ewha.ac.kr

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ABSTRACT

The aim of this study was to investigate the molecular mechanisms of ertapenem resistance among Enterobacteriaceae isolates in a clinical microbiology laboratory at a tertiary university hospital. A total of 40 clinical isolates including 20 resistant and 20 intermediate isolates were collected from August 2012 to July 2013. Ertapenem susceptibility was confirmed by the broth microdilution method. PCR and sequencing analysis of carbapenemase, AmpC β-lactamase, and extended-spectrum β-lactamase (ESBL) genes were performed. Outer membrane proteins (OMPs) were examined by urea-sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Molecular epidemiology studies were performed by pulsed-field gel electrophoresis (PFGE). AmpC β-lactamases and ESBLs were found in 32 (80.0%) and 20 (50.0%) of the 40 isolates with ertapenem non-susceptibility, respectively. Distributions of β-lactamase genes differed among the species. One *Citrobacter freundii* isolate among the 40 isolates with ertapenem non-susceptibility carrying the $bla_{\text{IMP-1}}$ associated class 1 integron was detected. SDS-PAGE of OMPs showed altered or greatly diminished expression of porins in all isolates of *Klebsiella pneumoniae* (n=5) and *Enterobacter cloacae* (n=11) with ertapenem resistance. Porin alterations were less common among the isolates with intermediate susceptibility (4/19). Integration of the results of molecular analysis of β-lactamases and OMP analysis revealed that most of the isolates with ertapenem resistance exhibited β-lactamase activity and porin alteration. PFGE revealed that most isolates were epidemiologically unrelated. Ertapenem resistance in clinical Enterobacteriaceae isolates was associated with β-lactamase activity and porin alteration. Even though carbapenemase-producing Enterobacteriaceae are still rare, continuous monitoring and infection control for carbapenem-resistant Enterobacteriaceae are necessary.

Significance of this study

What is already known about this subject?

- There are few data on ertapenem resistance among Enterobacteriaceae isolated from clinical microbiology laboratories.
- Ertapenem resistance in Enterobacteriaceae is known to be primarily caused by mechanisms other than carbapenemases.
- Ertapenem-resistant Enterobacteriaceae infections are associated with poor prognosis when compared to ertapenem-susceptible Enterobacteriaceae infections.

What are the new findings?

- The resistance rate to ertapenem among all Enterobacteriaceae isolates was 2.0% as determined using VITEK2. It was highest in *Enterobacter* spp. (14.0%), and was 2.5% and 0.4% in *Klebsiella pneumoniae* and *Escherichia coli*, respectively. In comparison, the resistance rate to imipenem was 0.1%.
- Most of the ertapenem-resistant isolates had porin alteration. A combination of β-lactamase activity and porin alteration tended to result in higher MICs of ertapenem compared to isolates with β-lactamase activity alone. This suggests that ertapenem resistance in Enterobacteriaceae might result from the accumulation of multiple carbapenem-resistance determinants.
- PFGE revealed genetic diversity among most of the isolates, suggesting that ertapenem resistance in clinical Enterobacteriaceae isolates was reflected independent of its emergence in different strains. Nonetheless, the phenomenon of the same clone spreading in the same ward during a single period was observed in this study.

How might these results change the focus of research or clinical practice?

- It provides useful information about ertapenem resistance and would be helpful for treatment and infection control of erpenem-resistant Enterobacteriaceae.

INTRODUCTION

Ertapenem has been widely used since the early 2000s, but it was only recently added to the routine antimicrobial susceptibility tests for Enterobacteriaceae performed by automated systems. Since then, ertapenem-resistant Enterobacteriaceae isolates have been detected. Even though some reports of worldwide

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surveillance have been published,[1–4] there are few data on ertapenem resistance among Enterobacteriaceae isolated from clinical microbiology laboratories.[5 6]

Ertapenem resistance in Enterobacteriaceae is known to be primarily caused by mechanisms other than carbapenemases, the most common expression of β-lactamases such as an AmpC β-lactamase or an extended-spectrum β-lactamase (ESBL) combined with porin loss.[1 2 4 5 7–9] In general, carbapenems are stable to β-lactamases, but this stability varies between agents, and ertapenem appears to be less stable than other carbapenems: the MICs of ertapenem increased with up to three or four doubling dilutions in Enterobacteriaceae isolates producing ESBLs or AmpC β-lactamases, whereas the MICs of other carbapenems, such as imipenem and meropenem, changed within one or two doubling dilutions.[10–12] Imipenem and meropenem often remain moderately active against isolates with low-level ertapenem resistance.[5–9 13] Ertapenem-resistant Enterobacteriaceae infections are associated with higher mortality rates and poor clinical response rates when compared to ertapenem-susceptible Enterobacteriaceae infections.[14] Understanding the underlying resistance mechanisms is important for treatment and infection control of ertapenem-resistant Enterobacteriaceae.

In this study, we investigated the molecular mechanisms of ertapenem resistance for Enterobacteriaceae isolates in a clinical microbiology laboratory at a tertiary university hospital.

MATERIALS AND METHODS

Isolates

Clinical Enterobacteriaceae isolates were collected from the clinical microbiology laboratory of Ewha Womans University Mokdong Hospital. All of the isolates were resistant to ertapenem using the VITEK2 system between August 2012 and July 2013. The resistance rate to ertapenem among all clinical Enterobacteriaceae isolates was 2.0% (97/4876): 14.0%, 2.5%, and 0.4% in *Enterobacter* spp., *Klebsiella pneumoniae*, and *Escherichia coli*, respectively. A total of 72 Enterobacteriaceae isolates were collected. Among them, 40 clinical isolates, including 20 resistant and 20 intermediate isolates as confirmed by broth microdilution, were analyzed: 26 (65.0%) *Enterobacter* spp., 6 (15.0%) *K. pneumoniae*, 5 (12.5%) *E. coli*, 1 (2.5%) *Citrobacter freundii*, 1 (2.5%) *Providencia rettgeri*, and 1 (2.5%) *Cronobacter sakazakii*. Isolates were collected from various specimens. The surveillance study was not performed; this study was studied retrospectively, so the molecular epidemiological relatedness of the isolates had not been recognized at that time. Bacterial identification was performed using VITEK2 with GN card (bioMérieux Inc., Durham, North Carolina, USA). Clinical features of patients were reviewed through electronic medical records. This study was approved by the Institutional Review Board of Ewha Womans University Mokdong Hospital.

Antimicrobial susceptibility test

Antimicrobial susceptibility test for various agents was performed using VITEK2 with AST-N224 card (bioMérieux Inc.), according to the manufacturer's instructions. Tested antimicrobial agents included ertapenem, imipenem, ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefazolin, cefotaxime, ceftazidime, cefepime, cefoxitin, aztreonam, amikacin, gentamicin, ciprofloxacin, and trimethoprim/sulfamethoxazole. The antimicrobial susceptibility test for the newer antimicrobial agent was not included. The meropenem susceptibility test was performed using MicroScan (Siemens Healthcare Diagnostics Inc., West Sacramento, California, USA).

The broth microdilution method was performed using 96-well broth microdilution panels according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[15 16] The solvent and diluent for preparation of stock solutions of ertapenem were prepared in the cation-adjusted Mueller-Hinton broth (Becton Dickinson, Sparks, Maryland, USA) as described in the CLSI document. The broth microdilution MIC test range was 0.25 μg/mL to 128 μg/mL. Susceptibility results were interpreted using the CLSI guideline recommended in 2013 as follows: ≤0.5 μg/mL for susceptible, 1 μg/mL for intermediate, and ≥2 μg/mL for resistant.[15] *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were used as controls.

Investigation of β-lactamases

Carbapenemase and ampC β-lactamase screening tests

For carbapenemase detection, a modified Hodge test and a carbapenemase inhibition test (Rosco Diagnostica, Taastrup, Denmark) were performed. Chromogenic agar for screening of carbapenemase-producing isolates was also used (bioMérieux Inc). AmpC β-lactamase detection was performed by a cefoxitin-boronic acid disk synergy test.[17] Previously confirmed positive isolates were used as controls.

Molecular analysis of β-lactamase genes

Detection of genes coding for carbapenemases (IMP, SPM, AIM, VIM, OXA, GIM, BIC, SIM, NDM, DIM, and KPC) and AmpC β-lactamases (CMY-1-like, CMY-2-like, DHA-1/2, and MIR-1T/ACT-1) was performed by multiplex PCR as described previously[18 19] with some modification. An additional primer pair was used to amplify the chromosomal *ampC* gene from *E. coli*.[20] Detection of genes coding for ESBL (SHV, TEM, CTX-M-1 group, CTX-M-2 group, CTX-M-8 group, and CTX-M-9 group) was performed by PCR as described previously.[21 22] Carbapenemase in *C. freundii* was confirmed by PCR and sequencing analysis using primers targeting the $bla_{IMP\text{-}1}$ gene and class 1 integron.[23] PCR was performed with PreMix (Bioneer, Daejeon, Korea) containing 1 U of Taq DNA polymerase in a total volume of 20 μL. The total amount of the DNA template in each reaction tube was adjusted to between 50 and 100 ng except for multiplex PCR of the carbapenemase gene, which used 10 ng of the DNA template. The typical PCR program consisted of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of DNA denaturation at 94°C for 30 s, primer annealing at 50°C for 40 s, and primer extension at 72°C for 1 min. Annealing temperatures in multiplex PCR of carbapenemase and AmpC β-lactamase genes were increased to 57°C and 64°C, respectively, to increase stringency. After the last cycle, a final extension step at 72°C for 7 min was performed in all reactions. Five-microliter aliquots of the PCR product were analyzed by gel electrophoresis in 2% agarose. Previously confirmed positive isolates were used as controls.

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Outer membrane protein analysis using sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to investigate alterations in outer membrane proteins (OMPs) as described previously with some modification.[24] Briefly, bacterial cells were disrupted by ultrasonic disintegration and the supernatants were treated with 8 M urea. After incubation for 30 min, OMPs were collected by centrifugation at 25,000 g for 1 h and analyzed by SDS-PAGE on a Mini-PROTEAN 3 Cell apparatus (Bio-Rad, Hercules, California, USA) using 10% (wt/vol) polyacrylamide gels. The gels were stained with Coomassie Brilliant Blue and alteration of porins was determined by comparison of electrophoretic migration patterns between isolates and control strains. OMP analysis was performed for *Enterobacter cloacae*, *K. pneumoniae*, and *E. coli* isolates. *E. cloacae* ATCC 13047, *K. pneumoniae* ATCC 13883, and *E. coli* ATCC 25922 were used as controls.

Molecular epidemiology study using pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) patterns of *Xba*I-restricted genomic DNA were compared to determine the relatedness of the 72 isolates that were identified as ertapenem-resistant by VITEK2. *Xba*I-restricted genomic DNA from isolates was separated by PFGE using a CHEF-DR II system (Bio-Rad) according to the manufacturer's protocol. Dendrograms were generated by the unweighted pair group method with the arithmetic average method, and DNA relatedness was calculated on the basis of the criteria suggested by Tenover *et al*.[25]

RESULTS

Antimicrobial susceptibilities and clinical features of the isolates

Resistance rates for various antimicrobial agents are shown in table 1. For carbapenems, only two ertapenem-resistant isolates were also resistant to imipenem and one was resistant to meropenem. The isolates showed high resistance rates for almost all β-lactams excluding carbapenems such as ampicillin, amoxicillin/clavulanic acid, third-generation cephalosporin (cefotaxime and ceftazidime), cefoxitin, and aztreonam. Isolates were relatively susceptible to non-β-lactams including amikacin, gentamicin, trimethoprim/sulfamethoxazole and ciprofloxacin.

Clinical characteristics of the patients carrying Enterobacteriaceae with a high MIC for ertapenem (≥8 μg/mL) are shown in table 2. All but one isolate with a high MIC for ertapenem were considered to be possible pathogens. Four patients were aged over 70 years. Three patients had malignant disease and one patient was bedridden. One patient was treated with meropenem and showed clinical improvement.

Investigation of β-lactamases

Detection of ESBL and AmpC β-lactamase

All ertapenem non-susceptible isolates (n=40) were positive in the AmpC β-lactamase screening test. AmpC β-lactamases were found in 32 (80.0%) isolates; 24 isolates with $bla_{MIR/ACT}$-like, 5 isolates with bla_{CMY-2}-like, and 4 isolates

Table 1 Antimicrobial susceptibility of the ertapenem non-susceptible Enterobacteriaceae isolates to various agents

Antimicrobial	Resistance rate (%) (Non-susceptibility rate, %)														
	MEM*	IPM	AMP	AMC	TZP	CFZ	CTX	CAZ	FEP	FOX	AZT	AMK	GEN	SXT	CIP
Resistant (n=20)	5 (20)	10 (30)	100 (100)	100 (100)	75 (95)	100 (100)	100 (100)	85 (85)	30 (35)	95 (100)	85 (90)	15 (20)	20 (35)	40 (40)	40 (40)
Intermediate (n=20)	0 (0)	0 (5)	100 (100)	100 (100)	90 (95)	100 (100)	95 (95)	95 (95)	20 (20)	95 (100)	90 (90)	8 (10)	25 (35)	35 (35)	38 (38)

*Meropenem susceptibility test was performed using MicroScan; all other tests were performed using VITEK2.
AMC, amoxicillin/clavulanic acid; AMK, amikacin; AMP, ampicillin; AZT, aztreonam; CAZ, ceftazidime; CFZ, cefazolin; CIP, ciprofloxacin; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; GEN, gentamicin; IPM, imipenem; MEM, meropenem; SXT, trimethoprim/sulfamethoxazole; TZP, piperacillin/tazobactam.

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Table 2 Clinical characteristics of patients carrying Enterobacteriaceae with a high MIC for ertapenem

Isolate	Specimen (duration of hospital stay prior to isolation)	Age/sex (Dept)	Clinical illness	Comorbidity	Previous hospital stay	Previous use of carbapenem	Pathogen	Treatment	Outcome	MIC* (μg/mL) ETP	IPM	MEM
EU-E-12-36	Urine (1 day)	3 m/F (PED)	Bronchiolitis	None	Yes	None	Not likely	AMC	Improved	16	2	2
EU-E-12-34	Wound (17 days)	70 yr/M (GS)	Wound infection	CBD cancer, s/p PPPD	Yes	Yes	Possible pathogen	CRO	Recurred	8	1	2
EU-E-12-27	Sputum (3 days)	82 yr/M (MP)	Aspiration pneumonia	HTN, old CVA, T-SAH, Lt. hemiplegia	Yes	Yes	Possible pathogen	MEM	Improved	128	4	>8
EU-E-13-35	Bile (10 days)	70 yr/F (MG)	Sepsis	CBD cancer	Yes	Yes	Pathogen	MEM	Improved	128	2	≤1
EU-E-13-22	Blood (11 days)	80 yr/F (MH)	Listeria septicemia	MDS	Yes	None	Pathogen	CAZ AMP	Expired	16	2	≤1

*MICs of ertapenem, imipenem, and meropenem were measured by the broth microdilution method, VITEK2, and MicroScan, respectively.
AMC, amoxicillin/clavulanic acid; AMP, ampicillin; CAZ, ceftazidime; CBD, common bile duct; CRO, ceftriaxone; CVA, cerebral vascular accident; ETP, ertapenem; GS, general surgery; HTN, hypertension; IPM, imipenem; m, month; MDS, myelodysplastic syndrome; MEM, meropenem; MG, gastroenterology; MH, hematoncology; MP, pulmonology; PED, pediatrics; PPPD, pylorus-preserving pancreatico-duodenectomy (Whipple procedure); T-SAH, traumatic subarachnoid hemorrhage; yr, year.

with bla_{DHA} (table 3). ESBL genes were detected in 20 (50.0%) isolates; 13 isolates with $bla_{CTX\text{-}M}$ (9 CTX-M-1 group and 4 CTX-M-9 group), 10 isolates with bla_{SHV}, and 12 isolates with bla_{TEM}. Distributions of β-lactamase genes were different among the species. $bla_{MIR/ACT}$-like genes were almost exclusively detected in *Enterobacter* spp. Most of the *K. pneumoniae* isolates had bla_{DHA} and bla_{SHV}, whereas most of the *E. coli* isolates had bla_{TEM}, $bla_{CTX\text{-}M}$, and $bla_{CMY\text{-}2}$-like genes. bla_{SHV} genes were detected more frequently in resistant isolates (8/20) than intermediate isolates (2/20), and in most of the *K. pneumoniae* isolates. Otherwise, there were no significant differences in the prevalence of β-lactamases between the resistant and the intermediate isolates.

Detection of carbapenemase

One *C. freundii* isolate among the 40 isolates with ertapenem non-susceptibility was positive for carbapenemase in the modified Hodge test, carbapenemase inhibition test, and chromogenic agar test for screening of carbapenemase-producing isolates. The MIC of ertapenem determined by broth microdilution was 4 μg/mL. The MIC of imipenem and meropenem determined by automated systems was 0.5 μg/mL and 2 μg/mL, respectively. Multiplex PCR for carbapenemase detected bla_{IMP} PCR and sequencing analysis using primers targeting the $bla_{IMP\text{-}1}$ gene and class 1 integron confirmed the $bla_{IMP\text{-}1}$-associated class 1 integron.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis for OMP

According to the SDS-PAGE analysis, all of the *K. pneumoniae* (n=5) and *E. cloacae* (n=11) isolates with ertapenem resistance showed altered or greatly diminished expression of porins compared to the control strains (table 4). Porin alterations were less common among the ertapenem-intermediate isolates (4/19). Among 14 ertapenem-intermediate isolates of *E. cloacae*, four had altered porins. All of the ertapenem non-susceptible *E. coli* isolates conserved porins compared to the control strain.

Table 3 Detection of AmpC β-lactamase and ESBL genes among the 40 Enterobacteriaceae isolates with ertapenem non-susceptibility

β-lactamase	Total (n=40) N (%)	Resistant (n=20) N (%)	Intermediate (n=20) N (%)
AmpC β-lactamase	32 (80.0)	14 (70.0)	18 (90.0)
MIR/ACT-like	24 (60.0)	11 (55.0)	13 (65.0)
CMY-2-like	5 (12.5)	1 (5.0)	4 (20.0)
DHA	4 (10.0)	3 (15.0)	1 (5.0)
ESBL	20 (50.0)	11 (55.0)	9 (45.0)
SHV	10 (25.0)	8 (40.0)	2 (10.0)
TEM	12 (30.0)	5 (25.0)	7 (35.0)
CTX-M	13 (32.5)	6 (30.0)	7 (35.0)
CTX-M-1 group	9 (22.5)	4 (20.0)	5 (25.0)
CTX-M-9 group	4 (10.0)	2 (10.0)	2 (10.0)

ESBL, extended-spectrum β-lactamase.

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Table 4 Resistance mechanisms associated with ertapenem non-susceptibility in Enterobacteriaceae

β-lactamase				No of isolates	
Carbapenemase	AmpC	ESBL	Porin alteration	R	I
Citrobacter freundii (n=1)				1	
IMP-1	CMY-2-like	SHV	ND	1	
Enterobacter cloacae (n=25)				11	14
	MIR/ACT-like		+	7	1
	MIR/ACT-like		–		7
	MIR/ACT-like	CTX-M-9 group	+	1	
	MIR/ACT-like	CTX-M-9 group	–		1
	MIR/ACT-like	CTX-M-1 group	+		1
	MIR/ACT-like	CTX-M-1 group, TEM	+		1
	MIR/ACT-like	CTX-M-1 group, TEM	–		1
	MIR/ACT-like	CTX-M-9 group, SHV	+	1	
	MIR/ACT-like, DHA	TEM	+	1	
		CTX-M-1 group, TEM, SHV	+	1	1
		TEM	–		1
Klebsiella pneumoniae (n=6)				5	1
		SHV	+	1	
	DHA	SHV	+	2	
	DHA	SHV, TEM	–		1
	DHA	SHV, TEM, CTX-M-1 group	+	2	
Escherichia coli (n=5)				1	4
	cAmpC, CMY-2-like		–		2
	cAmpC	TEM, CTX-M-1 group	–	1	
	cAmpC, CMY-2-like	TEM, CTX-M-1 group	–		1
	CMY-2-like	TEM, CTX-M-9 group	–		1
Others (n=3)				2	1
Enterobacter amnigenus					
	MIR/ACT-like		ND	1	
Providencia rettgeri					
	MIR/ACT-like		ND	1	
Cronobacter sakazakii					
			ND		1

cAmpC, chromosomal AmpC; ESBL, extended-spectrum β-lactamase; ND, not done; R, resistant isolates; I, intermediate isolates.

Mechanisms associated with ertapenem non-susceptibility in Enterobacteriaceae

The results of molecular analysis of β-lactamases and OMP analysis are integrated in table 4. Ertapenem resistance caused by carbapenemase was observed in one isolate. Most of the other isolates with ertapenem resistance were associated with β-lactamase activity and porin alterations. The presence of β-lactamases without porin alteration was observed in most of the intermediate isolates.

Molecular epidemiology

All of the *K. pneumoniae* and *E. coli* isolates showed distinct individual pulsotypes and were considered to be epidemiologically unrelated (figure 1 and table 5). Among the *E. cloacae* isolates, pulsotypes A, B, and C were observed in 4, 2, and 2 different patients, respectively. All four isolates of pulsotype A were isolated from patients in the neonatal intensive care unit during a 3-month period; the two isolates of pulsotype B were isolated from patients in the same ward from the same specimen during a 3-month period; and the two isolates of pulsotype C were isolated from respiratory specimens of patients in the same ward during a 1-week period. The two isolates of pulsotype A with porin alteration showed a relatively high MIC of ertapenem compared to the other two isolates.

DISCUSSION

Increasing resistance to ertapenem among Enterobacteriaceae is becoming a major therapeutic problem. The resistance rates of Enterobacteriaceae to ertapenem have been variously reported, depending on the species. The Study for Monitoring Antimicrobial Resistance Trends (SMART) reported that *E. coli* isolated in Europe and Latin America during 2008 and 2009 had high susceptibility to ertapenem with resistance rates of 0.2% and 0.3%, respectively.[2 4] *K. pneumoniae*, on the other hand, showed a relatively high resistance rate of 6.5% globally.[1] Obviously, there are also regional differences. The rates of susceptibility to ertapenem of *K. pneumoniae* varied between different geographical regions, from 82.3% in the Middle East to 100% in Africa. In Taiwan, the ertapenem susceptibility rate was 92.9%, 80.9%, and 67.9% for *E. coli*, *K. pneumoniae,* and *E. cloacae*, respectively.[3]

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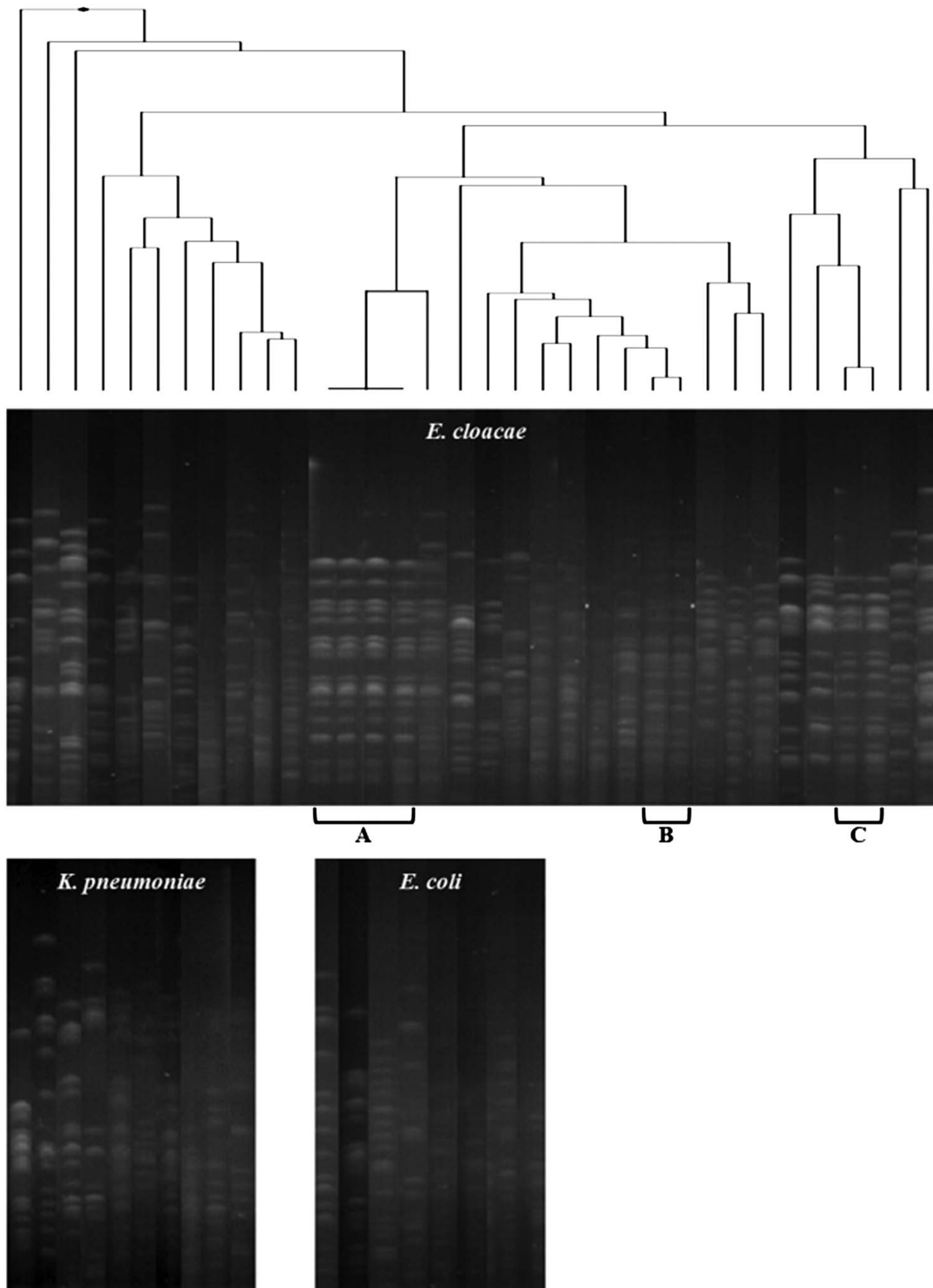

Figure 1 Pulsed-field gel electrophoresis patterns of *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Escherichia coli* isolates. Among the *E. cloacae* isolates, three pulsotypes of related isolates were identified (A–C). Other isolates were considered to be unrelated.

In this study, the resistance rate to ertapenem among all Enterobacteriaceae isolates was 2.0% as determined using VITEK2. It was highest in *Enterobacter* spp. (14.0%), and was 2.5% and 0.4% in *K. pneumoniae* and *E. coli*, respectively. In comparison, the resistance rate to imipenem was 0.1%. Even though there were differences in the antimicrobial susceptibility testing methods and the criteria used for interpretation, in general the resistance rates to ertapenem were higher than those for other carbapenems, and *Enterobacter* spp. and *K. pneumoniae* showed higher resistance rates than *E. coli*.[1–4]

As reported in previous studies,[5–9 13] susceptibility to imipenem and meropenem was retained in most of the isolates with ertapenem non-susceptibility. This may reflect relative penetration rates through minor porins, differential susceptibility to efflux, or relative susceptibility to slow hydrolysis by AmpC β-lactamases or ESBLs, and may perhaps be related to the larger size and more negative charge of ertapenem.[7 13] Further investigation of this differential susceptibility is warranted to determine the optimal antimicrobial therapy.

Previous studies showed that ertapenem resistance in Enterobacteriaceae is mainly due to mechanisms other than carbapenemases, especially expression of β-lactamases, such as an AmpC β-lactamase or an ESBL, combined with porin loss.[1 2 4 5 7–9] Similarly, in this study, most of the isolates

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Table 5 Characteristics of patients with *Enterobacter cloacae* isolates with the same pulsotypes

Isolate	Specimen	Date	Age/sex	Dept	ESBL	Porin alteration	Pulsotype	ETP MIC (μg/mL)
EU-E-12-42	Gastric juice	December 3, 2012	19 d/female	PED	CTX-M-9 group	+	A	1
EU-E-12-45	Gastric juice	December 12, 2012	9 d/female	PED	CTX-M-9 group	–	A	0.5
EU-E-13-01	Transtracheal aspiration	January 8, 2013	1 m/male	PED	CTX-M-9 group	–	A	0.5
EU-E-13-08	Gastric juice	February 12, 2013	14 d/female	PED	CTX-M-9 group	+	A	4
EU-E-12-18	Bile	August 23, 2012	66 yr/male	GS	CTX-M-1 group	+	B	1
EU-E-12-14	Bile	October 2, 2012	79 yr/male	GS	CTX-M-1 group	+	B	2
EU-E-13-06	Sputum	February 11, 2013	28 yr/male	NS	–	–	C	0.5
EU-E-13-12	Transtracheal aspiration	February 18, 2013	81 yr/male	RM	CTX-M-1 group	–	C	1

d, day; ESBL, extended-spectrum β-lactamase; ETP, ertapenem; GS, general surgery; NS, neurosurgery; PED, pediatrics; RM, rehabilitation medicine; yr, year.

with ertapenem non-susceptibility were associated with β-lactamase activity and/or porin alteration. Most of the ertapenem-resistant isolates had porin alteration, whereas porin alterations were less common among the ertapenem-intermediate isolates. A combination of β-lactamase activity and porin alteration tended to result in higher MICs of ertapenem compared to isolates with β-lactamase activity alone. This suggests that ertapenem resistance in Enterobacteriaceae might result from the accumulation of multiple carbapenem-resistance determinants.

PFGE revealed genetic diversity among most of the isolates, suggesting that ertapenem resistance in clinical Enterobacteriaceae isolates was not the result of dissemination of resistant clones in the hospital but rather reflected independent emergence in different strains. Nonetheless, the phenomenon of the same clone spreading in the same ward during a single period was observed in this study. Interestingly, isolates of the same pulsotype with porin alteration showed relatively high MICs of ertapenem compared to the other isolates, suggesting that the non-susceptibility to ertapenem in Enterobacteriaceae might result from the accumulation of multiple carbapenem-resistance determinants in different isolates.

The patients in this study carrying Enterobacteriaceae with a high MIC of ertapenem (MIC≥8 μg/mL) were older and had underlying diseases. The clinical course and outcome differed from individual to individual. One patient who was treated with meropenem showed clinical improvement, indicating the therapeutic potential of meropenem treatment for ertapenem-resistant Enterobacteriaceae without carbapenemase. Further clinical studies are needed to evaluate the use of other carbapenems for ertapenem-resistant Enterobacteriaceae.

Even though carbapenemase-producing Enterobacteriaceae are still rare in Korea, there has recently been an increase in their detection.[26–29] In this study, one *C. freundii* isolate was confirmed to be a carbapenemase-producing Enterobacteriaceae and screening tests, including the modified Hodge test, carbapenemase inhibition test, and chromogenic agar test, were shown to be helpful for detection. Even though it carried IMP-1, this isolate was not resistant to other carbapenems (imipenem and meropenem) tested using the two automated systems. In this case, ertapenem was only sensitive for detecting carbapenemase-producing isolates. Ertapenem susceptibility has been recommended as a sensitive indicator of KPC,[30–33] although its low specificity and positive predictive value have been persistent problems.[34–36] In this study, the positive predictive value of ertapenem resistance for detection of carbapenemase-producing isolates was very low (1/20). The low specificity and positive predictive value observed in tests for detection of carbapenemase-producing Enterobacteriaceae were due in part to the low prevalence of these isolates. Therefore, accurate detection of carbapenemase-producing Enterobacteriaceae is still challenging.

In summary, ertapenem resistance in clinical isolates was associated with β-lactamase activity and porin alteration. Even though carbapenemase-producing Enterobacteriaceae are still rare, continuous monitoring and infection control for carbapenem-resistant Enterobacteriaceae is necessary.

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