isoleucine alteration in the naturally occurring enzyme.[6] A single amino acid substitution not corresponding to any naturally occurring β-lactamase was identified in the mutants derived from OXA-10 (Table 1). Danel *et al*.[4] also generated (with non-hypermutable *P. aeruginosa*) an OXA-derived ESBL from R151 that does not correspond to a naturally occurring enzyme.

Although ceftazidime resistance has been associated with hypermutability in clinical isolates of *P. aeruginosa* from chronic lung infections[2] it is not known whether these strains contain ESBLs. Indeed on the basis of data reported here, we suggest that hypermutable *P. aeruginosa* may not be the source of those ESBLs found in clinical isolates of this organism. This situation may reflect the relative ease by which derepression of AmpC in *P. aeruginosa* provides a route for resistance to expanded-spectrum β-lactams.

Transparency declarations

None to declare.

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Co-production of 16S rRNA methylases and extended-spectrum β-lactamases in AmpC-producing *Enterobacter cloacae*, *Citrobacter freundii* and *Serratia marcescens* in Korea

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Sir,

Enterobacter cloacae, Citrobacter freundii and *Serratia marcescens* are important nosocomial pathogens. In Korea, extended-spectrum β-lactamase (ESBL) prevalence in AmpC-producing *E. cloacae, C. freundii* and *S. marcescens* is quite high (10.9–23.6%), and these ESBL-producers show higher resistance rates to aminoglycosides than do the ESBL-non-producers.[1] While ribosomal protection mediated by methylation of 16S rRNA has been known as a self-defense mechanism for aminoglycoside-producing actinomycetes, it was not reported in other species until 2003. However, a series of methylases have been identified in several nosocomial pathogens, including *Pseudomonas aeruginosa*,[2] *S. marcescens*,[3] *Proteus mirabilis*[4] and *Klebsiella pneumoniae*.[5] The existence of these enzymes (RmtA, RmtB, RmtC and ArmA) is of great concern because they are capable of conferring an extraordinary high level of resistance (MIC > 512 mg/L) against most clinically important aminoglycosides, and they were often associated with ESBLs.[6]

In the present study, a total of 413 consecutive, non-duplicate isolates, including *E. cloacae* (158), *C. freundii* (126) and *S. marcescens* (129), were collected during March–July 2003 at 11 university hospitals in Korea. The isolates were from wound (37%), urine (35%), respiratory specimen (20%), blood (4%) and body fluid (4%). The MICs of amikacin (8–512 mg/L) and arbekacin (8–512 mg/L) were determined by an agar dilution method in accordance with the CLSI guideline. The detection of ESBL was based on the double disc synergy test (DDST) using discs containing 30 μg of ceftazidime, cefotaxime, aztreonam and cefepime. They were placed 2 cm from a disc containing amoxicillin/clavulanic acid (20/10 μg) (BBL, Cockeysville, MD, USA).

For the isolates that showed high-level resistance (MICs of >512 mg/L) to amikacin or arbekacin, a search for the 16S rRNA methylase genes (*rmtA, rmtB, rmtC* and *armA*) was performed by PCR. The total DNAs were extracted from isolates by boiling and the PCR was carried out with the *Taq* DNA polymerase (Takara Shuzo, Shiga, Japan) and the following sets of primers: rmtA-F, 5′-CTA GCG TCC ATC CTT TCC TC-3′; rmtA-R, 5′-TTT GCT

Table 1. Distribution of ESBLs among 16S rRNA methylase-producing *E. cloacae*, *C. freundii* and *S. marcescens*

	E. cloacae (13)	*C. freundii* (13)	*S. marcescens* (21)
ESBL producers	13	10	14
CTX-M-3	9	4	12
CTX-M-9	0	1	0
CTX-M-14	0	1 (*rmtB*)	1
TEM-52	1	0	0
SHV-12	2	1	1
PER-1	0	0	0
undetermined	1	3	0

TCC ATG CCC TTG CC-3′; rmtB-F, 5′-CCC AAA CAG ACC GTA GAG GC-3′; rmtB-R, 5′-CTC AAA CTC GGC GGG CAA GC-3′; rmtC-F, 5′-CGA AGA AGT AAC AGC CAA AG-3′; rmtC-R, 5′-ATC CCA ACA TCT CTC CCA CT-3′; armA-F, 5′-AGG TTG TTT CCA TTT CTG AG-3′; armA-R, 5′-TCT CTT CCA TTC CCT TCT CC-3′. Plasmids harbouring each gene were used as positive controls. For the detection of the ESBL genes, primers specific for bla_{TEM} (TEM-F, 5′-ATA AAA TTC TTG AAG AAA-3′; TEM-R, 5′-GAC AGT TAC CAA TGC TTA ATC-3′), bla_{SHV} (SHV-F, 5′-TGG TTA TGC GTT ATA TTC GCC-3′; SHV-R, 5′-GGT TAG CGT TGC CAG TGC T-3′), $bla_{\text{CTX-M}}$ (CTX-M-F, 5′-CGC TTT GCG ATG TGC AG-3′; CTX-M-R, 5′-ACC GCG ATA TCG TTG GT-3′), $bla_{\text{CTX-M-9}}$ (CTX-M-9-F, 5′-CGC TTT ATG CGC AGA CGA-3′; CTX-M-9-R, 5′-GAT TCT CGC CGC TGA AGC-3′) and $bla_{\text{PER-1}}$ (PER-1-F, 5′-AAT TTG GGC TTA GGG CAG AA-3′; PER-1-R, 5′-ATG AAT GTC ATT ATA AAA GC-3′) were used. PCR products were purified with a QIAquick PCR purification kit (QIAGEN, Hilden, Germany) and sequenced on a 3730 DNA analyser (Applied Biosystems, Foster City, CA, USA). The nucleotide and deduced protein sequences were analysed with software available from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Of the total 413 isolates, 58 were resistant to amikacin, and the majority (49/58, 84.5%) of these 58 isolates showed high-level resistance (MIC > 512 mg/L) to both amikacin and arbekacin. One *E. cloacae* isolate was highly resistant to arbekacin (MIC > 512 mg/L), but it was susceptible to amikacin (16 mg/L). The frequency of the high-level resistance to amikacin or arbekacin was 9.5% (15/158), 10.3% (13/126) and 17.1% (22/129) for *E. cloacae, C. freundii* and *S. marcescens* isolates, respectively, and almost all of them (13 *E. cloacae*, 12 *C. freundii* and 21 *S. marcescens* isolates) harboured the *armA* gene. One *C. freundii* isolate harboured the *rmtB* gene. The 16S rRNA methylase-harbouring isolates were isolated from nine hospitals distributed nationwide. All of them were highly resistant to both arbekacin and amikacin. None harboured an *rmtA* or *rmtC* gene. The ESBL production rate was significantly higher in 16S rRNA methylase-producers (100%, 76.9% and 66.7% among *E. cloacae, C. freundii* and *S. marcescens*, respectively), compared with 16S rRNA methylase-non-producers (25.0%, 12.4% and 10.2%, respectively) ($P = 0.002$, $P < 0.001$ and $P < 0.001$, respectively). Most of the ArmA producers co-harboured various ESBLs (CTX-M-3, CTX-M-9, CTX-M-14, TEM-52 and SHV-12), among which CTX-M-3 was the most common (Table 1).

This finding corroborates the previous reports that the *armA* was frequently associated with $bla_{\text{CTX-M}}$ and they were co-transferred by conjugation.[6,7] Although only one isolate harboured the *rmtB* gene in the present study, it also harboured CTX-M-14; this coincides with the report by Yan *et al*.[6] where six out of the seven *rmtB*-positive isolates harboured CTX-M-14. In Korean medical practice, arbekacin is only rarely used for the treatment of methicillin-resistant *Staphylococcus aureus*. Nevertheless, the prevalence of high-level resistance to amikacin and arbekacin was similarly high, suggesting that *armA* can confer resistance to arbekacin as in other 16S rRNA methylases.[2,3]

In conclusion, the *armA* gene is widespread in Korean isolates of *E. cloacae, C. freundii* and *S. marcescens*, and an *rmtB* producer was also found. Given the multiresistance in these isolates, prudent antibiotic use, accurate detection of this resistance and strict infection control are urgently needed to prevent the spread of these organisms.

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Transparency declarations

None to declare.

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