

recipient at 22°C and 37°C (selection of transconjugants in MacConkey agar with 2 mg/L of ceftazidime and 130 mg/L of azide)⁷ failed to yield transconjugants either for *bla*_{VIM-34} or *bla*_{SHV-12}. The location of *bla* (*bla*_{VIM-34}, *bla*_{SHV-12}) genes and plasmid characterization were accomplished by S1- and I-CeuI-PFGE, and identification of incompatibility groups.⁷ In both isolates, *bla*_{VIM-34}, *bla*_{SHV-12} and *repH12* probes hybridized in the same chromosomal band (I-CeuI-PFGE) whereas no signals were observed in the S1 gel, suggesting the acquisition of both *bla* genes by an IncHI2 plasmid and subsequent plasmid (whole or in part) integration. A chromosomal location for *bla* genes, including *bla*_{VIM}, has been occasionally observed in different Enterobacteriaceae species.¹

The linkage of *bla*_{VIM-34} to class 1 integrons and Tn402 derivatives was investigated by PCR (*intI1*, 5'CS-3'CS region, *orf5*, *orf6*, IS1326, IS1353, IS6100) and sequencing.^{4,11} *bla*_{VIM-34} was located within an ~6 kb class 1 integron named In817 by INTEGRALL (<http://integrall.bio.ua.pt/>) (GenBank accession number JX185132), with an original array of gene cassettes comprising *bla*_{VIM-34}, *aacA4'*, *aphA15*, *aadA1b* and *catB2* (Figure S1; available as Supplementary data at JAC Online). The absence of *tni402* sequences and the high similarity detected with In70 and In113, identified in VIM-1-producing *Achromobacter xylosoxidans*, *K. pneumoniae* and *E. coli* isolates, suggests that the In817 integron might have arisen by both recombination and *in vivo* evolution events (Figure S1; available as Supplementary data at JAC Online).⁴

In summary, we present the first report of VIM-34, a VIM-1-like variant embedded in the novel integron type In817 on the chromosome of the intercontinental ST15 *K. pneumoniae* clone, associated with carbapenem susceptibility profiles similar to those observed for VIM-1. This study highlights the risk of further dissemination of the multidrug-resistant ST15 *K. pneumoniae* clone and genetic backgrounds containing metallo-β-lactamase genes in our country, which deserves future monitoring.

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Transparency declarations

None to declare.

Supplementary data

Figure S1 is available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

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Mutant prevention concentrations of colistin for *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* clinical isolates

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Sir,
Although colistin resistance is rare, colistin-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolates have been reported worldwide, including Korea.¹ The mutant selection window (MSW) hypothesis has been suggested as a new strategy to investigate the emergence of antimicrobial resistance. The mutant prevention concentration (MPC) is the drug concentration required to prevent the emergence of all single-step mutations from a susceptible population of more than 10¹⁰ cells; MPC as a measure of antibiotic potency allows the probability of resistance selection during the treatment of infected patients to be predicted.² *In vitro* time-kill experiments have suggested that the MPCs of colistin are high.³ We determined the MPCs of colistin for *A. baumannii*, *P. aeruginosa* and *K. pneumoniae* isolates.

Forty *A. baumannii* (20 imipenem resistant and 20 susceptible), 40 *P. aeruginosa* (20 imipenem resistant and 20 susceptible) and 33 *K. pneumoniae* (11 imipenem resistant and 22 susceptible) isolates from Korean hospitals were investigated. The MICs of imipenem and colistin were determined by a broth microdilution method according to CLSI guidelines.⁴ The MPC of colistin was determined as previously described.⁵ All resulting mutants grown

within a colistin concentration range known as the MSW (8 mg/L) were stocked and stored at -80°C for further study. Nucleotide sequences of *pmrAB*, *phoPQ*, *parRS* and *lpxACD* (*pmrAB* and *lpxACD* for *A. baumannii*, *pmrAB*, *phoPQ* and *parRS* for *P. aeruginosa*, and *pmrAB* and *phoPQ* for *K. pneumoniae*) were determined in single-step mutants to understand their association with the emergence of colistin resistance.

All isolates were susceptible to colistin: the MIC ranges of colistin were 0.5–1 mg/L, 1–2 mg/L and 0.06–4 mg/L for *A. baumannii*, *P. aeruginosa* and *K. pneumoniae*, respectively. The MPCs for the three Gram-negative species were very high and were unrelated to their colistin and imipenem MICs. All isolates except two *P. aeruginosa* isolates and one *K. pneumoniae* isolate showed an MPC ≥64 mg/L. In particular, the MPCs for 25 *A. baumannii* (62.5%) and 25 *K. pneumoniae* (75.8%) isolates were >128 mg/L. The high MPC values in this study suggest the possibility of enriching colistin-resistant mutant subpopulations during treatment with colistin monotherapy.⁶ Thus, our MPC data may explain the emergence of colistin resistance in Gram-negative pathogens. In reality, the emergence of colistin resistance or heteroresistance after colistin treatment has been reported in *A. baumannii*,⁷ and colistin-resistant mutants can be easily selected *in vitro*.⁸ The recommended dose regimen of colistin methanesulphonate of approximately 4–6 mg/kg/day lies within the MSW,⁹ which may be linked to the generation of colistin resistance. Since the use of high concentrations of colistin would involve a risk of toxicity,

Table 1. Amino acid alterations of PmrAB, PhoPQ and ParRS between the parental isolate and first-step mutant

Species/isolate	Colistin MIC (mg/L)	Colistin MPC (mg/L)	Amino acid alterations				
			PmrB	PhoP	PhoQ	ParR	ParS
<i>A. baumannii</i>							
C072	0.5	>128	P233S	NA	NA	NA	NA
<i>P. aeruginosa</i>							
P1	2	64				N24S	
P10	2	>128					Q232E
P29	1	64					G361R
P70	2	>128	V281I		K123E		
P83	1	>128				L18I, S24N	
P88	2	64		N188H			G361R
P112	2	64				Syn	
P147	2	128			Syn		
P155	1	>128			R214H		
P179	2	128			V184G		
P185	2	128	V281I		Q133E		
P199	2	128			A207R		
P206	2	128	Syn		N104I		
P213	2	128	F237L				
<i>K. pneumoniae</i>							
513 BTB	0.5	>128	Δ3 nt position 14			NA	NA
507 BTB	0.5	>128	T157P			NA	NA
08-u-899		>128	S208N, Δ3 nt position 209			NA	NA
K08-Bact-08-039	4	>128			S174N	NA	NA
YDJ	0.5	>128	T157P			NA	NA

NA, not applicable; Syn, synonymous mutation; Δ3 nt, three nucleotide deletion.

combination therapy would be recommended to prevent the emergence of colistin resistance by mutant selection.

The emergence of resistance after exposure to antimicrobial agents may be due to the selection of antimicrobial-resistant subpopulations or the occurrence of new mutants through antibiotic stress. Mutations in two-component systems and LpxACD cause colistin resistance due to the modification of lipopolysaccharides in Gram-negative bacteria.^{8,9} Among 40 *A. baumannii* isolates, an amino acid alteration was identified only in a single-step mutant of one isolate, which showed a P233S substitution in PmrB in C072 (Table 1), as has been reported in a previous study.¹⁰ No LpxACD mutations were found in this study. In *P. aeruginosa*, amino acid alterations of PmrAB, PhoPQ and ParRS were observed in 12 single-step mutants (Table 1). Double amino acid substitutions were observed in the single-step mutants of four *P. aeruginosa* isolates (P70, P83, P88 and P185). V281I in PmrB and G361R in ParS were each present in two mutants. In *K. pneumoniae*, three kinds of amino acid substitution were observed: S174N in PhoQ and T157P and S208N in PmrB. A T157P substitution was identified in the single-step mutants of two *K. pneumoniae* isolates, 507 BTB and YDJ. Unlike *A. baumannii* and *P. aeruginosa*, amino acid deletions were identified in *pmrB* of two single-step mutants of *K. pneumoniae*. In the single-step mutant of 08-u-899, amino acid substitution and deletion were both identified.

Our results indicate that amino acid alterations of PmrAB, PhoPQ and ParRS occurred *in vitro* within the period of selection of single-step mutants. This suggests that colistin treatment can provoke genetic mutations related to resistance as a mutagen within a short period in addition to the selection of resistant subpopulations. In short, colistin resistance may occur very easily during drug use.

In summary, we identified high MPCs of colistin for imipenem-susceptible and imipenem-resistant *A. baumannii*, *P. aeruginosa* and *K. pneumoniae* clinical isolates. In single-step mutants, several amino acid substitutions of two-component regulatory systems, PmrAB, PhoPQ or ParRS, were also identified. These findings suggest the possibility of the rapid emergence and spread of colistin resistance by a single mutation. Thus, combination therapy for colistin treatment of non-fermenter and Enterobacteriaceae infections would be necessary to prevent or slow the emergence of colistin resistance.

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Transparency declarations

None to declare.

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Quality control ranges for tylosin 30 µg and 15 µg discs applicable to *Staphylococcus aureus* ATCC® 25923

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