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SHORT REPORT
Emergence of an extended-spectrum β-lactamase-producing serotype K1 *Klebsiella pneumoniae* ST23 strain from Asian countries

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SUMMARY

Extended-spectrum β-lactamase (ESBL) production has been very rare in serotype K1 *Klebsiella pneumoniae* ST23 strains, which are well-known invasive community strains. Among 92 ESBL-producing strains identified in 218 isolates from nine Asian countries, serotype K1 *K. pneumoniae* strains were screened. Two ESBL-producing *K. pneumoniae* isolates from Singapore and Indonesia were determined to be serotype K1 and ST23. Their plasmids, which contain CTX-M-15 genes, are transferable rendering the effective transfer of ESBL resistance plasmids to other organisms.

Key words: Extended-spectrum β-lactamase, *Klebsiella pneumoniae*, serotype K1.

Klebsiella pneumoniae is an important pathogen causing hospital-acquired infections and multidrug resistance in these bacteria has increased [1, 2]. Treatment of multidrug-resistant *K. pneumoniae* infections has been a big challenge and there is an increasing concern about the clinical impact of these bacteria [3]. Invasive *K. pneumoniae* strains belonging to serotype K1 have emerged as important causes of community-acquired infections including liver abscess in many Asian countries [4–6]. Despite a high prevalence of community infections by these invasive *K. pneumoniae* strains has been characterized by ST23, which appears to be susceptible to most antibiotic classes except ampicillin and piperacillin [6]. Only a few case reports of Extended-spectrum β-lactamase (ESBL)-producing serotype K1 *K. pneumoniae* have been reported [7–9]. In this study, we molecularly characterized two serotype K1 ESBL-producing *K. pneumoniae* strains and investigated the transferability of plasmid DNA from these isolates.

As part of a multinational Asian Network for Surveillance of Resistant Pathogens (ANSORP) surveillance study on hospital-acquired pneumonia during 2008 and 2009 [10], a total of 218 *K. pneumoniae* isolates were collected from nine Asian countries. Of the 218 strains, 92 isolates were identified as ESBL producers [11]. In this study, the 92 ESBL-producing *K. pneumoniae* isolates were tested for serotype K1, and serotype K1 ESBL-producers were included for further characterization. *In vitro* antimicrobial susceptibility testing was performed by a broth microdilution

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method and double-disk synergy test following Clinical and Laboratory Standards Institute (CLSI) guidelines. Double-disk synergy test-positive isolates were further tested by polymerase chain reaction (PCR) and sequence analyses to determine the gene responsible for the ESBL phenotype. PCR for bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ was conducted using previously described PCR primers and conditions [12]. All PCR products were sequenced and the sequences were compared using the database available at http://www.lahey.org/studies/.

K1 serotyping was tested by PCR using a primer pair specific for *magA*, which is specific for the K1 antigen. The primers used were as described previously [13]. Multilocus sequence typing was performed on serotype K1 *K. pneumoniae* by determining the nucleotide sequences of seven housekeeping genes as described previously [14]. For pulsed-field gel electrophoresis (PFGE), agarose embedded bacterial genomic DNA was digested with 20 U *Xba*I. The restriction fragments were separated by electrophoresis in 0·5× Tris-borate-EDTA buffer. The PFGE patterns were analysed using GelCompar II v. 6.1 (Applied Maths, Belgium). Conjugation experiments were performed using azide-resistant *E. coli* J53 as a recipient strain. Mixed strains at a ratio of 1:10 (donor:recipient) were used for broth and filter mating assays. Transconjugants were selected on lysogeny broth medium containing cefotaxime (4 mg/l) and sodium azide (200 mg/l). Isolated DNA plasmids from serotype K1 ESBL transconjugants were used for transformation in the *E. coli* DH5α strain. All transconjugants and transformants were tested for ESBL production using the disk and broth microdilution method followed by PCR amplification of the ESBL genes.

Among the 92 ESBL-producing *K. pneumoniae* isolates, only two were determined as serotype K1. The two isolates were from patients in Singapore and Indonesia named, SGP-1 and INS-2, respectively. The results of antimicrobial susceptibility testing of these isolates are shown in Table 1. Both SGP-1 and INS-2 belonged to ST23 and carried the $bla_{CTX\text{-}M\text{-}15}$ gene. Moreover, those isolates were negative for TEM and SHV ESBL genes and fully susceptible to carbapenems. SGP-1 was isolated in a patient with ventilator-associated pneumonia (VAP) after neurosurgery. The patient was treated with meropenem and discharged. INS-2 was isolated in a patient with VAP after surgery. Ceftazidime was given empirically and replaced by meropenem. Although VAP improved, the patient died due to cardiac arrest 77 days after admission. The PFGE analysis showed that the PFGE patterns of these strains and serotype K1 non-ESBL *K. pneumoniae* isolates of ST23 from Asian countries clustered into a major group with a similarity of >75% (Fig. 1). To determine whether ESBL resistance in serotype K1 ST23 *K. pneumoniae* strains was transferable, a conjugation experiment was performed. The plasmids carrying $bla_{CTX\text{-}M\text{-}15}$ from SGP-1 and INS-2 were successfully transferred into recipient J53 *E. coli* isolates. The transfer of the plasmid carrying $bla_{CTX\text{-}M\text{-}15}$ from the JI-14 transconjugant was accomplished using an *E. coli* DH5α strain. All transconjugants (JS-1, JS-2, JS-4, JS-5, JS-6, JS-7, and JS-8 from SGP-1; JI-2, JI-3, JI-5, JI-10, JI-11, and JI-15 from INS-2) and transformants (FI14-1 and FI14-2) were tested for ESBL production using the disk and broth microdilution method followed by PCR amplification of ESBL genes. All transconjugants and transformants were found to express $bla_{CTX\text{-}M\text{-}15}$ genes. Antimicrobial resistance patterns did not differ between the donors and transconjugants; however, all of the transformants showed decreased minimum inhibitory concentrations for tested antimicrobials (Table 1).

In this study, we investigated the molecular characteristics of two serotype K1 ESBL-producing *K. pneumoniae* strains belonging to ST23 and the transferability of plasmid DNA from these isolates. The increasing prevalence of ESBL-producing *K. pneumoniae* is becoming a serious worldwide problem. Over the past decade, the global emergence of multidrug-resistant strains of *K. pneumoniae* that produce ESBL of CTX-M types has been of considerable concern. In particular, CTX-M-15-producing *K. pneumoniae* is highly prevalent in Asian counties and worldwide [11, 15]. Although serotype K1 *K. pneumoniae* strains have emerged as important pathogens causing community-acquired infections in many countries, those strains have shown good susceptibility to most antimicrobials to date. However, our report of two serotype K1 ESBL-producing *K. pneumoniae* strains carrying $bla_{CTX\text{-}M\text{-}15}$ on transferrable plasmids suggests that dissemination of CTX-M-15 producers in highly pathogenic serotype K1 *K. pneumoniae* strains may become a serious threat to the treatment of community-acquired *K. pneumoniae* infections in the near future. Furthermore, two isolates in this study were found in the Asian Hospital Acquired Pneumonia surveillance study in the absence of antimicrobial pressure. The results

Table 1. *Antimicrobial susceptibilities and molecular characteristics of recipient, donor and transconjugants*

	Strain	Description	ESBL	Molecular characteristics	MICs and antimicrobial susceptibility											
					CTX		CAZ		CPM		P/T		CIP		AZT	
Recipient	J53	*E. coli* J53	–	–	0·12	S	0·12	S	0·12	S	2/4	S	0·12	S	0·06	S
Donor 1	SGP-1	ST23 serotype K1 *K. pneumoniae*	+	CTX-M-15	>128	R	64	R	>128	R	8/4	S	2	I	>64	R
Transconjugants	JS-1	*E. coli* J53/ESBL	+	CTX-M-15	>128	R	64	R	128	R	32/4	I	1	S	>64	R
	JS-2	*K. pneumoniae* SGP-1	+	CTX-M-15	>128	R	64	R	>128	R	32/4	I	0·5	S	>64	R
	JS-4		+	CTX-M-15	>128	R	64	R	32	R	32/4	S	1	S	>64	R
	JS-5		+	CTX-M-15	>128	R	64	R	32	R	64/4	I	1	S	>64	R
	JS-6		+	CTX-M-15	>128	R	128	R	128	R	64/4	I	2	I	>64	R
	JS-7		+	CTX-M-15	>128	R	32	R	32	R	16/4	S	1	S	>64	R
	JS-8		+	CTX-M-15	>128	R	64	R	128	R	32/4	I	1	S	>64	R
Donor 2	INS-2	ST23 serotype K1 *K. pneumoniae*	+	CTX-M-15	>128	R	64	R	128	R	8/4	S	2	I	>64	R
Transconjugants	JI-2	*E. coli* J53/ESBL	+	CTX-M-15	>128	R	32	R	128	R	8/4	S	2	I	>64	R
	JI-3	*K. pneumoniae* INS-2	+	CTX-M-15	>128	R	16	R	64	R	8/4	S	2	I	>64	R
	JI-5		+	CTX-M-15	>128	R	64	R	16	I	16/4	S	0·12	S	64	R
	JI-10		+	CTX-M-15	>128	R	16	R	128	R	8/4	S	2	I	>64	R
	JI-11		+	CTX-M-15	>128	R	16	R	128	R	8/4	S	2	I	>64	R
	JI-15		+	CTX-M-15	>128	R	32	R	128	R	8/4	S	2	I	>64	R
Recipient	DH5α	*E. coli* DH5α	–	–	0·12	S	0·12	S	0·12	S	0·5/4	S	0·12	S	0·06	S
Transformants	FI14-1	*E. coli* DH5α/ESBL	+	CTX-M-15	64	R	16	R	4	S	4/4	S	0·25	S	16	R
	FI14-2	JI14 plasmid	+	CTX-M-15	64	R	16	R	2	S	4/4	S	0·25	S	16	R

MIC, Minimum inhibitory concentration; CTX, cefotaxime; CAZ, ceftazidime; CPM, cefepime; P/T, piperacillin-tazobactam; CIP, ciprofloxacin; AZT, aztreonam; R, resistant; I, intermediate; S, susceptible.

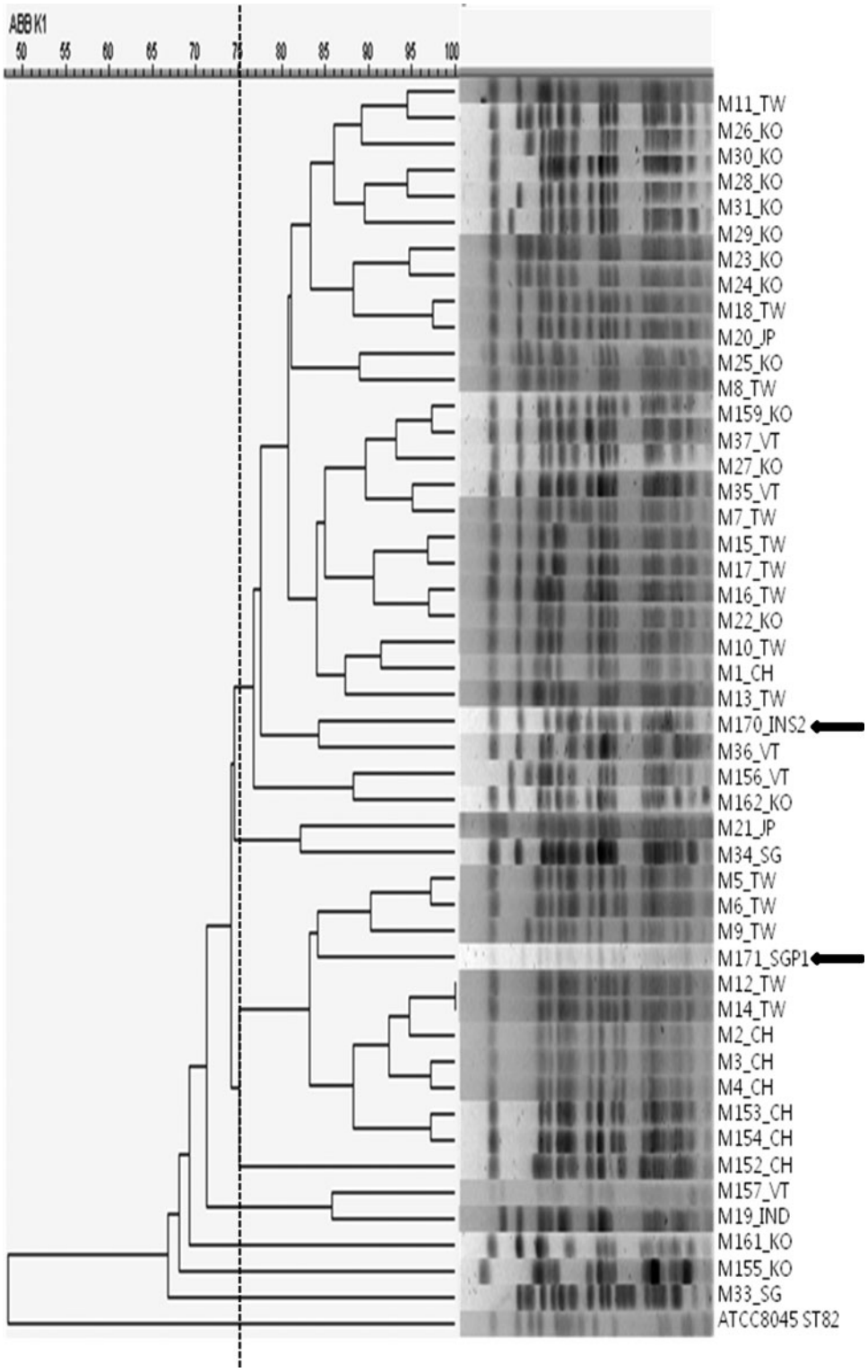

Fig. 1. PFGE dendrogram of ST23 serotype K1 *K. pneumoniae* isolates from Asian countries.

presented here are in agreement with the observation from Shin & Ko [16]. Moreover, we found that ST23 K1 *K. pneumoniae* showed a similar PFGE pattern regardless of ESBL. Previous reports support this result that the ST23 ESBL *K. pneumoniae* might have disseminated from a single strain [16, 17].

In summary, we report two clinical isolates of serotype K1 ESBL-producing *K. pneumoniae* carrying

transferable CTX-M-15 plasmids that could transfer resistance of antimicrobials to other clinical isolates.

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DECLARATION OF INTEREST

None.

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