Predominance of an ST11 extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* clone causing bacteraemia and urinary tract infections in Korea

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To investigate the antimicrobial resistance, extended-spectrum β-lactamases (ESBLs) and clones of *Klebsiella pneumoniae* isolates causing bacteraemia or urinary tract infection (UTI) in Korea, a total of 406 *K. pneumoniae* isolates from patients with bacteraemia (221 isolates) and UTI (185 isolates) were collected from 10 tertiary-care Korean hospitals from July 2006 to October 2007. *In vitro* antimicrobial susceptibility testing was performed for all isolates and ESBL production was tested. Multilocus sequence typing (MLST) analyses were performed to characterize genotypes of ESBL-producing *K. pneumoniae* isolates. PFGE was performed for sequence type 11 (ST11) isolates. Forty-seven UTI isolates (25.4 %) produced ESBLs, while 30 bacteraemia isolates (13.6 %) produced ESBLs (P=0.002). Among 77 ESBL-producing isolates, thirty-two (41.6 %) produced SHV-type ESBLs. $bla_{\text{CTX-M}}$ genes such as $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$ were detected

Abbreviations: ConNUH, Chonnam National University Hospital; CubNUH, Chungbuk National University Hospital; CunNUH, Chungnam National University Hospital; DFH, Daegu Fatima Hospital; ESBL, extended-spectrum β-lactamase; GNUH, Gyeongsang National University Hospital; JNUH, Jeju National University Hospital; KNUH, Kyungpook National University Hospital; KSH, Kangbuk Samsung Hospital; KUH, Konkuk University Hospital; MLST, multilocus sequence typing; SMC, Samsung Medical Center; ST, sequence type; UTI, urinary tract infection.

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in 36.4 %. MLST and PFGE analyses showed that ST11 was dominant in ESBL-producing *K. pneumoniae* isolates causing UTI (57.4 %) and in those causing bacteraemia (70.0 %) and has been prevalent in Korean hospitals. ST11 isolates harbour a combination of different ESBL genes. The ST11 clone of ESBL-producing *K. pneumoniae* isolates prevails in Korea, but most isolates might acquire ESBL genes independently or several different clones might be distributed in Korea.

INTRODUCTION

In Korea, the prevalence of extended-spectrum β-lactamases (ESBLs) in clinical *Klebsiella pneumoniae* isolates has been reported to be 17.7–30.0 % (Jeong *et al.*, 2004; Kim *et al.*, 2005; Ko *et al.*, 2008a). SHV-12 is the most common ESBL in *K. pneumoniae* isolates from Korean hospitals followed by SHV-31, SHV-32 and TEM-52 and also CTX-M-type ESBLs, CTX-M-3, CTX-M-12, CTX-M-14 and CTX-M-15 (Bae *et al.*, 2007; Jeong *et al.*, 2004; Kim *et al.*, 2005; Ko *et al.*, 2008a; Ryoo *et al.*, 2005). However, most of the previous studies in Korea have been performed on clinical isolates from patients irrespective of whether they cause disease.

Previously, we reported the clonal dissemination of ESBL-producing *K. pneumoniae* isolates in an intensive care unit in a Korean hospital based on PFGE and multilocus sequence typing (MLST) (Ko *et al.*, 2008b). Although PFGE has been widely used for epidemiological investigation, MLST has been developed to overcome the limitations of the band-based typing methods. Because it is highly discriminative and easy to standardize, store and exchange the information, it has been applied successfully for the epidemiological studies of many clinically important pathogens (Maiden *et al.*, 1998). Several studies have investigated the epidemiology and characteristics of antimicrobial-resistant or virulent *K. pneumoniae* isolates using MLST (Diancourt *et al.*, 2005; Damjanova *et al.*, 2008; Brisse *et al.*, 2009). In particular, Damjanova *et al.* (2008) showed dissemination of CTX-M-15-producing *K. pneumoniae* isolates belonging to limited clones in Hungary and suggested convergent population evolution in *K. pneumoniae* as in meticillin-resistant *Staphylococcus aureus* using MLST.

In this study, we investigated the antimicrobial resistance, prevalence of ESBL, type of ESBL and genotype of *K. pneumoniae* isolates causing bacteraemia and urinary tract infections (UTIs) from 10 university hospitals in various regions of Korea from July 2006 to October 2007.

METHODS

Collection of *K. pneumoniae* isolates causing bacteraemia and UTIs. As part of a multicentre surveillance study, a total of 406 *K. pneumoniae* isolates were obtained from in- and outpatients with UTIs (185 isolates) or bacteraemia (221 isolates) in 10 university hospitals in various regions of Korea: Samsung Medical Center (SMC, Seoul), Kangbuk Samsung Hospital (KSH, Seoul), Konkuk University Hospital (KUH, Seoul), Kyungpook National University Hospital (KNUH, Daegu), Daegu Fatima Hospital (DFH, Daegu), Chonnam National University Hospital (ConNUH, Gwangju), Chungnam National University Hospital (CunNUH, Daejeon), Chungbuk National University Hospital (CubNUH, Cheongju), Gyeongsang National University Hospital (GNUH, Jinju) and Jeju National University Hospital (JNUH, Jeju). The number of isolates recovered from each hospital is described in Table 1. Only the first isolate per patient was included in the study. UTI was diagnosed according to the definitions by the Center for Disease Control and Prevention (CDC). A bacterial isolate of $\geqslant 10^5$ c.f.u. ml^{-1} in urinary culture or $\geqslant 10^8$ c.f.u. ml^{-1} in suprapubic puncture was identified as a causative pathogen of UTI. Bacteraemia was diagnosed if there were two or more of the following conditions of systemic inflammatory response syndrome: temperature of >38 °C or <36 °C, heart rate of >90 beats min^{-1}, respiratory rate of >20 beats min^{-1} or $PaCO_2$ of <32 mmHg, and white blood cell count of $>12\,000$ or <4000 cells μl^{-1}, >10 % bands.

Antimicrobial susceptibility testing. *In vitro* antimicrobial susceptibility testing was performed by a broth microdilution method according to CLSI guidelines (CLSI, 2009). Nine antimicrobial agents were tested: ampicillin, gentamicin, ciprofloxacin, ceftazidime, cefotaxime, aztreonam, imipenem, trimethoprim–sulfamethoxazole and piperacillin–tazobactam. Susceptibility interpretive criteria used were those established in CLSI standard M100-S19 (CLSI, 2009). *Escherichia coli* ATCC 25922, *K. pneumoniae* ATCC 700603 and *S. aureus* ATCC 29213 were used as control strains. For ESBL-positive candidates, which were screened as ceftazidime, cefotaxime or aztreonam MIC $\geqslant 2$ mg l^{-1} (CLSI, 2009), production of ESBL was confirmed by a double-disc synergy test using BD BBL Sensi-Disk (Becton Dickinson), according to CLSI guidelines (CLSI, 2009). Quality control for the production of ESBL test was performed using *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603.

Detection of *bla* genes. PCR and sequencing of PCR products were performed to determine the gene responsible for the ESBL activity in the ESBL producers. PCR for bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, bla_{OXA} and bla_{VEB} was conducted using previously described PCR primers and conditions (Kim *et al.*, 2005; Girlich *et al.*, 2001). Both strands of all PCR fragments were sequenced and the types of β-lactamase genes were identified by comparing the sequences to those in the database of G. Jacoby and K. Bush (http://www.lahey.org/Studies/). IS*Ecp1* was also detected for $bla_{CTX\text{-}M}$-positive isolates by PCR as previously described (Damjanova *et al.*, 2008).

MLST and PFGE. MLST was performed as described by Diancourt *et al.* (2005) (http://www.pasteur.fr/recherche/genopole/PF8/mlst/). Briefly stated, PCR fragments of the seven housekeeping genes *rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB* and *tonB* were obtained from chromosomal DNA and directly sequenced. Allelic profiles and sequence types (STs) were designated at the website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). New alleles and STs were submitted to the MLST website and approved. Clonal complexes were determined by including whole *K. pneumoniae* MLST data using the program eBURST v3 (Feil *et al.*, 2004). For PFGE, agarose-embedded bacterial genomic DNA was digested with 20 U *Xba*I. The restriction fragments were separated by electrophoresis in 0.5× TBE buffer (Chung *et al.*, 2008). Electrophoresis was performed using CHEF Mapper XA (Bio-Rad).

Table 1. *K. pneumoniae* isolates included in this study

Hospital*	UTI		Bacteraemia	
	No. of isolates	No. of ESBL-producing isolates	No. of isolates	No. of ESBL-producing isolates
SMC	58	12	157	20
KSH	19	5	2	1
KUH	2	−†	11	1
KNUH	4	−†	3	−†
DFH	10	3	NA	NA
ConNUH	39	11	18	2
CunNUH	30	8	9	1
CubNUH	5	−†	1	1
GNUH	7	4	2	−†
JNUH	11	4	18	4
Total	185	47	221	30

NA, Not available.
*SMC, Samsung Medical Center; KSH, Kangbuk Samsung Hospital; KUH, Konkuk University Hospital; KNUH, Kyungpook National University Hospital; DFH, Daegu Fatima Hospital; ConNUH, Chonnam National University Hospital; CunNUH, Chungnam National University Hospital; CubNUH, Chungbuk National University Hospital; GNUH, Gyeongsang National University Hospital; JNUH, Jeju National University Hospital.
†No isolates.

Statistical analysis. Fisher's exact *t*-test was used to determine the significant differences in resistance and production of ESBL using SPSS for Windows (version 11.5 software package).

RESULTS AND DISCUSSION

This study is the first nationwide surveillance and molecular characterization of invasive *K. pneumoniae* isolates from patients with bacteraemia or UTI in Korea.

Antimicrobial resistances and ESBL prevalence

The first finding from this study was that *K. pneumoniae* isolates from bloodstream infections showed lower antimicrobial resistance rates than isolates from urine cultures, although this was not significant for ampicillin, imipenem and trimethoprim–sulfamethoxazole. While resistance rate was the highest for ampicillin (93.1 %) and the lowest for imipenem (0.3 %) among 406 invasive *K. pneumoniae* isolates, the resistance rates to the other antimicrobial agents ranged from 18.2 % (cefotaxime) to 24.9 % (ceftazidime) (Table 2). Although all ESBL-producing members of the *Enterobacteriaceae* are considered resistant to third-generation cephalosporins, three and five *K. pneumoniae* isolates had MICs in the susceptibility range of cefotaxime (8 mg l^{-1}) and ceftazidime (4–8 mg l^{-1}), respectively. *K. pneumoniae* isolates from UTIs were more frequently ESBL producers than those from bacteraemia (25.4 % vs 13.6 %; $P=0.002$). Among 47 ESBL-producing isolates from patients with UTIs, 12 isolates were from SMC, 11 from ConNUH, eight from CunNUH, five from KSH, four each from GNUH and JNUH, and three from DFH. Among 30 ESBL-producing isolates from patients with bacteraemia, 20 isolates were from SMC, four from JNUH, two from ConNUH, and one each from KSH, KNUH, CunNUH and CubNUH (Table 1). Resistance rates of clinical *K. pneumoniae* isolates in a previous (June–August 2005) investigation were similar to those in the present study (Ko *et al.*, 2008a).

For gentamicin, ciprofloxacin, ceftazidime, cefotaxime, aztreonam and piperacillin–tazobactam, antimicrobial resistance rates were significantly lower in isolates from blood than in those from urine (Table 2). The lower resistance rates in isolates from blood may be associated with lower ESBL production rates in them. Lower resistance rates and lower prevalence rate of ESBL in *K. pneumoniae* isolates from bloodstream infections have been documented in previous studies (Jones *et al.*, 2004; Kolar *et al.*, 2006). However, the reason for lower resistance rates in *K. pneumoniae* isolates from blood is unclear.

As shown in Table 2, one *K. pneumoniae* isolate from blood showed imipenem resistance. This isolate was resistant to all antimicrobial agents tested in this study except trimethoprim–sulfamethoxazole. However, it did not produce ESBL. A modified Hodge test was performed for this imipenem-resistant isolate to screen for carbapenemase activity (Lee *et al.*, 2001). As a result, the imipenem-resistant isolate was positive for carbapenemase activity.

Table 2. Prevalence of antimicrobial resistance and ESBLs in *K. pneumoniae* UTI and bacteraemia isolates

Results are given as the number of resistant isolates (%).

Antimicrobial	UTI (n=185)		Bacteraemia (n=221)		P*
	ESBL	Non-ESBL	ESBL	Non-ESBL	
Ampicillin	47 (100)	129 (93.5)	30 (100)	172 (90.1)	0.139
Gentamicin	27 (57.4)	21 (15.2)	23 (76.7)	13 (6.8)	**0.017**
Ciprofloxacin	33 (70.2)	27 (19.6)	26 (86.7)	13 (6.8)	**0.001**
Ceftazidime†	40 (85.1)	22 (15.9)	29 (96.7)	10 (5.2)	**0.000**
Cefotaxime†	33 (70.2)	9 (6.5)	25 (83.3)	7 (3.7)	**0.033**
Aztreonam	42 (89.4)	21 (15.2)	29 (96.7)	8 (4.2)	**0.000**
Imipenem	–	–	–	1 (0.5)	1.000
Trimethoprim–sulfamethoxazole	23 (48.9)	25 (18.1)	17 (56.7)	26 (15.2)	0.118
Piperacillin–tazobactam	32 (68.1)	17 (12.3)	26 (86.7)	9 (4.7)	**0.008**
ESBL production	47 (25.4)		30 (13.6)		**0.002**

*P-value compared between total isolates from UTI and bacteraemia. Bold indicates significance.
†Although several ESBL-producing *K. pneumoniae* isolates have MICs in the susceptible range for ceftazidime and cefotaxime, they are considered resistant to them.

Types of *bla* genes

Among 77 ESBL-producing *K. pneumoniae* isolates, nine different types of ESBL genes were identified: one TEM, four SHVs and four CTX-Ms (Table 3). The most prevalent ESBL gene was SHV-12 (17 isolates, 22.1 %), followed by CTX-M-15 (14 isolates, 18.2 %), SHV-31 (13 isolates, 16.9 %) and CTX-M-14 (9 isolates, 11.7 %). While CTX-M-15 was identified in 10.6 % of isolates from UTIs, it was the most prevalent type in bacteraemia isolates (30.0 %). Although TEM-135, SHV-22 and CTX-M-27 were not found in bacteraemia isolates, there were no significant differences in distribution of ESBL genes between UTI and bacteraemia isolates. Although most ESBL-producing isolates contained one ESBL gene, SHV-12 and CTX-M-14, SHV-26 and CTX-M-15, SHV-31 and CTX-M-15, and SHV-31 and CTX-M-22 were identified simultaneously in each isolate. Nineteen isolates possessed SHV-11, but no ESBL genes were found in them. Their ESBL activities may be due to different ESBLs or yet-to-be identified ESBL genes. Alternatively, it is possible that we could not detect the *bla* genes due to nucleotide changes at the primer sites.

Table 3. Prevalence of *bla* genes

bla gene	Total (n=77) (%)	UTI (n=47) (%)	Bacteraemia (n=30) (%)
bla_{TEM}			
bla_{TEM-1}	23 (29.9)	13 (27.7)	10 (33.3)
$bla_{TEM-135}$	1 (1.3)	1 (2.1)	–
bla_{SHV}			
bla_{SHV-1}	11 (14.3)	4 (8.5)	7 (23.3)
bla_{SHV-11}	32 (41.6)	22 (46.8)	10 (33.3)
bla_{SHV-12}	17 (22.1)	10 (21.3)	7 (23.3)
bla_{SHV-26}	2 (2.6)	2 (4.3)	–
bla_{SHV-31}	13 (16.9)	8 (17.0)	5 (16.7)
bla_{SHV-93} variant*	2 (2.6)	1 (2.1)	1 (3.3)
bla_{CTX-M}			
$bla_{CTX-M-14}$	9 (11.7)	7 (14.9)	2 (6.7)
$bla_{CTX-M-15}$	14 (18.2)	5 (10.6)	9 (30.0)
$bla_{CTX-M-22}$	4 (5.2)	3 (6.4)	1 (3.3)
$bla_{CTX-M-27}$	1 (1.3)	1 (2.1)	–
None of the above	19 (24.7)	13 (27.7)	6 (20.0)

*Differs from SHV-93 by A79T.

It has been suggested that IS*Ecp1* may play an important role in the mobility of the bla_{CTX-M} genes as a promoter (Kolar *et al.*, 2006). IS*Ecp1* was detected in all but one bla_{CTX-M}-positive *K. pneumoniae* isolates: a single isolate with CTX-M-27 was not positive for PCR assay of IS*Ecp1*. However, when association of IS*Ecp1* with CTX-M was screened by a combination of an IS*Ecp1* forward primer and CTX-M reverse primer (Poirel *et al.*, 2005), additional seven bla_{CTX-M}-positive isolates showed no PCR products: four CTX-M-14-producing isolates, two CTX-M-15-producing isolates and one CTX-M-22-producing isolate. This may imply that diverse bla_{CTX-M} genes have been incorporated in *K. pneumoniae* isolates from Korea.

Genotypes

Genotypes of 77 ESBL-producing *K. pneumoniae* isolates were determined using MLST. As a result, a total of 22 different STs were identified in this study (Table 4). Of these, 19 STs belonged to 14 clonal complexes, but the other 3 STs were designated singletons. The most prevalent was ST11 (allelic profile 3-3-1-1-1-1-4). ST11 accounted for 57.4 % and 70.0 % of isolates from UTIs

Table 4. Distribution of sequence types in ESBL-producing *K. pneumoniae* isolates

Clonal complex	Sequence type	Allelic profile*	No. of isolates		ESBL gene (no. of isolates)
			UTI (n=47)	Bacteraemia (n=30)	
CC11	ST11	3-3-1-1-1-1-4	27 (57.4 %)	21 (70.0 %)	SHV-12 (12)
					SHV-12 + CTX-M-14 (1)
					SHV-31 (9)
					SHV-31 + CTX-M-15 (1)
					SHV-31 + CTX-M-22 (1)
					CTX-M-15 (4)
					CTX-M-14 (1)
					CTX-M-22 (3)
					Unknown (16)
	ST258	3-3-1-1-1-1-79	1	1	CTX-M-14 (2)
	ST270	3-3-1-1-1-1-23	−†	1	SHV-31 (1)
CC14	ST15	1-1-1-1-1-1-1	−	1	CTX-M-15 (1)
	ST265	31-1-1-1-1-1	1		SHV-12 (1)
CC37	ST37	2-9-2-1-13-1-16	2	−	TEM-35 + SHV-12 (1)
					CTX-M-15 (1)
	ST271	2-9-2-1-13-1-81	−	1	CTX-M-15 (1)
CC17	ST17	2-1-1-1-4-4-4	−	1	CTX-M-15 (1)
	ST261	2-1-1-1-4-27-12	2	−	CTX-M-14 (2)
CC292	ST268	2-1-2-1-7-1-81	1	−	Unknown (1)
CC65	ST262	2-1-1-1-10-4-43	1	1	SHV-93 var. (2)
CC122	ST34	2-3-6-1-9-7-4	2	−	SHV-26 + CTX-M-15 (1)
					SHV-26 (1)
CC219	ST107	2-1-2-17-27-1-39	1	−	CTX-M-14 (1)
CC163	ST218	2-3-1-1-9-4-12	2	1	CTX-M-15 (3)
CC133	ST269	12-1-1-2-5-1-18	−	1	SHV-12 (1)
CC285	ST264	16-24-21-27-58-22-80	1	−	SHV-12 (1)
CC39-224‡	ST39	2-1-2-4-9-1-14	1	−	CTX-M-14 (1)
CC43-101‡	ST101	2-6-1-5-4-1-6	1	−	SHV-31 (1)
CC190-263-286‡	ST263	2-3-1-20-7-1-16	1	−	CTX-M-14 (1)
Singleton	ST48	2-5-2-2-7-1-10	−	1	CTX-M-15 (1)
	ST266	2-1-1-13-16-1-4	1	−	CTX-M-27 (1)
	ST267	3-5-20-1-16-1-12	2	−	Unknown (2)

*Allelic profiles are in the following order: *gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB*.
†No isolates.
‡Ancestor of CC was not designated.

and bacteraemia, respectively. It was found in most hospitals except DFH; 25 isolates from SMC, nine isolates from ConNUH, four isolates from JNUH, three isolates from KSH, three isolates from CunNUH, two isolates from GNUH, one isolate from KUH and one isolate from CubNUH. Although these ST11 isolates showed similar PFGE results, they may diversify into several subtypes. Specific PFGE patterns were not related with a specific hospital or a specific ESBL gene. Only one to three isolates belonged to the other 21 STs. Thirty-two ST11 isolates contained one or more ESBL genes, consisting of eight combinations (Table 4). Although it was investigated whether other ESBL genes such as OXA and VEB were present in them, we could not find additional ESBL genes. ST11 has been described in Europe such as Hungary, the Netherlands, France and Spain (http://www.pasteur.fr/recherche/genopole/PF8/mlst/) and designated epidemic clone III (EC III) (Damjanova *et al.*, 2008). Dissemination in both Europe and Asia may indicate that ST11 is one of the pandemic clones although there are limited epidemiological data on the worldwide distribution of *K. pneumoniae*. These ST11 isolates from Korea are characterized by all being ciprofloxacin-resistant, while only 25.0 % of other ESBL-producing isolates were resistant to ciprofloxacin (data not shown). However, the combination of different ESBL genes with ST11 isolates may indicate that the ESBL-producing isolates with ST11 have diverse origins. In addition, it can be emphasized that further characterization of ESBL-producing isolates such as assay of *bla* genes is needed, instead of relying only on the MLST, ribotyping, PFGE, etc.

The most prevalent ESBL in ST11 isolates was SHV-12 (13 isolates), followed by SHV-31 (11 isolates). Among 17 ESBL-producing isolates with SHV-12, 13 isolates (76.5 %) belonged to ST11. Isolates with CTX-M-15 showed divergent genotypes. Another prevalent CTX-M enzyme, CTX-M-14, was also found in diverse clones: nine isolates with CTX-M-14 belonged to six STs. CTX-M-type ESBLs, especially CTX-M-15, have recently emerged as the prevailing ESBL enzyme in Gram-negative bacteria including *K. pneumoniae* (Livermore *et al.*, 2007). In the present study, CTX-M-15-producing isolates showed eight different STs. Although outbreaks of clonal strains producing CTX-M-15 have been reported worldwide, several reports also documented clonal diversity of CTX-M-15 based on PFGE type (Abbassi *et al.*, 2008; Livermore *et al.*, 2007), which suggests horizontal transfer of the $bla_{\text{CTX-M-15}}$ gene. In addition, MLST analyses also indicated that CTX-M-15-producing *K. pneumoniae* isolates belonged to diverse STs (Elhani *et al.*, 2010; Oteo *et al.*, 2009). Moreover, different STs were positive for $bla_{\text{CTX-M-15}}$ in each country: STs 1, 11, 14, 17, 20, 35 and 36 in Spain (Oteo *et al.*, 2009); and STs 42, 101, 104, 133, 147 and 321 (Elhani *et al.*, 2010), and STs 11, 15, 17, 34, 37, 48, 218 and 271 in Korea (this study). Therefore, $bla_{\text{CTX-M-14}}$ or $bla_{\text{CTX-M-15}}$ genes might be incorporated frequently into several *K. pneumoniae* strains including the inter-continently disseminated clone ST11.

Although it was recently reported that most *K. pneumoniae* isolates causing liver abscess in Korea were ST23, serotype K1 and *magA*-positive (Chung *et al.*, 2008), no ST23 ESBL-producing isolates were found in this study, which suggests that different *K. pneumoniae* infections might be caused by different clones.

Our study may have a few limitations: (i) sufficient *K. pneumoniae* isolates were not included although 10 hospitals participated in the surveillance; and (ii) the numbers of included isolates were not equal among participating hospitals. However, we propose that ST11 is prevalent in ESBL-producing *K. pneumoniae* isolates causing bacteraemia and UTIs in Korean hospitals, although a large study including more isolates from more hospitals is required. Isolates of the same ST such as ST11 carried different ESBL genes, which may indicate that more tools employed in the epidemiological study would guarantee better understanding of the evolution of ESBL-producing isolates and tracking of them. In addition, CTX-M-type ESBLs belonged to diverse STs, indicating their frequent introduction into *K. pneumoniae*.

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REFERENCES

Abbassi, M. S., Torres, C., Achour, W., Vinué, L., Sáenz, Y., Costa, D., Bouchami, O. & Ben Hassen, A. (2008). Genetic characterization of CTX-M-15-producing *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from stem cell transplant patients in Tunisia. *Int J Antimicrob Agents* **32**, 308–314.

Bae, I. K., Lee, Y. N., Jeong, S. H., Lee, K., Lee, H., Kwak, H. S. & Woo, G. J. (2007). High prevalence of SHV-12 and the emergence of CTX-M-12 in clinical isolates of *Klebsiella pneumoniae* from Korea. *Int J Antimicrob Agents* **29**, 362–364.

Brisse, S., Fevre, C., Passet, V., Issenhuth-Jeanjean, S., Tournebize, R., Diancourt, L. & Grimont, P. (2009). Virulent clones of *Klebsiella pneumoniae*: identification and evolutionary scenario based on genomic and phenotypic characterization. *PLoS One* **4**, e4982.

Chung, D. R., Lee, H. R., Lee, S. S., Kim, S. W., Chang, H. H., Jung, S. I., Oh, M. D., Ko, K. S., Kang, C. I. & other authors (2008). Evidence for clonal dissemination of the serotype K1 *Klebsiella pneumoniae* strain causing invasive liver abscesses in Korea. *J Clin Microbiol* **46**, 4061–4063.

CLSI (2009). *Performance Standards for Antimicrobial Susceptibility Testing*; 19th Informational Supplement. M100-S19. Wayne, PA: Clinical and Laboratory Standards Institute.

Damjanova, I., Tóth, A., Pászti, J., Hajbel-Vékony, G., Jakab, M., Berta, J., Milch, H. & Füzi, M. (2008). Expansion and countrywide dissemination of ST11, ST15, and ST147 ciprofloxacin-resistant CTX-M-15-type β-lactamase-producing *Klebsiella pneumoniae* epidemic clones in Hungary in 2005 – the new 'MRSAs'? *J Antimicrob Chemother* **62**, 978–985.

Diancourt, L., Passet, V., Verhoef, J., Grimont, P. A. & Brisse, S. (2005). Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol* **43**, 4178–4182.

Elhani, D., Bakir, L., Aouni, M., Passet, V., Arlet, G., Brisse, S. & Weill, F. X. (2010). Molecular epidemiology of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* strains in a university hospital in Tunis, Tunisia, 1999–2005. *Clin Microbiol Infect* **16**, 157–164.

Feil, E. J., Li, B. C., Aanensen, D. M., Hanage, W. P. & Spratt, B. G. (2004). eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing. *J Bacteriol* **186**, 1518–1530.

Girlich, D., Poirel, L., Leelaporn, A., Karim, A., Tribuddharat, C., Fennewald, M. & Nordmann, P. (2001). Molecular epidemiology of the integron-located VEB-1 extended spectrum β-lactamase in nosocomial enterobacterial isolates in Bangkok, Thailand. *J Clin Microbiol* **39**, 175–182.

Jeong, S. H., Bae, I. K., Kwon, S. B., Lee, J. H., Jung, H. I., Song, J. S., Jeong, B. C., Kim, S. J. & Lee, S. H. (2004). Investigation of extended-spectrum β-lactamases produced by clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Korea. *Lett Appl Microbiol* **39**, 41–47.

Jones, M. E., Karlowsky, J. A., Draghi, D. C., Thornberry, C., Sahm, D. F. & Bradley, J. S. (2004). Rates of antimicrobial resistance among common bacterial pathogens causing respiratory, blood, urine, and skin and soft tissue infections in pediatric patients. *Eur J Clin Microbiol Infect Dis* **23**, 445–455.

Kim, J., Lim, Y. M., Rheem, I., Lee, Y., Lee, J. C., Seol, S. Y., Lee, Y. C. & Cho, D. T. (2005). CTX-M and SHV-12 β-lactamases are the most common extended-spectrum enzymes in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* collected from 3 university hospitals within Korea. *FEMS Microbiol Lett* **245**, 93–98.

Ko, K. S., Lee, M. Y., Song, J. H., Lee, H., Jung, D. S., Jung, S. I., Kim, S. W., Chang, H. H., Yeom, J. S. & other authors (2008a). Prevalence and characterization of extended-spectrum β-lactamase-producing

Enterobacteriaceae isolated in Korean hospitals. *Diagn Microbiol Infect Dis* **61**, 453–459.

Ko, K. S., Yeom, J. S., Lee, M. Y., Peck, K. R. & Song, J. H. (2008b). Clonal dissemination of extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* isolates in a Korean hospital. *J Korean Med Sci* **23**, 53–60.

Kolar, M., Latal, T., Cermak, P., Bartonikova, N., Chmelarova, E., Sauer, P. & Kesselova, M. (2006). Prevalence of extended-spectrum β-lactamase-positive *Klebsiella pneumoniae* isolates in the Czech Republic. *Int J Antimicrob Agents* **28**, 49–53.

Lee, K., Chong, Y., Shin, H. B., Kim, Y. A., Yong, D. & Yum, J. H. (2001). Modified-Hodge and EDTA-disk synergy tests to screen metallo-beta-lactamase-producing strains of *Pseudomonase* and *Acinetobacter* species. *Clin Microbiol Infect* **7**, 88–91.

Livermore, D. M., Canton, R., Gniadkowski, M., Nordmann, P., Rossolini, G. M., Arlet, G., Ayala, J., Coque, T. M., Kern-Zdanowicz, I. & other authors (2007). CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother* **59**, 165–174.

Maiden, M. C., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K. & other authors (1998). Multilocus sequence typing: a portable approach to identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci U S A* **95**, 3140–3145.

Oteo, J., Cuevas, O., López-Rodríguez, I., Banderas-Florido, A., Vindel, A., Pérez-Vázquez, M., Bautista, V., Arroyo, M., García-Caballero, J. & other authors (2009). Emergence of CTX-M-15-producing *Klebsiella pneumoniae* of multilocus sequence types 1, 11, 14, 17, 20, 35 and 36 as pathogens and colonizers in newborns and adults. *J Antimicrob Chemother* **64**, 524–528.

Poirel, L., Lartigue, M.-F., Decousser, J.-W. & Nordmann, P. (2005). ISEcp1B-mediated transposition of bla_{CTX-M} in *Escherichia coli*. *Antimicrob Agents Chemother* **49**, 447–450.

Ryoo, N. H., Kim, E. C., Hong, S. G., Park, Y. J., Lee, K., Bae, I. K., Song, E. H. & Jeong, S. H. (2005). 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* **56**, 698–702.