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Clonal Dissemination of Extended-Spectrum β-Lactamase (ESBL)-Producing *Klebsiella pneumoniae* Isolates in a Korean Hospital

In this study, we investigated the molecular characteristics of extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* isolates that were recovered from an outbreak in a Korean hospital. A new multilocus sequence typing (MLST) scheme for *K. pneumoniae* based on five housekeeping genes was developed and was evaluated for 43 ESBL-producing isolates from an outbreak as well as 38 surveillance isolates from Korea and also a reference strain. Overall, a total of 37 sequence types (STs) and six clonal complexes (CCs) were identified among the 82 *K. pneumoniae* isolates. The result of MLST analysis was concordant with that of pulsed-field gel electrophoresis. Most of the outbreak isolates belonged to a certain clone (ST2), and they produced SHV-1 and CTX-M14 enzymes, which was a different feature from that of the *K. pneumoniae* isolates from other Korean hospitals (ST20 and SHV-12). We also found a different distribution of CCs between ESBL-producing and -nonproducing *K. pneumoniae* isolates. The MLST method we developed in this study could provide unambiguous and well-resolved data for the epidemiologic study of *K. pneumoniae*. The outbreak isolates showed different molecular characteristics from the other *K. pneumoniae* isolates from other Korean hospitals.

Key Words : *Klebsiella pneumoniae; Extended-Spectrum β-Lactamase (ESBL); Multilocus Sequence Typing (MLST); Pused-Field Gel Electrophoresis (PFGE)*

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INTRODUCTION

Klebsiella pneumoniae is a relevant opportunistic pathogen that accounts for up to 10% of all nosocomial infections (1, 2). It is a frequent causal agent of urinary tract infections, septicemia, and pneumonia in immunocompromised patients, and it is also an important pathogen for community-acquired infections. Its importance in clinical settings is increasing due to its ability to produce extended-spectrum β-lactamase (ESBL) (3). It has been reported that ESBL-producing organisms tend to be multidrug-resistant and they have an increased risk of treatment failure (3, 4). Thus, it is indispensable to detect these ESBL-producing isolates and to control their emergence and spread.

Several molecular methods have been used for characterizing *K. pneumoniae* isolates such as randomly amplified polymorphic DNA analysis, pulsed-field gel electrophoresis, and amplified fragment length polymorphism (5-7). However, these methods are band-based and so the results may be ambiguous, but generally these methods are well suited for local epidemiologic investigation. Yet, it is very difficult to compare the results generated at different laboratories for conducting a global epidemiologic analysis. To overcome the limitations of the band-based typing methods, multilocus sequence typing (MLST) has been developed, which is a nucleotide sequence-based method for bacterial or fungal typing (8-10). It is highly discriminative and it is easy to standardize, store, and exchange information electronically. It has been applied successfully for the epidemiologic characterization of a variety of clinically important bacterial pathogens, such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecium*, *Neisseria meningitidis*, and *Campylobacter jejuni* (9-13). Very recently, a MLST method for *K. pneumoniae* by using seven housekeeping genes, such as *rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB*, *and tonB*, has also been developed (14).

We developed another MLST scheme for *K. pneumoniae* isolates based on five housekeeping genes, and we characterized the ESBL-producing *K. pneumoniae* isolates from an outbreak in a Korean hospital.

MATERIALS AND METHODS

Bacterial isolates

From August 2004 to May 2005, a total of 66 ESBL-producing *K. pneumoniae* were isolated from patients in an intensive care unit (ICU) of the Kangbuk Samsung Hospital (Seoul, Korea), which is a tertiary care hospital with 645 beds. Of these, 43 isolates were preserved and included in this study (Table 1). Twenty-nine isolates were from sputum, and 11 isolates were from urine; the others were isolated from blood or bile. Thirty-eight *K. pneumoniae* isolates, which were isolated at another three Korean tertiary hospitals as a part of a surveillance study that was conducted in 1999 and 2004, were also investigated in this study for comparisons (Table 2). Thirty-one isolates were collected from the Samsung Medical Center (Seoul, Korea), and others were from the Dong-A University Hospital (Busan, Korea) and the Chungbuk Nation-

Table 1. Characteristics of 43 ESBL-producing *K. pneumoniae* isolates from the Kangbuk Samsung Hospital

Isolate No.	Month/year	Source	Allelic profile*	ST	CC	PFGE	*bla* gene		
							SHV†	CTX-M	TEM
KS409-1	9/2004	Blood	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS409-2	9/2004	Urine	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS411-1	11/2004	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	TEM-1
KS411-2	11/2004	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS411-3	11/2004	Sputum	2-9-2-12-3	2	2	A	SHV-5	CTX-M14	TEM-1
KS411-4	11/2004	Sputum	2-9-2-12-3	2	2	A	SHV-5	CTX-M14	TEM-1
KS411-5	11/2004	Sputum	2-9-2-12-3	2	2	A	SHV-5	CTX-M14	TEM-1
KS411-6	11/2004	Sputum	2-2-8-3-9	9	36	B	SHV-11	CTX-M14	–
KS411-7	11/2004	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS411-8	11/2004	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS411-9	11/2004	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS411-10	11/2004	Urine	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS411-11	11/2004	Sputum	2-11-2-4-2	10	–	C	SHV-14	–	–
KS411-12	11/2004	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	TEM-1
KS412-1	12/2004	Sputum	2-8-5-2-3	11	11-14	D	SHV-11	CTX-M14	–
KS412-2	12/2004	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS412-3	12/2004	Sputum	2-2-8-3-9	9	36	B	SHV-11	CTX-M14	–
KS412-4	12/2004	Sputum	8-12-9-13-14	12	–	E	–	–	–
KS412-5	12/2004	Urine	2-2-8-3-9	9	36	B	SHV-11	CTX-M14	–
KS412-6	12/2004	Sputum	2-2-2-12-3	13	2	AA	SHV-1	CTX-M14	–
KS412-7	12/2004	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS412-8	12/2004	Sputum	2-8-5-2-3	11	11-14	D	SHV-11	CTX-M14	–
KS412-9	12/2004	Sputum	2-2-8-3-9	9	36	B	SHV-11	CTX-M14	–
KS501-1	1/2005	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS501-2	1/2005	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS501-3	1/2005	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS501-4	1/2005	Urine	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS501-5	1/2005	Sputum	2-4-5-2-3	14	11-14	DD	SHV-1	CTX-M14	–
KS501-6	1/2005	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS501-7	1/2005	Sputum	7-9-2-12-3	15	2	A	SHV-1	CTX-M14	TEM-1
KS501-8	1/2005	Urine	7-9-2-12-3	15	2	A	SHV-1	CTX-M14	–
KS502-1	2/2005	Sputum	7-9-2-12-3	15	2	A	SHV-1	CTX-M14	–
KS502-2	2/2005	Urine	7-9-2-12-3	15	2	A	SHV-1	CTX-M14	TEM-1
KS502-3	2/2005	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	TEM-1
KS502-4	2/2005	Urine	7-9-2-12-3	15	2	A	SHV-1	CTX-M14	–
KS502-5	2/2005	Urine	5-10-2-10-6	16	3-16	F	SHV-12	–	TEM-1
KS502-6	2/2005	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	TEM-1
KS502-7	2/2005	Bile	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS502-8	2/2005	Urine	4-7-8-11-1	17	20	G	SHV-11	CTX-M14	–
KS504-1	4/2005	Urine	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS505-1	5/2005	Sputum	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–
KS505-2	5/2005	Bile	4-3-8-11-1	18	20	G	SHV-11	–	–
KS505-3	5/2005	Urine	2-9-2-12-3	2	2	A	SHV-1	CTX-M14	–

*, *rpoB-gyrA-gapA-groEL-gyrB*; †, SHV-1 is present in the chromosome of *K. pneumoniae*.
ESBL, extended-spectrum β-lactamase; ST, sequence type; CC, clonal complex; PFGE, pulsed-field gel electrophoresis.

al University Hospital (Cheongju, Korea). Of these 38 surveillance isolates, 15 produced ESBL and the others did not. For screening for ESBL, the GNS-650 card (Vitek system, bioMérieux, Hazelwood, MO, U.S.A.), which is based on the use of cefotaxime and ceftazidime with or without clavulanic acid, was used. For a reference, *K. pneumoniae* ATCC 13883 was also analyzed. Thus, a total of 82 *K. pneumoniae* isolates were analyzed.

Multilocus sequence typing

We selected five housekeeping genes to establish the MLST scheme; *rpoB* (RNA polymerase β-subunit), *gyrA* (DNA gyrase subunit A), *gapA (*glyceraldehydes 3-phosphate dehydrogenase A), *groEL* (GroEL protein), and *gyrB* (DNA gyrase subunit B). The primers we used for amplification of the gene fragments are shown in Table 3. PCR amplifications were carried out under the following conditions: 35 cycles of denaturation at 94℃ for 30 sec, annealing at 50-55℃ for 30 sec, and extension at 72℃ for 1 min; this was all preceded by a 5 min denaturation at 94℃ and then it was all followed by a 5 min final extension at 72℃. The PCR products were purified using a PCR purification kit (Bioneer, Daejeon, Korea), according to the manufacturer's recommendations, and then the products were sequenced on an ABI3700 DNA sequencer (Applied Biosystems, Foster City, CA, U.S.A.).

Table 2. Characteristics of *K. pneumoniae* isolates from other Korean hospitals

Isolate No.	Hospital	Year	Source	Allelic profile*	ST	CC	ESBL	*bla* gene		
								SHV	CTX-M	TEM
ATCC13883				1-1-1-1-6	1	-				
01-40	SMC	2004	Transtracheal aspirate	4-7-6-11-1	20	20	P	SHV-12	CTX-M14	–
01-41	SMC	2004	Catheterized urine	4-17-6-11-1	21	20	P	SHV-12	CTX-M14	TEM-1
01-43	SMC	2004	Tracheal aspirate	4-7-6-11-1	20	20	P	SHV-12	CTX-M14	–
01-84	SMC	2004	Discharged ear	4-7-6-11-1	20	20	P	SHV-12	–	TEM-1
01-86	SMC	2004	Catheterized urine	4-17-6-11-1	21	20	P	SHV-11	–	TEM-1
01-88	SMC	2004	Others	2-8-5-2-3	11	11-14	P	SHV-12	–	TEM-1
01-114	SMC	2004	Sputum	4-18-6-11-1	26	20	P	SHV-12	–	–
01-129	SMC	2004	Others	4-7-6-11-1	20	20	P	SHV-2	–	–
01-144	SMC	2004	Tracheal aspirate	4-7-6-11-1	20	20	P	SHV-12	–	–
01-163	SMC	2004	Others	2-8-5-2-3	11	11-14	P	SHV-12	–	TEM-1
01-175	SMC	2004	Catheterized urine	4-19-2-5-8	28	–	P	SHV-2	–	–
01-184	SMC	2004	Sputum	2-2-2-5-7	29	–	P	SHV-27	CTX-M14	TEM-1
05-412	DAUH	2004	-	2-15-6-2-4	30	–	P	SHV-1	CTX-M14	TEM-1
10-347	CNUH	2004	-	2-14-6-3-9	31	36	P	SHV-12	–	TEM-1
10-352	CNUH	2004	-	4-7-6-11-1	20	20	P	SHV-12	–	–
K1	SMC	1999	Pus	4-4-2-4-13	5	–	N			
K2	SMC	1999	Others	4-2-2-8-6	6	–	N			
K3	SMC	1999	Others	6-3-5-9-6	7	–	N			
K77	SMC	2000	Sputum	5-4-2-10-6	3	3-16	N			
K78	SMC	2000	Blood	4-3-4-2-6	8	–	N			
01-39	SMC	2004	Voided urine	4-7-6-6-1	19	–	N			
01-42	SMC	2004	Peritoneal fluid	4-7-6-11-1	20	20	N			
01-44	SMC	2004	Sputum	3-6-7-7-1	22	22-27	N			
01-45	SMC	2004	Others	2-2-2-12-3	13	2	N			
01-46	SMC	2004	Others	2-2-2-12-3	13	2	N			
01-47	SMC	2004	Throat swab	2-2-6-3-10	4	36	N			
01-85	SMC	2004	Others	2-4-5-2-3	14	11-14	N			
01-87	SMC	2004	Sputum	3-6-7-7-3	27	22-27	N			
01-89	SMC	2004	Voided urine	2-4-2-4-1	23	–	N			
01-112	SMC	2004	Open pus	9-16-3-14-11	24	–	N			
01-113	SMC	2004	Sputum	4-3-2-3-1	25	–	N			
01-115	SMC	2004	Sputum	4-7-6-11-1	20	20	N			
01-116	SMC	2004	Nasal/Nasopharynx	4-5-2-5-5	35	–	N			
01-117	SMC	2004	Closed pus	2-20-2-12-3	37	2	N			
05-408	DAUH	2004	-	2-2-6-3-9	36	36	N			
10-351	CNUH	2004	-	10-7-6-11-1	32	20	N			
10-356	CNUH	2004	-	4-13-2-3-6	33	–	N			
10-357	CNUH	2004	-	9-16-3-15-12	34	–	N			

SMC, Samsung Medical Center; DAUH, Dong-A University Hospital; CNUH, Chungbuk National University Hospital; ST, sequence type; CC, clonal complex; ESBL, extended-spectrum β-lactamase; P, positive; N, negative. **rpoB-gyrA-gapA-groEL-gyrB.*

The raw sequences were concatenated and edited by using the EditSeq and MegAlign programs (DNASTAR, Madison, WI, U.S.A.). For each locus, distinct allele sequences were assigned as an arbitrary allele number. Each isolate was characterized by its allelic profile, which was represented as a series of 5 integers corresponding to the alleles at each of the loci, in the order of *rpoB*, *gyrA*, *gapA*, *groEL*, and *gyrB*. The sequence type (ST) was designated for each unique allelic profile. Clusters of related STs sharing three or more identical alleles out of the five were defined as clonal complexes (CCs), which were determined by the eBURST program (http://eburst.mlst.net/loci.asp) (15). The d_N/d_S ratios, with d_N being the number of nonsynonymous substitutions per nonsynonymous site and d_S being the number of synonymous substitutions per synonymous site (16), were determined by using the START program (http://outbreak.ceid.ox.ac.uk/software.htm). The index of association (I_A) was also calculated with the START program to test to what extent the *K. pneumoniae* population was clonal (17). I_A is a measure of the variance in the number of allelic mismatches relative to that expected under the hypothesis of panmixia (random association), where the mean number of pairwise allelic mismatches is a measure of genetic distance. When the I_A value does not deviate significantly from zero, the alleles are in linkage disequilibrium, i.e., there is frequent recombination or non-clonal population structure.

Pulsed-field gel electrophoresis (PFGE)

The 43 ESBL-producing *K. pneumoniae* isolates recovered from an outbreak at the Kangbuk Samsung Hospital were characterized by PFGE as described elsewhere (18, 19). Agarose plugs containing genomic DNA were digested with *Sma*I (Gibco, BRL, Gaithersburg, MD, U.S.A.), according to the manufacturer's recommendations. The DNA restriction fragments were separated with using a CHEF-Mapper apparatus (Bio-Rad Lab.), at 6 V/cm for 22 hr. The PFGE patterns were interpreted with using the published criteria (20). A divergence in more than three bands was interpreted as indicative of different PFGE types.

Table 3. Primers for multilocus sequence typing of *K. pneumoniae*

Primers	Sequences (5′ to 3′)	Reference
rpoB (RNA polymerase β-subunit)		29
CM81	CAG TTC CGC GTT GGC CTG	
CM32b	CGG AAC GGC CTG ACG TTG CAT	
gyrA (DNA gyrase subunit A)		30
gyrA1	ATG AGC GAC CTT GCG AGA GAA AT	
gyrA2	CTC GTC ACG CAG CGC GCT GAT GCC	
gapA (glyceraldehydes 3-phosphate dehydrogenase A)		30
gapA1	AGA ACA TCA TCC CGT CCT CTA CC	
gapA2	CCA GAA CTT TGT TGG AGT AAC C	
groEL (GroEL protein)		30
groEL1	GAC GCT CGY GTR AAA ATG CTS C	
groEL2	GCA GTG CAA CTT TGA TAC CCA CG	
gyrB (DNA gyrase subunit B)		This study
gyrB1	GCC TCG AAA CCT TCA CCA	
gyrB2	CGC GAC GTG CGG CCT CAC GG	

Detection of *bla* genes

The *bla* genes related to ESBL enzymes were assayed by PCR with the corresponding primers for the TEM, SHV, CTX-M, and OXA ESBL types, as described previously (21). To specify the subtype of the *bla* genes, the amplified PCR products were sequenced on both strands as was done in the MLST analysis. The amino acid sequences were deduced from the nucleotide sequences using the MegAlign program (DNASTAR), and they were compared with the database of the website (http://www.lahey.org/Studies/).

Nucleotide sequence accession numbers

The allele sequences of *K. pneumoniae* ATCC13883 have deposited in the GenBank database under accession numbers DQ673324 to DQ673328.

RESULTS

MLST scheme for *K. pneumoniae* isolates

Sequence data were obtained for the five selected loci from all 82 *K. pneumoniae* isolates included in this study. The nucleotide sequences at each locus ranged from 271 bp (*gapA*) to 752 bp (*gyrA*). The number of different alleles ranged from 9 (*gapA*) to 20 (*gyrA*). The proportion of sites at which nucleotide variation was observed within each locus that was examined ranged from 3.93% (*rpoB*) to 8.49% (*groEL*) (Table 4). By calculating the d_N/d_S ratio, the degree of selection operating on each locus was evaluated. No nonsynonymous substitutions were found in the *gapA* and *gyrB* loci, so the d_N/d_S ratios could not obtained for them. The d_N/d_S ratios for the other loci were significantly less than 1 (Table 4), indicating there was no strong positive selective pressure on the selected genes. Thus, these five loci could be appropriate for the

Table 4. Variation in loci used in the present *K. pneumoniae* MLST scheme

Locus	Size (bp)	No. of alleles	No. of polymorphic sites (%)	d_N/d_S
rpoB	585	10	23 (3.93)	0.0911
gyrA	752	20	30 (3.99)	0.1068
gapA	271	9	11 (4.06)	–*
groEL	636	15	54 (8.49)	0.0078
gyrB	542	14	32 (5.90)	–*

*No nonsynonymous substitutions.
MLST, multilocus sequence typing.

K. pneumoniae MLST scheme. The index of association (I_A) was used to test for linkage disequilibrium between the alleles at the five loci. The I_A value was 1.951 for all the *K. pneumoniae* isolates. This value was reduced to 0.524 when including only one representative isolate for each sequence type (STs), and this reflects the moderate linkage equilibrium or clonal population structure of the *K. pneumoniae* isolates included in this study.

Based on the nucleotide variations of the five genetic loci, a total of 37 different STs could be identified among 82 *K. pneumoniae* isolates (Table 1, 2). The majority of these (29 out 37 STs, 78.4%) were represented by single isolate. Among the STs shared by multiple isolates, the most frequently encountered were ST2 (25 isolates, 30.5%), ST20 (8 isolates, 9.8%), and ST15 (5 isolates, 6.1%). Other STs (ST9, -11, -13, -14, and -21) represented two to four isolates. These STs were grouped, using the eBURST program, into CCs that consisted of all the isolates of the STs that shared three or more identical alleles (Fig. 1). As a result, six CCs were identified among the 82 *K. pneumoniae* isolates. CC20 included six STs (ST17, -18, -20, -21, -26, and -32), of which ST20 was assumed as a founder. Fourteen isolates (17.1%) were included in the CC20. CC2 included four STs (ST2, -13, -15, and -37), of which ST2 was assumed as a founder. Thirty-four isolates (41.5%) belonged to the CC2, but most of them were isolated at the Kangbuk Samsung Hospital during an outbreak. CC36 also included 7 isolates (8.5%) of four STs (ST4, -9, -31, and -36), of which ST36 was assumed as a founder. The other three CCs each consisted of two STs (Table 1, 2, Fig. 1).

PFGE and MLST

PFGE was performed for 43 outbreak isolates from Kangbuk Samung Hospital. Among the 43 outbreak isolates, seven PFGE types (A to G) were identified (Fig. 2 and Table 1). The PFGE patterns were well concordant with the STs in the MLST. Two subtypes, AA and DD, could be differentiated from A and D, respectively. All 31 isolates of the PFGE type A belonged to CC2, which were differentiated into ST2 or ST15. ST2 and ST15 were single-locus variants of each other, differing at *rpoB*. One isolate (KS412-6) of PFGE type AA belonged to ST13, a single-locus variant of ST2 with a different allele in *gyrA*. PFGE type B was correlated with ST36 of CC36. Isolates of the PFGE type D and DD showed ST11 and ST14, respectively, both of which belonged to CC11-14. Both isolates of the PFGE type G belonged to CC20, but they could be differentiated into ST17 and ST18, which are different at *gyrA*. The other three PFGE types, C, E, and F, represented single isolates.

ESBL-producing *K. pneumoniae* isolates with outbreak

Of the 43 ESBL-producing *K. pneumoniae* isolates recovered from the Kangbuk Samsung Hospital, all but one produced SHV enzymes (29 isolates of SHV-1, 5 of SHV-5, 8 of SHV-11, and one each of SHV-12 and SHV-14). All but four isolates produced CTX-M-14 and 10 isolates were positive to *bla*$_{TEM-1}$ (Table 1). One isolate (KS412-4) was negative on PCR assays for the four *bla* genes tested in this study.

All 33 outbreak isolates of CC2 and PFGE type A produced SHV and CTX-M enzymes. All the isolates produced CTX-M-14, and all but three isolates produced SHV-1. The other three isolates produced SHV-5. Nine *K. pneumoniae* isolates produced TEM-1. Except for one isolate (KS502-5) of CC3-16 and PFGE type F, all the TEM-1-producing isolates belonged to CC2 and they showed PFGE type A. Four isolates

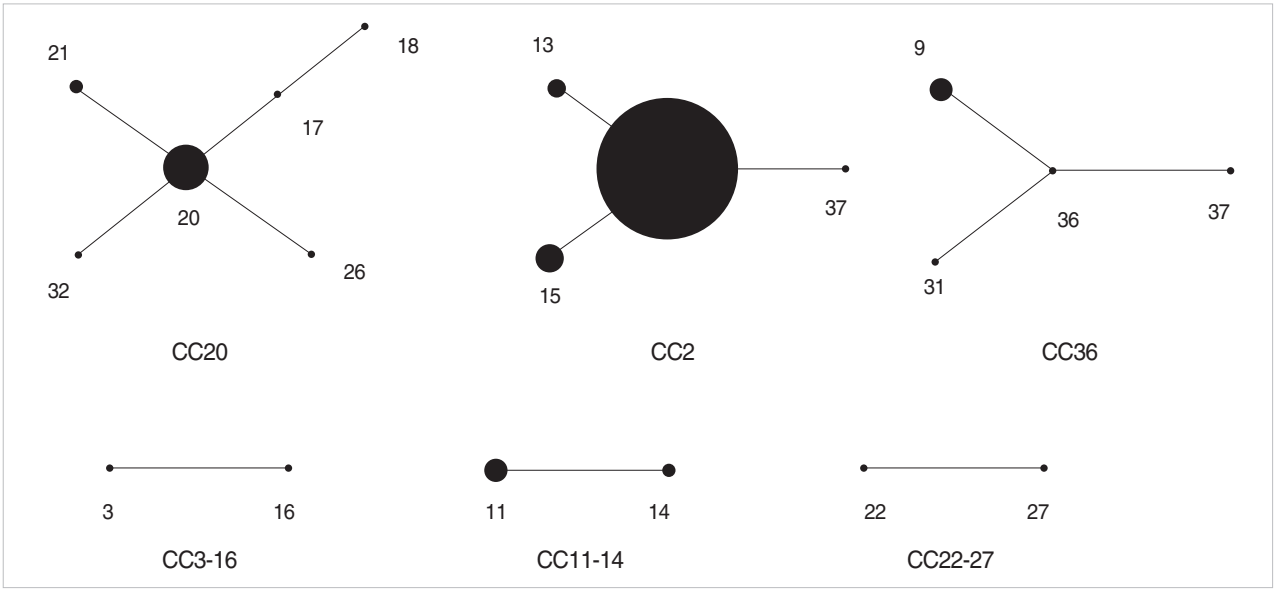

Fig. 1. eBURST analysis of MLST.

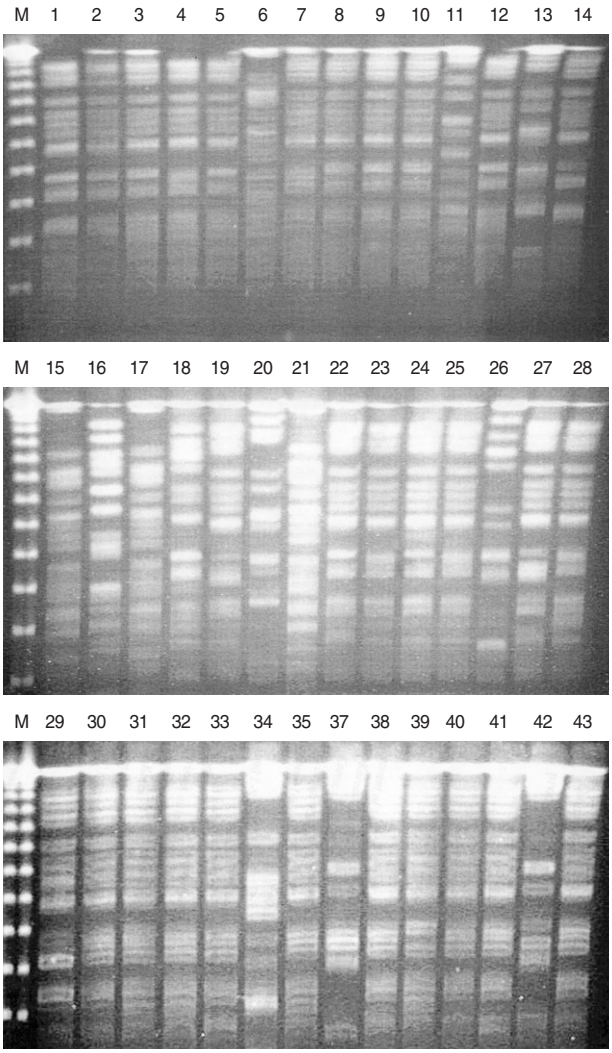

Fig. 2. PFGE patterns of outbreak isolates from Kangbuk Samsung Hospital. M, size marker; 1, KS409-1; 2, KS409-2; 3, KS411-3; 4, KS411-4; 5, KS411-5; 6, KS411-6; 7, KS411-7; 8, KS411-8; 9, KS411-9; 10, KS411-10; 11, KS411-11; 12, KS411-12; 13, KS421-1; 14, KS412-2; 15, KS412-3; 16, KS412-4; 17, KS412-5; 18, KS412-6; 19, KS412-7; 20, KS412-8; 21, KS412-9; 22, KS501-1; 23, KS501-2; 24, KS501-3; 25, KS501-4; 26, KS501-5; 27, KS501-6; 28, KS501-7; 29, KS501-8; 30, KS502-1; 31, KS502-2; 32, KS502-3; 33, KS502-4; 34, KS502-5; 35, KS502-6; 37, 502-8; 38, KS411-2; 39, KS411-1; 40, KS504-1; 41, KS505-1; 42, KS505-2; 43, KS506-3.

of CC36 and PFGE type B produced SHV-11 and CTX-M-14. Of the three isolates of CC11-14, two of ST11 and the PFGE type D produced SHV-11 and CTX-M-14, while one of ST13 and PFGE type DD produced SHV-1 and CTX-M-14. Two isolates of CC20 and PFGE type G produced both SHV-11 and CTX-M-14, or only SHV-11.

K. pneumoniae isolates from other hospitals

Of the 38 *K. pneumoniae* isolates collected from three Korean hospitals as a part of a surveillance program, 15 isolates produced ESBL. All 15 ESBL-producing isolates produced SHV enzymes. Among these surveillance isolates, SHV-12 was the most frequent (10 isolates), and this was in contrast to the outbreak isolates, of which SHV-1 was the most common. Five isolates produced CTX-M-14, and 8 isolates were positive to *bla*$_{TEM-1}$ (Table 2). Of the 15 ESBL-producing isolates, nine belonged to CC20, six isolates belonged to ST20, two belonged to ST21, and one belonged to ST26. There might be no obvious correlation between CC and the *bla* genes. Twenty-three *K. pneumoniae* isolates did not produce ESBL. These ESBL-negative isolates were more diverse than were the ESBL-producers. Only three isolates belonged to CC20 (ST20 and ST32), and three isolates belonged to CC2 (ST13 and ST37). Most of the isolates formed singletons in MLST analysis.

DISCUSSION

The objectives of this study were to demonstrate development of a typing method for *K. pneumoniae* and to investigate the characteristics of the ESBL-producing isolates recovered during an outbreak within a Korean hospital. Molecular typing is prerequisite for elucidating the epidemiology and population structure of bacterial pathogens. Out of several molecular typing methods, MLST is becoming popular owing to its several advantages over the other typing methods, which have been discussed repeatedly elsewhere; a high level of discrimination, it is unambiguous and reproducible, and its scalable nature resulting from use of nucleotide sequences, it has electronic portability via the Internet, it can easily analyze the generated data, and it has a wide applicability (9, 10, 22, 23). Very recently, the MLST scheme for *K. pneumoniae* was reported on and this was based on seven housekeeping genes (14). Diancourt et al. (14) characterized 67 *K. pneumoniae* isolates that were collected from several European countries with using their MLST scheme and they compared the results with those from ribotyping.

In this study, we presented another MLST scheme for *K. pneumoniae* based on five housekeeping genes. The appropriate nucleotide variations of each gene fragment (3.93% to 8.49%) indicated that these genes were applicable for the MLST scheme. The low of d_N/d_S ratio (0 to 0.1068), which indicated the neutral feature of the nucleotide substitution, also suggested that the selected housekeeping genes are probably suitable for population genetic studies of *K. pneumoniae*. Although our MLST scheme could not be compared with that of Diancourt et al. (14) because of the different loci and isolates, it showed results concordant with the PFGE. That is, the isolates with a distinct PFGE pattern belonged to a different ST. Moreover, some PFGE types could be differentiated into two STs, for example, the PFGE type A was differentiated into ST2 and ST15. Thus, our MLST procedure presented here is probably useful for a short-term epidemio-

logic study as well as for a global epidemiologic study, although only five loci were included in the scheme.

Using the MLST scheme, we investigated the molecular characteristics of ESBL-producing *K. pneumoniae* isolates from an outbreak within a Korean hospital. At the Kangbuk Samsung Hospital, at most three or less ESBL-producing *K. pneumoniae* had been isolated per month by July 2004. From August 2004 to May 2005, we observed an outbreak of ESBL-producing *K. pneumoniae* isolates primarily at the ICU. During this period, a total of 66 ESBL-producing *K. pneumoniae* were isolated at up to 14 isolates a month. Although we did not preserve the isolates found in August 2004, we could include the 43 ESBL-producing *K. pneumoniae* isolates from September 2004 to May 2005 in this study.

The results of MLST indicated that most of the isolates collected during the outbreak period belonged to the same clone, ST2 (allelic profile, 2-9-2-12-3). Particularly, all the isolates except two belonged to ST2 early during the outbreak period (September and November, 2004). After November 2004, ST2 was still the most prevalent, but its proportion decreased. In addition, five isolates emerged with its single-locus variant, ST15. ST15 (7-9-2-12-3) differed from ST2 by a single nucleotide of *rpoB*, which was probably due to a point mutation, and so it must have diverged from ST2 (24, 25).

Most of these outbreak isolates used in this study produced SHV and CTX-M enzymes as the ESBL enzymes (Table 1). A previous study performed in other Korean hospitals also reported that SHV and CTX-M were the most common ESBL enzymes (21). SHV-12 was the most frequently encountered SHV enzyme in the previous studies (21, 24-26), which was also confirmed in this study by the investigation of ESBL-producing *K. pneumoniae* isolates from Korean hospitals other than the Kangbuk Samsung Hospital (Tabel 2). However, SHV-11 was the most common among the outbreak *K. pneumoniae* isolates. This indicates that the outbreak *K. pneumoniae* isolates that produce ESBL are different from the prevailing isolates among the other Korean hospitals. However, we could not conclude whether this clone had previously existed in the Kangbuk Samsung Hospital and had spread during the outbreak period or whether it was transferred from another hospital or the community because we did not investigate the characteristics of the ESBL-producing *K. pneumoniae* isolates prior to the outbreak.

One of the interesting findings in this study was probably the different distribution of CCs between the ESBL-producing isolates and the non-producing isolates. Of the 12 ESBL-producing *K. pneumoniae* isolates from the Samsung Medical Center in 2004, eight isolates (66.7%) belonged to CC20. However, only three out of 23 ESBL-nonproducing isolates (13.0%) that were collected from the same hospital during the same period belonged to CC20 (Table 2). Although this result was based on the small number of isolates and thus it might be premature to draw a conclusion, it may suggest that the ESBL genes prefer to incorporate into a particular clone (s) as in methicillin-resistant *S. aureus* and vancomycin-resistant *E. faecium* (12, 27, 28). Further investigation by including more isolates from diverse localities will be required to prove such a hypothesis.

In summary, we developed a new MLST scheme for *K. pneumoniae* based on five housekeeping genes. We investigated the molecular characteristics of ESBL-producing *K. pneumoniae* isolates from an outbreak within a Korean hospital with using the developed MLST method. Most of the outbreak isolates belonged to ST2 and they produced SHV-1 and CTX-M-14, which was different from the features of the *K. pneumoniae* isolates from other Korean hospitals.

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