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Chromosome-Encoded AmpC and CTX-M Extended-Spectrum β-Lactamases in Clinical Isolates of *Proteus mirabilis* from Korea▽

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Among 222 *Proteus mirabilis* clinical isolates collected from 17 hospitals in Korea in 2008, 28 (12.6%) and 8 (3.6%) isolates exhibited extended-spectrum β-lactamase (ESBL) and AmpC phenotypes, respectively. The most common type of ESBL gene identified by PCR and sequencing experiments was $bla_{\text{CTX-M-14a}}$ ($n = 12$). The $bla_{\text{CTX-M-90}}$ ($n = 4$), $bla_{\text{CTX-M-15}}$ ($n = 3$), $bla_{\text{CTX-M-12}}$ ($n = 3$), $bla_{\text{CTX-M-2}}$ ($n = 2$), $bla_{\text{CTX-M-14b}}$ ($n = 1$), $bla_{\text{TEM-52}}$ ($n = 5$), and $bla_{\text{SHV-12}}$ ($n = 1$) genes were also detected. Eight isolates carried an AmpC β-lactamase gene, such as $bla_{\text{CMY-2}}$ ($n = 6$) or $bla_{\text{DHA-1}}$ ($n = 2$). All *bla* genes encoding CTX-M-1- and CTX-M-9-type enzymes and all $bla_{\text{CMY-2}}$ genes were preceded by IS*Ecp1*-like elements. The $bla_{\text{CTX-M-2}}$ gene found in two isolates was located on a complex class 1 integron. The $bla_{\text{DHA-1}}$ gene was preceded by a transcriptional regulator gene and was followed by phage shock protein genes. The $bla_{\text{CTX-M}}$ genes were located on the chromosome in 21 isolates. A plasmid location for the $bla_{\text{CTX-M}}$ gene was found in only four isolates: the $bla_{\text{CTX-M-14a}}$ gene was located on ~150-kbp IncA/C plasmids in three isolates and on a ~50-kbp IncN plasmid in one isolate. The $bla_{\text{TEM-52}}$ gene was located on ~50-kbp IncN plasmids in all five isolates. The AmpC β-lactamase genes were located on the chromosome in seven of eight isolates; one isolate carried the $bla_{\text{CMY-2}}$ gene on a ~150-kbp IncA/C plasmid. Our results show that a chromosomal location of CTX-M ESBL and AmpC β-lactamase genes in *P. mirabilis* is no longer an unusual phenomenon in hospital environments.

Proteus mirabilis is the second most common cause of urinary tract infections and an important cause of nosocomial infections (19). Oxyimino-cephalosporins have been used as the drugs of choice to treat infections caused by ampicillin-resistant *P. mirabilis*. However, as with other *Enterobacteriaceae*, *P. mirabilis* strains exhibiting resistance to expanded-spectrum β-lactam agents have been widely reported in many parts of the world (1, 5, 12, 18). Because *P. mirabilis* lacks species-specific chromosome-borne β-lactamases, resistance in this microorganism is wholly dependent on the acquisition of plasmid-encoded β-lactamases, such as extended-spectrum β-lactamases (ESBLs), AmpC enzymes, and metallo-β-lactamases (10, 15, 24).

Although uncommon, a chromosomal location for AmpC and CTX-M ESBL genes in *P. mirabilis* has been reported. Since the first report of a clinical strain of *P. mirabilis* carrying a chromosomally encoded CMY-2-like enzyme, designated CMY-3 (3), *P. mirabilis* strains harboring chromosomal CMY-4, CMY-12, CMY-14, CMY-15, or CMY-16 from France, Italy, and Poland have been described (9, 10, 14). Two *P. mirabilis* strains carrying chromosomally encoded CTX-M ESBL, CTX-M-25 or CTX-M-41 have also been reported (17).

Here we report a high prevalence of clinical *P. mirabilis* isolates carrying the chromosome-borne $bla_{\text{CTX-M}}$ and/or AmpC β-lactamase genes in Korea.

MATERIALS AND METHODS

Bacterial strains. In a nationwide survey conducted between July and September 2008, a total of 222 consecutive nonduplicate *P. mirabilis* isolates were collected from 17 hospitals in 11 cities in Korea. The isolates were recovered from urine ($n = 138$), blood ($n = 10$), respiratory specimens ($n = 18$), and pus ($n = 56$). The isolates were identified using a Vitek GNI card (bioMérieux, Marcy-l'Etoile, France).

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TABLE 1. Sequences of PCR primers used in a genetic environment study

Name	Target gene	Sequence (5′ to 3′)
CTX-M1-mF	$bla_{CTX-M-1}$ cluster	CGC GCT ACA GTA CAG CGA TA
CTX-M1-mR		AGC TTA TTC ATC GCC ACG TT
CTX-M2-mF	$bla_{CTX-M-2}$ cluster	ACAGAGCGAGAGCGATAAGC
CTX-M2-mR		TATTGCCCTTAAGCCACGTC
CTX-M9-mF	$bla_{CTX-M-9}$ cluster	ACG TGG CTC AAA GGC AAT AC
CTX-M9-mR		TCA ATT TGT TCA TGG CGG TA
CMY-2bF	bla_{CMY-2} cluster	GAT TAG GCT GGG AGA TGC TG
DHA-fF	bla_{DHA}	TTA CGG TCA TTA CGC CAC TG
DHA-mF		ATT TTC AGT GAC CGG CTG TT
DHA-fR		TAA TTC AGC GCA GAT TGT GG
DHA-mR		CAT TAA CGG TGT GAC CAA CG
DHA AmpR-mR		CAG GGT AAA GCG GTG AAC AT
IRL ISEcp1-F	IS*Ecp1*-like	CCT AGA TTC TAC GTC AGT ACT TCA AAA
ISEcp1-F		TCT GCT CCT TGA GAA TGC AA
ISEcp1-R		TCG CCC AAA ATG ACT TTA GC
ISEcp1-bmF		TCA AAG AAG CCA AAT ACG ACA
ISCR1-F	IS*CR1*	GCG AGT CAA TCG CCC ACT
ISCR1-R		CGA CTC TGT GAT GGA TCG AA
ISCR1-mF		GAT GCC GAG AAT ACG TGG TT
ISCR1-pR		CAG ACG CTC GTG ATG ACA AT
IS903-F	IS*903*	TGG CCC ACC TAC AAT AAA GC
IS903-R		GGC ATA CCT GCT TTC GTC AT
IS903-bF		CTG TTC GGG GGT TCA CTG AC
IS903-mR		CAG ATG CAG CTT ACG CCA TA
pspA-fF	*pap*	GCA TTG CCC GAT ATC AGA AC
pspB-F		TAT TTC TGG CCA TCC CGT TA
pspD-mF		ACG TCC GCT AAA AAT GTT GC
pspB-mR		CGA CTG GAA CGA TTG CTG TA
pspD-bR		TGC TTG CTT TTG TTG TTT CG
IntI1-F	*IntI1*	CCA AGC TCT CGG GTA ACA TC
IntI1-R		CAT GAA AAC CGC CAC TGC
5CS-R		CTT AAG GAG GCT ACG GCT TTC
qacEΔ1-F	*qacEΔ1-sul1*	GAA AGG CTG GCT TTT TCT TG
qacEΔ1-R		GCA GCG ACT TCC ACG ATG
3CS-mR		TGT GAA AGG CGA GAT CAT CA
sul1-F		TCA CCG AGG ACT CCT TCT TC
sul1-mF		GGG TTT CCG AGA AGG TGA TT
sul1-R		ACG AGA TTG TGC GGT TCT TC
orf1-F	*orf1*	GCT GTT CGT CGC TTT CTC TT
cat-F	*cat*	CCA AAA GGA CAC GGA GAT GT
cat-fF		AAG CAA GCT CAG GAG GTG AA
aadA1-mF	*aadA1*	ACA TCA TTC CGT GGC GTT AT
aadA1-fF		AGG TCC GTT TTT GGT CAC AG
aadA1-mR		AGG TTT CAT TTA GCG CCT CA
aadA1-fR		AGC CGA GGG AAG TTA AAA GC
ORF477-F	*orf477*	GAG CGA TAT GCA GCA ACA GA
ORF477-R		GCA TAC CAG GCC ATA AGC TC
blc-R	*blc*	GTA GGG GTC CGT GAA AGG AT

Antimicrobial susceptibility testing. The phenotypic confirmatory test for ESBL and/or AmpC β-lactamase production using boronic acid (BA) as an AmpC β-lactamase inhibitor was performed as described previously (21). MICs of β-lactams in the absence or presence of clavulanic acid (at a fixed concentration of 4 μg/ml; Sigma, St. Louis, MO) were determined by the agar dilution method according to CLSI guidelines (6).

Conjugal transfer experiments. The agar mating method was used to test the transferability of oxyimino-cephalosporin resistance by using azide-resistant *Escherichia coli* J53 as a recipient (13). Transconjugants were selected on Mueller-Hinton agar plates supplemented with either 2 μg/ml ceftazidime, 2 μg/ml cefotaxime, or 8 μg/ml cefoxitin and 100 μg/ml sodium azide.

Characterization of genes encoding β-lactamases. Genes coding for ESBLs and AmpC β-lactamases were detected by PCR amplification as described previously (20, 22). The templates for PCR amplification in clinical isolates were whole-cell lysates, and the PCR products were subjected to direct sequencing. Both strands of the PCR products were sequenced twice with an automatic sequencer (model 3730*xl*; Applied Biosystems, Weiterstadt, Germany).

Analysis of the genetic environments surrounding the *bla* genes. The genetic organization of the *bla* genes of CTX-M and AmpC enzymes was investigated by PCR mapping and sequencing of the regions surrounding the genes by using the primers listed in Table 1.

PCR-based replicon typing of plasmids. Plasmid replicon typing of the isolates carrying genes encoding CTX-M ESBLs and/or AmpC β-lactamases was performed using the PCR-base method with 18 pairs of primers described by Carattoli et al. (4).

Plasmid analysis. Plasmid DNA was purified from clinical *P. mirabilis* isolates using the NucleoBond Xtra Midi kit (Macherey-Nagel GmbH, Düren, Germany). Purified plasmids were digested with the restriction endonucleases HindIII, NdeI, and SpeI, and their restriction patterns were compared with one another by agarose gel electrophoresis.

PFGE and Southern blotting. Plugs containing whole genomic DNA of the *P. mirabilis* isolates were digested with SfiI, I-CeuI, and S1 nuclease separately. DNA fragments were separated by pulsed-field gel electrophoresis (PFGE) using a CHEF-DRII device (Bio-Rad, Hercules, CA). The PFGE conditions of SfiI macrorestriction analysis were 6 V/cm for 20 h, with pulse times ranging from 0.5 to 60 s, at a temperature of 14°C. Pulse times for I-CeuI and S1 nuclease restriction analysis were 9 to 90 s. DNA treated with I-CeuI or S1 nuclease was blotted onto a nylon membrane (Zeta-Probe blotting membranes; Bio-Rad) and was hybridized with probes specific for genes encoding AmpC β-lactamases or CTX-M ESBLs, various specific replicons, and 16S rRNA genes. The probes were obtained by PCR experiments as described above. Probe labeling, hybridization, and detection were performed with the DIG DNA labeling and detection kit (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's protocols.

Nucleotide sequence accession number. The nucleotide sequence data for the $bla_{CTX-M-90}$ gene are available in the GenBank nucleotide sequence database under accession no. GU065288.

RESULTS AND DISCUSSION

Prevalence and types of ESBLs and AmpCs. The phenotypic confirmatory test detected 28 (12.6%) ESBL producers and 8 (3.6%) AmpC producers among 222 *P. mirabilis* isolates (data not shown). Three isolates gave positive results for both an ESBL and an AmpC enzyme simultaneously. *P. mirabilis* isolates exhibiting the ESBL and AmpC phenotypes were recovered from 12 and 7 hospitals, respectively.

The most common type of class A ESBL gene identified using PCR and sequencing experiments was $bla_{CTX-M-14a}$ (n = 12). The $bla_{CTX-M-90}$ (n = 4), $bla_{CTX-M-15}$ (n = 3), $bla_{CTX-M-12}$ (n = 3), $bla_{CTX-M-2}$ (n = 2), $bla_{CTX-M-14b}$ (n = 1), bla_{TEM-52} (n = 5), and bla_{SHV-12} (n = 1) genes were also detected. Three isolates each carried two ESBL genes (Table 2). No *bla* genes of the PER, VE-, GES, or TLA type, or of the CTX-M-8 cluster, were detected in this survey.

Compared with that found by a survey carried out in 2004 (18), the prevalence of ESBL-producing *P. mirabilis* has increased from 9.7% to 12.6% in Korea. In 2004, 6.7% (9/134) of *P. mirabilis* clinical isolates were reported to produce CTX-M ESBLs, while in 2008, the prevalence of these enzymes showed a notable increase, reaching 11.3% (25/222). These results suggest that the significant increase in the incidence of ESBL-producing *P. mirabilis* has been due to the dissemination of strains producing CTX-M enzymes.

All eight isolates exhibiting the AmpC phenotype carried an AmpC β-lactamase gene, such as bla_{CMY-2} (n = 6) or bla_{DHA-1} (n = 2). Moreover, *bla* genes encoding ESBLs and AmpC β-lactamases were detected simultaneously in three isolates. The phenotypic characteristics of ESBL- and/or AmpC-producing isolates and their transconjugants are shown in Table 2.

Analysis of the genetic contexts of the *bla* genes. All *bla* genes encoding CTX-M-1- and CTX-M-9-type enzymes were preceded by IS*Ecp1*-like elements (Fig. 1). The IS*Ecp1*-like elements were observed 48 bp upstream of the open reading

TABLE 2. Characteristics of *P. mirabilis* isolates producing ESBLs and/or AmpC β-lactamases

Isolate	Hospital	Ward[a]	Specimen	β-Lactamase		MIC (μg/ml)[b]				
				ESBL	AmpC	CTX	CTX-CA	CAZ	CAZ-CA	FOX
SSM6	Seoul-1	ER	Urine	TEM-52		8	<0.06	4	<0.06	2
SSM10	Seoul-1	GW	Pus	CTX-M-90, TEM-52		32	0.12	8	0.12	2
SSM12	Seoul-1	ER	Urine	CTX-M-14, SHV-12	DHA-1	32	0.25	16	0.12	16
trcSSM12				CTX-M-14		16	<0.06	0.25	<0.06	2
KNS26	Seoul-2	GW	Urine	CTX-M-12		16	<0.06	2	<0.06	4
KNS34	Seoul-2	GW	Urine		CMY-2	8	8	8	4	32
KDS43	Seoul-3	GW	Pus		CMY-2	16	16	16	16	64
YS126	Seoul-4	GW	Pus	CTX-M-12		32	0.12	0.5	0.12	4
YS132	Seoul-4	GW	Sputum	CTX-M-90, TEM-52		64	0.12	4	0.5	2
trcYS132				TEM-52		32	<0.06	16	0.25	2
YS143	Seoul-4	GW	Pus	CTX-M-14		32	<0.06	0.25	<0.06	4
JYS190	Seoul-5	GW	Pus	CTX-M-14		32	0.12	0.25	<0.06	2
JYS195	Seoul-5	GW	Pus	CTX-M-14		16	<0.06	0.25	<0.06	2
JYS201	Seoul-5	ER	Pus	CTX-M-14		16	<0.06	0.25	<0.06	4
JHS209	Seoul-6	GW	Pus	CTX-M-12		16	0.12	2	0.25	2
JHS214	Seoul-6	GW	Urine	CTX-M-15		32	<0.06	8	<0.06	4
JHS215	Seoul-6	ICU	Pus	CTX-M-14	CMY-2	32	16	4	4	32
JHS218	Seoul-6	ICU	Urine	CTX-M-14	CMY-2	16	16	4	4	32
trcJHS218					CMY-2	16	8	16	16	64
JHS222	Seoul-6	GW	Urine	CTX-M-14		32	<0.06	0.25	<0.06	2
MG63	Goyang	ER	Blood	CTX-M-14		32	<0.06	0.25	<0.06	2
trcMG63				CTX-M-14		16	<0.06	0.12	<0.06	2
MG67	Goyang	ICU	Blood		CMY-2	8	8	8	4	32
MG73	Goyang	GW	Pus	CTX-M-15		>128	<0.06	8	<0.06	2
MG74	Goyang	GW	Urine	CTX-M-15		128	0.12	4	0.12	2
MG77	Goyang	ER	Urine	CTX-M-14		32	<0.06	0.25	<0.06	8
BDC115	Sungnam	GW	Pus	CTX-M-14		32	0.12	0.5	0.12	4
WJC161	Wonju	GW	Sputum	CTX-M-2		64	0.25	2	0.12	8
WJC164	Wonju	GW	Urine	CTX-M-2		64	0.25	1	0.12	8
WJC168	Wonju	GW	Urine		DHA-1	1	2	1	2	64
GM58	Daegu	ICU	Sputum		CMY-2	4	8	2	2	32
GS56	Jinju	GW	Sputum	CTX-M-14		32	0.12	2	0.12	4
PS79	Busan-1	ER	Blood	TEM-52		16	<0.06	32	<0.06	2
IJP86	Busan-2	GW	Pus	CTX-M-14		32	<0.06	0.12	<0.06	4
trcIJP86				CTX-M-14		16	<0.06	0.12	<0.06	2
IJP88	Busan-2	GW	Urine	TEM-52		32	<0.06	>128	<0.06	4
IJP92	Busan-2	ER	Urine	CTX-M-90		32	0.12	0.25	0.12	4
JJH178	Jeju	GW	Urine	CTX-M-90		128	0.25	0.25	<0.06	4

[a] ER, emergency room; GW, general ward; ICU, intensive-care unit.
[b] CTX, cefotaxime; CA, clavulanic acid; CAZ, ceftazidime; FOX, cefoxitin.

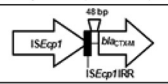
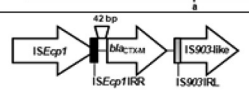
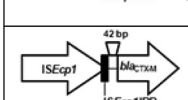
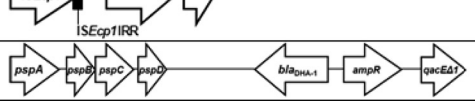
Genotype	Genetic environment (500 bp)	Identical sequences (GenBank accession no.)	Isolate
bla_{TEM-52}		EF141186 (2,231 bp)	SSM6, SSM10, PS79, IJP88, YS132
bla_{SHV-12}		EU855787 (2,181 bp)	SSM12
$bla_{CTX-M-1}$ type ($bla_{CTX-M-12}$ and $bla_{CTX-M-15}$)		DQ821704 (2,284 bp)	KNS26, MG73, MG74, YS126, JHS209, JHS214
$bla_{CTX-M-2}$		a. DQ520941 (4,632 bp) b. AY079169 (4,007 bp)	WJC161, WJC164
$bla_{CTX-M-9}$ type ($bla_{CTX-M-14a}$, $bla_{CTX-M-14b}$, and $bla_{CTX-M-90}$)		EU418916 (2,408 bp)	GS56, GM63, MG77, IJP86, IJP92, BDC115, YS132, YS143, JJH178, JYS201, JHS215, JHS218, JHS222
		AJ416341 (2,256 bp)	SSM10, SSM12, JYS190, JYS195
bla_{CMY-2}		AB525688 (4,234 bp)	KNS34, KDS43, GM58, MG67, JHS215, JHS218
bla_{DHA-1}		AJ971345 (5,446 bp)	SSM12, WJC168

FIG. 1. Schematic representation of the genetic environments surrounding the ESBL and AmpC genes in *Proteus mirabilis* clinical isolates. IRR, right inverted repeat sequences; IRL, left inverted repeat sequences.

frames (ORFs) encoding CTX-M-1-type enzymes or 42 bp upstream of the ORFs encoding CTX-M-9-type enzymes, as reported previously (11). The IS*903*-like element was detected downstream of the $bla_{CTX-M-9}$-type genes in 13 isolates (Fig. 1).

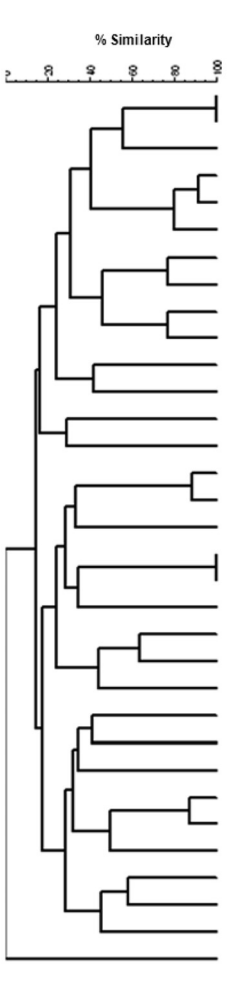
The $bla_{CTX-M14b}$ gene was first detected in *E. coli* strains isolated in Spain, and it exhibits three silent mutations compared with the $bla_{CTX-M-14a}$ gene (16). Navarro et al. (16) reported that the $bla_{CTX-M-14a}$ gene was associated with IS*Ecp1* and IS*903* located on an IncK plasmid, whereas the $bla_{CTX-M-14b}$ gene was associated with In*60* located on an IncHI2 plasmid, like $bla_{CTX-M-9}$. In this study, one isolate (JY195) was found to contain a $bla_{CTX-M-14b}$ gene, but it was linked to IS*Ecp1*, like the $bla_{CTX-M-14a}$ gene, and was located on the chromosome (see below).

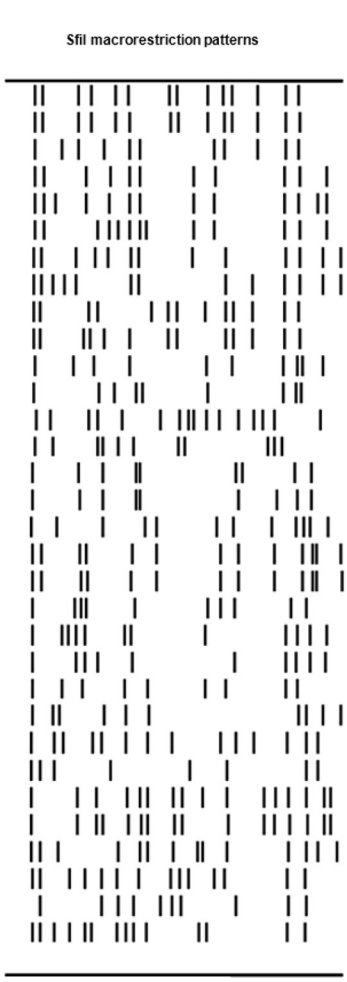
The $bla_{CTX-M-2}$ gene in two isolates was located on a complex class 1 integron. The 4,632-bp region from the 5′ conserved segment (5′-CS) to the first copy of 3′-CS (3′-CS1) showed 100% homology with a class 1 integron previously characterized from a *P. mirabilis* strain (12). The 4,007-bp region from IS*CR1* to the second copy of 3′-CS (3′-CS2) also showed 100% homology to that of an unusual class 1 integron, In*35* (2) (Fig. 1).

The bla_{TEM-52} gene in all five isolates was preceded by resolvase (*tnpR*) and transposase (*tnpA*) genes identical to those of transposon Tn*3* (7). The bla_{SHV-12} gene in isolate SSM12 was preceded by IS*26* and was followed by a transcriptional regulator gene (*ygbI*), showing the same arrangement as that found on plasmid pE19 from an *Enterobacter cloacae* strain (GenBank accession no. GU205813) (Fig. 1).

The bla_{CMY-2} gene in all six isolates was also preceded by IS*Ecp1*-like elements. The IS*Ecp1*-like elements were observed 116 bp upstream of the ORF encoding the CMY-2 enzyme. The bla_{DHA-1} gene in two isolates was preceded by a transcriptional regulator gene (*ampR*) and followed by phage shock protein genes (*pspA*, *pspB*, *pspC*, and *pspD*), as reported previously (23) (Fig. 1).

Locations of the *bla* genes. Despite repeated attempts, transconjugants were obtained from only five isolates, with transfer of $bla_{CTX-M-14}$ in three cases and of bla_{TEM-52} and bla_{DHA-1} in the remaining cases (Table 2). The probe specific for the $bla_{CTX-M-14a}$ gene hybridized with a ~50-kbp IncN plasmid and ~150-kbp IncA/C plasmids in one and three isolates, respectively (Fig. 2). IncA/C plasmids carrying the $bla_{CTX-M-14a}$ gene from three isolates exhibited restriction patterns identical to one another when digested with endonucleases of HindIII, NdeI, and SpeI (Fig. 3Ab). The probe specific for the bla_{TEM-52} gene hybridized with ~150-kbp IncA/C plasmids in all five isolates. The IncN plasmids also showed restriction patterns identical to one another (Fig. 3Bb). The probe specific for the bla_{CMY-2} gene hybridized with a ~50-kbp IncN plasmid in only one isolate. However, probes specific for the CTX-M and AmpC genes did not bind to any plasmids in the remaining isolates. Probes specific for CTX-M genes hybridized strongly with I-CeuI macrorestriction fragments (250 to >800 kbp) from 21 CTX-M ESBL-producing *P. mirabilis* isolates (2 isolates with CTX-M-2, 3 with CTX-M-12, 9 with CTX-M-14, 3 with CTX-M-15, and 4 with CTX-M-90). Furthermore, probes specific for the bla_{DHA-1} and bla_{CMY-2} genes also hybridized with I-CeuI macrorestriction fragments (450 to >800 kbp) from two and





Isolates	Gene encoding: TEM/SHV variant	TEM/SHV hybridization	CTX-M variant	CTX-M hybridization	AmpC variant	AmpC hybridization
MG73			CTX-M-15	250 kb chromosome		
MG74			CTX-M-15	250 kb chromosome		
JHS214			CTX-M-15	800 kb chromosome		
JHS209			CTX-M-12	800 kb chromosome		
KNS26			CTX-M-12	800 kb chromosome		
YS126			CTX-M-12	700 kb chromosome		
JJH178			CTX-M-90	800 kb chromosome		
IJP92			CTX-M-90	800 kb chromosome		
SSM10	TEM-52	50 kb IncN plasmid	CTX-M-90	800 kb chromosome		
YS132	TEM-52	50 kb IncN plasmid	CTX-M-90	800 kb chromosome		
SSM6	TEM-52	50 kb IncN plasmid				
IJP88	TEM-52	50 kb IncN plasmid				
GS56			CTX-M-14	800 kb chromosome		
PS79	TEM-52	50 kb IncN plasmid				
KNS34					CMY-2	450 kb chromosome
MG67					CMY-2	450 kb chromosome
GM58					CMY-2	800 kb chromosome
WJC161			CTX-M-2	300 kb chromosome		
WJC164			CTX-M-2	300 kb chromosome		
KDS43					CMY-2	800 kb chromosome
GM63			CTX-M-14	150 kb IncA/C plasmid		
IJP86			CTX-M-14	150 kb IncA/C plasmid		
MG77			CTX-M-14	800 kb chromosome		
WJC168					DHA-1	800 kb chromosome
JYS190			CTX-M-14	50 kb IncN plasmid		
JHS218			CTX-M-14	800 kb chromosome	CMY-2	150 kb IncA/C plasmid
JHS215			CTX-M-14	800 kb chromosome	CMY-2	800 kb chromosome
JHS222			CTX-M-14	800 kb chromosome		
JYS201			CTX-M-14	800 kb chromosome		
YS143			CTX-M-14	800 kb chromosome		
JYS195			CTX-M-14	800 kb chromosome		
BDC115			CTX-M-14	800 kb chromosome		
SSM12	SHV-12	150 kb IncA/C plasmid	CTX-M-14	150 kb IncA/C plasmid	DHA-1	150 kb IncA/C plasmid

FIG. 2. Dendrogram based on SfiI macrorestriction patterns of *P. mirabilis* isolates producing ESBLs and/or AmpC β-lactamases, indicating the similarity (expressed as a percentage) of PFGE profiles. Isolate SSM12 repeatedly failed to yield SfiI macrorestriction fragments. Three CTX-M-12-producing isolates showed >80% similarity, despite being recovered from three different hospitals, while other types of ESBL- or AmpC-producing isolates showed lower percentages of similarity. The locations of the *bla* genes of TEM-, SHV-, and CTX-M-type ESBLs and AmpC enzymes are given on the right.

five isolates, respectively. Finally, the hybridization of macrorestriction fragments with the probe specific for 16S rRNA genes confirmed the chromosomal location of the *bla* genes encoding CTX-M ESBLs and AmpC enzymes (data not shown).

Strain typing. Of 13 CTX-M-14-producing isolates, 1 (SSM12) repeatedly failed to yield SfiI macrorestriction fragments. Among 12 CTX-M-14-producing isolates, only 2 (JHS215 and JHS222), which were recovered from the same hospital, were clonally related to one another, with 80% similarity; others were not clonally related (Fig. 2).

Our results revealed that the spread of the $bla_{\text{CTX-M-14a}}$ and $bla_{\text{TEM-52}}$ genes among *P. mirabilis* might be driven by horizontal transfer of IncA/C and IncN plasmids, respectively. Notably, however, most of the CTX-M ESBL and AmpC β-lactamase genes were found on the chromosome and not on plasmids. A chromosomal location for CTX-M ESBL and AmpC β-lactamase genes in *Enterobacteriaceae* other than *P. mirabilis* has rarely been reported, though Coque et al. (8) recently described eight *E. coli* clinical isolates carrying the chromosome-borne $bla_{\text{CTX-M-15}}$ gene. In contrast to other species, *P. mirabilis* containing CTX-M ESBL and/or AmpC β-lactamase genes on the chromosomes has been reported uncommonly elsewhere. It is noteworthy that the chromosomal location of CTX-M ESBL and AmpC β-lactamase genes in *P. mirabilis* is no longer an unusual phenomenon in the hospital environment. Chromosomally located genes are surrounded by a genetic environment similar to that of plasmids, and this supports the idea that the CTX-M ESBL and AmpC β-lactamase genes are integrated into the chromosome via transposable elements in the *P. mirabilis* species.

In conclusion, the present data suggested that the incidence of ESBL-producing *P. mirabilis* has increased due to the dissemination of *bla* genes encoding CTX-M enzymes in Korea. The most common types of ESBL and AmpC β-lactamase in *P. mirabilis* were CTX-M-14 and CMY-2, respectively. Interestingly, these enzymes were chromosomally located in most *P.*

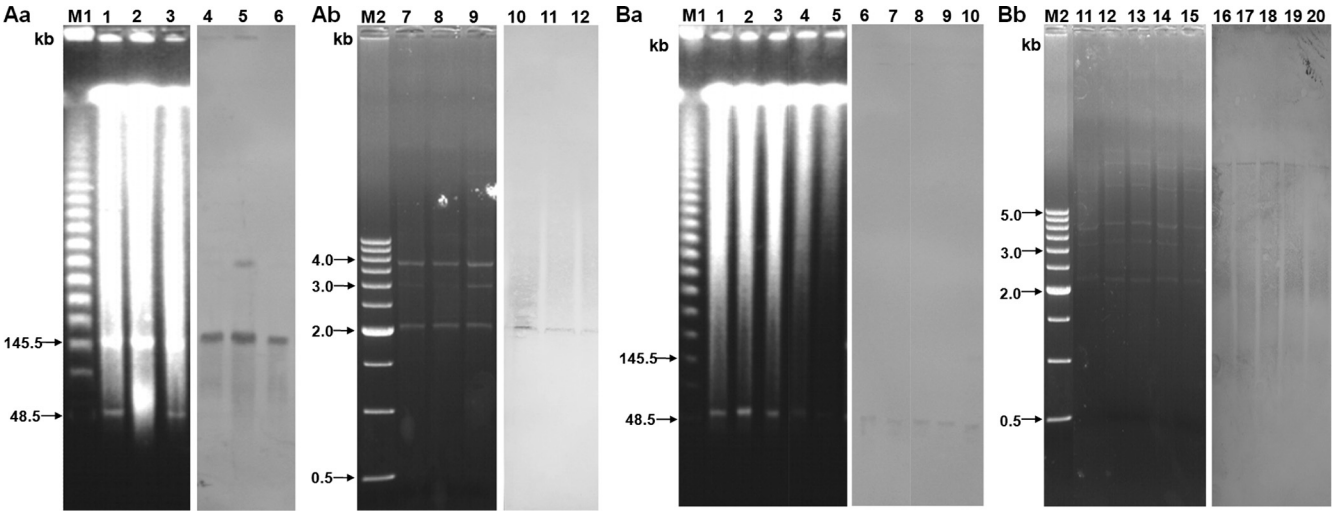


FIG. 3. (Aa) Banding patterns of S1 nuclease-digested linearized plasmids from three *P. mirabilis* clinical isolates carrying the $bla_{\text{CTX-M-14a}}$ gene (lanes 1 to 3) and Southern blot analysis of plasmid DNA with a probe specific to the gene (lanes 4 to 6). (Ab) Restriction patterns of ~150-kbp plasmids carrying the $bla_{\text{CTX-M-14a}}$ gene digested with the HindIII, NdeI, or SpeI restriction endonuclease (lanes 7 to 9) and Southern blot analysis of plasmid DNA with a probe specific to the gene (lanes 10 to 12). (Ba) Banding patterns of S1 nuclease-digested linearized plasmids from five *P. mirabilis* clinical isolates carrying the $bla_{\text{TEM-52}}$ gene (lanes 1 to 5) and Southern blot analysis of plasmid DNA with a probe specific to the gene (lanes 6 to 10). (Bb) Restriction patterns of ~50-kbp plasmids carrying the $bla_{\text{TEM-52}}$ gene (lanes 11 to 15) and Southern blot analysis of plasmid DNA with a probe specific to the gene (lanes 16 to 20). Lanes M1, Lambda ladder (Promega Co., Madison, WI); lanes M2, 500-bp DNA ladder (Takara Inc., Tokyo, Japan).

mirabilis isolates. The mechanism and significance of this phenomenon need to be elucidated.

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