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ScienceDirect
Diagnostic Microbiology and Infectious Disease 61 (2008) 453–459
DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
www.elsevier.com/locate/diagmicrobio

Prevalence and characterization of extended-spectrum β-lactamase–producing Enterobacteriaceae isolated in Korean hospitals

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Received 23 January 2008; accepted 18 March 2008

Abstract

Prevalence and characteristics of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in Korean hospitals were assessed. A total of 1484 clinical Enterobacteriaceae isolates were collected from 8 tertiary-care hospitals in various regions of Korea over a 3-month period (June to August) in 2005. Among 546 *Klebsiella pneumoniae* isolates, 123 isolates (22.4%) showed ESBL-producing activity, and 47 (10.2%) of 460 isolates of *Escherichia coli* were ESBL producers. Of the *Enterobacter cloacae* isolates, 16.2% (17/105) evidenced ESBL-producing activity. The most prevalent ESBLs were SHV-12 and CTX-M-14 in *K. pneumoniae* and *E. coli*, respectively. In *E. cloacae*, SHV-12 was also the most prevalent. Prevalence of ESBL production differed among the specimens. Although the *K. pneumoniae* isolates from urine and aspirates evidenced high ESBL production rates (35.4% and 57.1%, respectively), those from sputum, blood, and pus showed relatively low ESBL production rates (17.0%, 14.8%, and 5.3%, respectively). However, *E. coli* isolates obtained from sputum showed significantly higher ESBL production rates (37.5%) than were seen in samples obtained from other sources, but those obtained from urine showed lower ESBL production rates (8.3%). These significant differences in ESBL-producing *K. pneumoniae* and *E. coli* isolates among the isolated specimens should be examined further, with an eye toward the implications of this research in clinical settings.

Keywords: Extended-spectrum β-lactamases (ESBLs); *Klebsiella pneumoniae*; *Escherichia coli*

1. Introduction

Extended-spectrum β-lactamases (ESBLs) capable of degrading the extended-spectrum cephalosporins and monobactams are among the most relevant determinants of resistance emerging worldwide in the Enterobacteriaceae (Bradford, 2001; Jacoby and Munoz-Price, 2005; Paterson and Bonomo, 2005). Because ESBL-generating strains often exhibit multidrug resistance, including resistance to

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doi:10.1016/j.diagmicrobio.2008.03.005

aminoglycosides and fluoroquinolones, the therapeutic options associated with these strains are fairly limited. In addition, infections with ESBL-producing strains have been associated with increased morbidity, mortality, and health care-associated costs (Cosgrove, 2006; Lautenbach et al., 2001).

ESBLs are derived through 1 or more amino acid substitutions from the parental enzymes TEM-1, TEM-2, and SHV-1, and other enzymes, including CTX-M and PER, have reportedly been detected with increasing frequency in many regions (Jacoby and Munoz-Price, 2005). A few previous studies have reported on the prevalence of ESBLs in Enterobacteriaceae from Korea. The prevalence of ESBLs in *Escherichia coli* and *Klebsiella pneumoniae* in Korea has been estimated as 9.2% to 11.8% and 17.7% to 30.0%, respectively (Jeong et al., 2004a, 2004b; Kim et al., 2005; Ryoo et al., 2005). Kim and Lim (2005) reported that 25.7% of *Citrobacter freundii*, *Enterobacter* sp., and *Serratia marcescens* isolates from Korea showed ESBL-producing activity. However, their studies investigated the prevalence of ESBL production in only a few species, and the isolation source was not considered. To delineate the prevalence and features of ESBLs in Korea more precisely, we recently collected clinical isolates of Enterobacteriaceae from university-affiliated tertiary-care hospitals in various regions of Korea and assessed the prevalence of ESBLs and antimicrobial resistances with regard to bacterial species and specimens.

2. Materials and methods

2.1. Bacterial isolates

As a component of a multicenter surveillance study conducted over a 3-month period (June to August) in 2005, a total of 1484 nonduplicate clinical Enterobacteriaceae isolates (Table 1) were collected from 8 tertiary-care hospitals in various regions of South Korea: Samsung Medical Center (Seoul), Konkuk University Hospital (Seoul), Dong-A University Hospital (Busan), Kyungpook National University Hospital (Daegu), Kangbuk Samsung Hospital (Seoul), Chungbuk National University Hospital (Cheongju), Chungnam National University Hospital (Daejeon), and Chonnam National University Hospital (Gwangju). Clinical isolates were collected both from inpatients and outpatients. Species identification was performed at the clinical laboratories of the respective hospitals.

2.2. Antimicrobial susceptibility testing

In vitro antimicrobial susceptibility testing of the isolates was performed in Mueller–Hinton broth (Becton Dickinson, Sparks, MD) by broth microdilution method in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) (formerly National Committee for Clinical Laboratory Standards) (CLSI, 2006). The following 8 antimicrobial agents were included in this study: cephalothin (Sigma-Aldrich, St. Louis, MO), cefotaxime (Sigma-Aldrich), ceftazidime (Sigma-Aldrich), cefepime (Boryung, Seoul, Korea), aztreonam (Sigma-Aldrich), ciprofloxacin (Fluka, Buchs, Switzerland), imipenem (Choongwae Pharma, Seoul, Korea), and piperacillin–tazobactam (Sigma-Aldrich). The interpretive criteria for susceptibility used in this study were also those established by the CLSI (2006). Quality control was performed using *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603. In vitro susceptibility testing for all isolates was performed at the central laboratory of the Asian-Pacific Research Foundation for Infectious Diseases, Seoul, Korea.

For 13 imipenem-nonsusceptible isolates, we tested whether imipenem nonsusceptibility was due to AmpC production or metallo-β-lactamase by using double-disk synergy test including 500 μg of cloxacillin (Neo-Sensitabs; Rosco Diagnostica, Taastrup, Denmark) and double-disk method combining imipenem and imipenem/EDTA (Becton

Table 1
Antimicrobial resistance and ESBL production rates

Bacterial species (no. of isolates)	Antimicrobial resistance rates (%)								ESBL production	
	CEP	CTX	CAZ	CPM	AZT	CIP	IMP	PTZ	Screening positive (%)[a]	Confirmed isolates (%)
E. coli (460)	41.1	11.1	6.3	8.7	12.2	30.9	0.2	9.6	56 (12.2)	47 (10.2)
K. pneumoniae (546)	42.3	23.1	26.6	6.9	31.0	28.2	0.5	19.7	195 (35.5)	123 (22.4)
K. oxytoca (56)	26.8	47.1	8.9	1.8	7.1	1.8	0	3.6	8 (14.3)	4 (7.1)
C. freundii (53)	98.1	32.1	45.3	5.7	43.4	13.2	3.8	30.2	31 (58.5)	6 (11.3)
E. cloacae (105)	99.0	31.4	37.1	2.9	34.3	12.4	0	15.2	55 (52.4)	17 (16.2)
E. aerogenes (67)	100	4.5	22.4	0	14.9	1.5	0	14.9	26 (38.8)	1 (1.5)
S. marcescens (84)	100	17.9	8.3	6.0	10.7	8.3	1.2	8.3	22 (26.2)	7 (8.3)
P. mirabilis (66)	34.8	6.1	9.1	6.1	3.0	13.6	0	3.0	7 (10.6)	3 (4.5)
P. vulgaris (10)	100	20.0	0	0	0	0	0	0	4 (6.1)	0
Morganella morganii (25)	100	12.0	20.0	4.0	4.0	12.0	0	4.0	16 (64.0)	0
Providencia sp. (12)	83.3	25.0	33.3	25.0	41.7	16.7	8.3	0	6 (50.0)	1 (8.3)
Total (1484)	54.2	17.7	18.9	6.6	21.3	22.9	0.5	13.5	426 (28.7)	209 (14.1)

CEP = cephalothin; CTX = cefotaxime; CAZ = ceftazidime; CPM = cefepime; AZT = aztreonam; CIP = ciprofloxacin; IMP = imipenem; PTZ = piperacillin–tazobactam.

[a] Isolates of cefotaxime, ceftazidime, or aztreonam, MIC ≥2 mg/L.

Dickinson, Franklin Lakes, NJ), respectively (Cardoso et al., 2008; Ruppé et al., 2006).

2.3. Confirmation of ESBL production

Screening-positive *E. coli*, *K. pneumoniae*, *Klebsiella oxytoca*, and *Proteus mirabilis* isolates, all of which produced ESBL, were defined by a ceftazidime, aztreonam, or cefotaxime MIC of ≥2 mg/L (CLSI, 2006). Production of ESBL activity was confirmed in screening-positive isolates via a double-disk synergy test using BD BBL Sensi-Disk (Becton Dickinson). Disks containing 30 μg of cefotaxime and ceftazidime, either alone or coupled with 10 μg of clavulanate, were placed at distances of 20 mm (center to center). When the inhibition zone differed by ≥5 mm between at least 1 of the combination disks and its corresponding single antibiotic disk, the strain was identified as an ESBL producer. Quality control was performed using *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603. The same procedure for the screening and confirmation of ESBL production was also applied to the other Enterobacteriaceae isolates.

2.4. Molecular characterization of β-lactamase determinants

Polymerase chain reactions (PCRs) and sequence analyses were conducted to determine the gene responsible for the ESBL phenotype in the ESBL producers. PCRs for bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ were conducted using the previously described PCR primers and conditions (Kim et al., 2005). We sequenced both strands of all PCR products and identified kinds of β-lactamase genes. We also screened bla_{VEB} and bla_{PER} for ESBL-positive isolates with no ESBL determinants (Pasterán et al., 2006).

2.5. Statistical analysis

Fisher exact *t* test and χ^2 test were used to determine the significance of differences in resistance, where appropriate.

3. Results

3.1. Antimicrobial resistance rates

Susceptibility of 1484 clinical Enterobacteriaceae isolates from 8 Korean hospitals for 8 antimicrobial agents was tested (Table 1). As a whole, the antimicrobial resistance rate was the highest in cephalothin (54.2%). In this study, only 8 isolates were found to be resistant to imipenem: 3 isolates of *K. pneumoniae*, 2 of *C. freundii*, and 1 each of *E. coli*, *S. marcescens*, and *Providencia* sp. With regard to cefotaxime, *K. oxytoca*, *C. freundii*, and *Enterobacter cloacae* showed resistance rates of >30%. *C. freundii* and *E. cloacae* also showed resistance rates of >30% for ceftazidime, along with *Providencia* sp. With regard to ciprofloxacin, the highest resistance rates were detected in *E. coli* isolates (30.9%), whereas the majority of *K. oxytoca*, *Enterobacter aerogenes*, and *Proteus vulgaris* isolates were found to be susceptible to ciprofloxacin.

Among 13 imipenem-nonsusceptible isolates, 2 isolates (1 each of *K. pneumoniae* and *E. coli*) were positive for metallo-β-lactamase, and imipenem nonsusceptibility in 2 isolates (1 each of *C. freundii* and *Providencia* spp.) was related to AmpC production.

3.2. Prevalence of ESBL production

We tested ESBL production in the screening-positive isolates evidencing MIC values of ≥2 mg/L for ceftazidime, aztreonam, or cefotaxime. Altogether, a total of 426 isolates (28.7%) were screened as ESBL screening positives (Table 1). Approximately half of these screening-positive isolates (209/425 isolates, 49.2%) showed ESBL activity in our double-disk synergy tests. That is, approximately 14.0% of the Enterobacteriaceae isolates analyzed in this study evidenced ESBL-producing activity. *K. pneumoniae* had the highest ESBL production rate (123/546 isolates, 22.4%), and *E. coli* showed 10.2% ESBL production rate (47/460 isolates). Of the *E. cloacae* isolates, 16.2% (17/105) were ESBL producer.

Table 2
Comparison of antimicrobial resistance rates between ESBL-producing and non–ESBL–producing isolates

Antimicrobials	Resistance rates (%)											
	Total			*E. coli*			*K. pneumoniae*			*E. cloacae*		
	ESBL (n = 209)	Non-ESBL (n = 1275)	P	ESBL (n = 47)	Non-ESBL (n = 413)	P	ESBL (n = 123)	Non-ESBL (n = 423)	P	ESBL (n = 17)	Non-ESBL (n = 88)	P
Cefotaxime	75.1	8.2	<0.001	100	1.0	<0.001	64.2	11.3	<0.001	76.5	23.5	<0.001
Ceftazidime	67.0	11.0	<0.001	38.3	2.7	<0.001 [a]	74.8	12.7	<0.001	88.2	27.3	<0.001
Ciprofloxacin	58.4	17.1	<0.001	74.5	25.9	<0.001	63.4	18.1	<0.001	29.4	9.1	0.035 [a]
Cefepime	38.3	1.4	<0.001	83.0	1.0	<0.001 [a]	23.6	2.1	<0.001	17.6	0	0.004 [a]
Cephalothin	100	46.7	<0.001 [a]	100	34.4	<0.001	100	24.4	<0.001 [a]	100	98.9	1.000 [a]
Imipenem	1.0	0.5	0.314 [a]	2.1	0	0.102 [a]	0	0.7	1.000 [a]	0	0	–
Aztreonam	84.7	10.9	<0.001	72.3	5.3	<0.001	88.6	14.3	<0.001	94.1	22.7	<0.001
Piperacillin–tazobactam	40.2	9.2	<0.001	38.3	6.3	<0.001 [a]	43.1	12.9	<0.001 [a]	35.3	9.5	0.022 [a]

[a] Fisher exact *t* test. The others are from χ^2 test.

3.3. Antimicrobial resistance rates in ESBL-producing isolates

As expected, the ESBL-producing isolates showed significantly higher resistance rates than the non–ESBL-producing isolates in the majority of antimicrobial agents, with the exception of imipenem (Table 2). Besides cefotaxime, ceftazidime, cefepime, and aztreonam, more than 50% of the ESBL-producing Enterobacteriaceae isolates showed cross-resistance with ciprofloxacin (58.4%), whereas the non–ESBL-producing isolates showed only a 17.1% ciprofloxacin resistance rate. In addition, piperacillin–tazobactam was also shown to have a higher resistance rate in the ESBL-producing isolates (40.2%) than in the non–ESBL-producing isolates (9.2%).

Such higher resistance rates in the ESBL-producing isolates were also observed in each species. The ciprofloxacin resistance rate reached 74.5% in the ESBL-producing *E. coli* isolates. However, the ceftazidime resistance rate in the ESBL-producing *E. coli* isolates was rather low (38.3%) as compared with the ESBL-producing isolates of *K. pneumoniae* and *E. cloacae* (74.8% and 88.2%, respectively), although this rate was significantly higher than those seen in the non–ESBL-producing *E. coli*. In *K. pneumoniae* and *E. cloacae*, cefepime showed relatively good in vitro activity against ESBL-producing isolates (resistance rate, 23.6% and 17.6%, respectively). However, the in vitro activity of cefepime decreased significantly in the ESBL-producing *E. coli* isolates (resistance rate, 83.0%).

3.4. Prevalence of ESBL production among isolation sources

To compare the prevalence of ESBL production among isolation sources, we identified and classified them into 10 representative sources. In addition, the urine isolates were classified further into the "catheterized urine" and "voided urine" (Table 3). For all Enterobacteriaceae isolates, the isolates from catheterized urine showed significantly higher ESBL production rates (24.3%) than were seen in the isolates from other sources ($P < 0.001$), but the isolates from voided urine did not. In addition to the catheterized urine, aspirates were the other isolation source associated with significantly higher ESBL production rates (41.4%) ($P < 0.001$).

In *E. coli*, approximately two-third of the isolates were from urine, and most of these were from voided urine. Blood was the 2nd most frequently encountered source of *E. coli* isolation (54 isolates, 11.7%). *E. coli* isolates from catheterized urine evidenced relatively high ESBL production rates (12.5%) than those from other sources (7.6%), but not significantly so ($P = 0.613$). Although the *E. coli* isolates from sputum and aspirates showed significantly higher ESBL production rates (37.5% and 75.0%, respectively), only 16 and 4 *E. coli* isolates were from those sources.

K. pneumoniae was the most frequently isolated species from sputum (188 isolates, 34.4%). Among 546 *K. pneumoniae* isolates, 158 (28.9%) and 61 (11.2%) of the isolates were derived from urine and blood, respectively. By way of contrast to the *E. coli* isolates, only 17.0% of the *K. pneumoniae* isolates from sputum were estimated to generate ESBL, and this rate was significantly lower than those of other sources ($P = 0.026$). The prevalence of ESBL production in the *K. pneumoniae* isolates from urine (35.4%), particularly from catheterized urine (46.4%), were significantly higher than those from *K. pneumoniae* isolates from other sources ($P < 0.001$).

As in *K. pneumoniae*, *E. cloacae* was isolated most frequently from sputum (21.0%), followed by urine (16.2%) and blood (13.3%). The rates of ESBL production in the

Table 3
ESBL production rates in *E. coli*, *K. pneumoniae*, and *E. cloacae* isolates with respect to source

Isolation source	Total			*E. coli*			*K. pneumoniae*			*E. cloacae*		
	No. of isolates (%)	ESBL (%)	P[a]	No. of isolates (%)	ESBL (%)	P[a]	No. of isolates (%)	ESBL (%)	P[a]	No. of isolates (%)	ESBL (%)	P[a]
Sputum	298 (20.1)	49 (16.4)	0.190	16 (3.5)	6 (37.5)	0.003	188 (34.4)	32 (17.0)	0.026	22 (21.0)	5 (22.7)	0.344
Blood	151 (10.2)	16 (10.6)	0.194	54 (11.7)	4 (7.4)	0.468	61 (11.2)	9 (14.8)	0.144	14 (13.3)	2 (14.3)	1.000
Bile	36 (2.4)	8 (22.2)	0.155	9 (2.0)	2 (22.2)	0.232	8 (1.5)	3 (37.5)	0.388	4 (3.8)	1 (25.0)	0.512
Aspirate	29 (2.0)	12 (41.4)	<0.001	4 (0.9)	3 (75.0)	0.004	14 (2.6)	8 (57.1)	0.005	2 (1.9)	1 (50.0)	0.299
Urine	598 (40.3)	90 (15.1)	0.379	312 (67.8)	26 (8.3)	0.053	158 (28.9)	56 (35.4)	<0.001	17 (16.2)	1 (5.9)	0.296
Urine catheterized	136 (9.2)	33 (24.3)	<0.001	48 (10.4)	6 (12.5)	0.613	56 (10.3)	26 (46.4)	<0.001	4 (3.8)	0	1.000
Urine voided	427 (28.8)	53 (12.4)	0.239	238 (51.7)	18 (7.6)	0.052	102 (18.7)	30 (29.4)	0.130	12 (11.4)	1 (8.3)	0.685
Pus	95 (6.4)	7 (7.4)	0.052	18 (3.9)	2 (11.1)	0.705	38 (7.0)	2 (5.3)	0.008	8 (7.6)	1 (12.5)	1.000
Peritoneal fluid	26 (1.8)	3 (11.5)	1.000	4 (0.9)	0	1.000	10 (1.8)	1 (10.0)	0.469	5 (4.8)	2 (40.0)	0.184
Skin	17 (1.1)	1 (5.9)	0.494	4 (0.9)	0	1.000	7 (1.3)	1 (14.3)	1.000	2 (1.9)	0	1.000
Nasal	47 (3.2)	1 (2.1)	0.017	1 (0.2)	0	1.000	11 (2.0)	1 (9.1)	0.470	9 (8.6)	0	0.349
Wound	25 (1.7)	2 (8.0)	0.563	6 (1.3)	0	1.000	3 (0.5)	0	1.000	6 (5.7)	2 (33.3)	0.249
Others	153 (10.3)	18 (11.8)	0.384	32 (7.0)	4 (12.5)	0.555	48 (8.8)	10 (23.8)	0.755	16 (15.2)	2 (12.5)	1.000
Total	1484	209 (14.1)		460	47 (10.2)		546	123 (22.4)		105	17 (16.2)	

[a] Fisher exact t test or χ^2 test. Statistical significance was analyzed to compare ESBL production rate in isolates from a particular source with those in other sources.

Table 4
Distribution of ESBLs among Enterobacteriaceae isolates

Species (no. of isolates tested)	TEM			SHV							CTX-M				No PCR detection	Multiple detection
	TEM-1[a]	TEM-17	TEM-52	SHV-1[a]	SHV-2a	SHV-5	SHV-11[a]	SHV-12	SHV-26	SHV-31	SHV-32	CTX-M-3	CTX-M-12	CTX-M-14	CTX-M-15		
E. coli (47)	29	1					1	4		1	1	1	1	26	8	1	26
K. pneumoniae (123)[b]	37		12	11		1	81	16	1	6	3	4	2	16	3		58
K. oxytoca (4)		1	1		1			1									
C. freundii (6)	1		1					1								3	
E. cloacae (18)	5							9				2		1		5	6
E. aerogenes (1)																1	
S. marcescens (7)	5	1						1									
P. mirabilis (4)	2	1											2			1	2
Providencia sp. (1)			1														
Total	79	4	15	11	2	1	82	32	1	7	4	7	3	45	11	11	92

[a] TEM-1, SHV-1, and SHV-11 are not ESBL enzymes.
[b] Included 2 new variants, 2 SHV, judged by sequence analysis: SHV of Kpn11-047 (GenBank accession no. EU525920) differs from SHV-1 by H24Y, S26G, L35Q, M69V, V75L, D88V, E92D, K94R, A114V, V119I, M129L, A135G, E169A, S196A, R198H, R205Q, S223A, N252D, N253G, A255P, and Q276H. SHV of Kpn07-423 (GenBank accession no. EU525919) differs from SHV-1 by H112Y and A146V.

E. cloacae isolates did not differ significantly among isolation sources. They were higher in those from sputum (22.7%), bile (25.0%), aspirates (50.0%), and peritoneal fluid (40.0%), which were not significant.

3.5. Types of ESBL genes detected

In this study, we identified 13 different types of ESBL genes detected: 2 TEMs, 7 SHVs, and 4 CTX-Ms (Table 4). In 11 isolates (1 isolate of *E. coli*, 1 of *E. aerogenes*, 5 of *E. cloacae*, 3 of *C. freundii*, and 1 of *P. mirabilis*), no TEM, SHV, or CTX-M genes were detected via PCR (Table 4). Probably, other ESBL-related genes than TEM, SHV, and CTX-M confer those ESBL activities. However, they were negative to screening for VEB and PER genes. In *E. coli*, the most prevalent ESBL was CTX-M-14, which was detected in 26 isolates of *E. coli* (55.3%) (Table 4). Next, CTX-M-15 and SHV-12 were detected in 8 and 4 *E. coli* isolates, respectively. Seventeen *E. coli* isolates (36.2%) evidenced both TEM-1 and CTX-M-14 enzymes (data not shown).

In *K. pneumoniae*, the most prevalent β-lactamase was SHV-11 (81 isolates, 65.9%), followed by SHV-12 (16 isolates, 13.0%), CTX-M-14 (16 isolates, 13.0%), and TEM-52 (12 isolates, 9.8%). In *E. cloacae*, the most prevalent ESBL was SHV-12 (9 isolates, 52.9%). With regard to ESBL enzymes, CTX-M-3 and CTX-M-14 were identified in 2 and 1 *E. cloacae* isolates, respectively.

TEM-17 was detected in 1 isolate each of *K. oxytoca*, *S. marcescens*, and *P. mirabilis*, as well as in 1 *E. coli* isolate. TEM-52 was generated by 1 isolate each of *K. oxytoca*, *C. freundii*, and *Providencia* sp. SHV-2a was detected only in an isolate of *K. oxytoca*. Besides *K. pneumoniae*, *E. coli*, and *E. cloacae*, SHV-12 was also detected in 1 isolate each of *K. oxytoca*, *C. freundii*, and *S. marcescens*. Two ESBL-producing *P. mirabilis* isolates were found to generate CTX-M-14.

4. Discussion

In this study, a similar proportion of ESBL-producing isolates in *E. coli* (10.2%) and *K. pneumoniae* (22.4%) was observed as has been reported in previous Korean studies (Jeong et al., 2004a, 2004b; Kim et al., 2005; Ryoo et al., 2005). On the basis of several recent reports from Korea, the prevalence of ESBL-producing isolates in *E. coli* and *K. pneumoniae* was reported as 9.2% to 11.7% and 17.7% to 30.0%, respectively (Jeong et al., 2004b; Kim et al., 2005; Ryoo et al., 2005). The prevalence of ESBL-producing isolates increased from 4.8% in the 1990s (Pai et al., 1999). Although the present ESBL production rates in *E. coli* isolates from Korea were lower than those in China and Hong Kong (24.5% and 14.3%, respectively), they were higher than those in other Asia-Pacific countries, including Australia, Japan, the Philippines, and Taiwan (0.5–5.9%) (Hirakata et al., 2005). The ciprofloxacin resistance rate reached 74.5% in the ESBL-producing *E. coli* isolates,

similar to what has been observed previously in Korea (Ko et al., 2007). In the isolates of *K. pneumoniae*, the prevalence of ESBL in Korea (22.4%) was similar to those from China, the Philippines, Singapore, and South Africa (21.9–35.6%). However, these rates were higher than those seen in Australia, Hong Kong, Japan, and Taiwan (3.7–13.5%) (Hirakata et al., 2005). Thus, Korea may be 1 of the regions with very high prevalence of ESBL production in *E. coli* and *K. pneumoniae*.

Previously, the most prevalent ESBLs in *E. coli* isolates from Korea were recognized as SHV-12 and CTX-M, as well as a prototype of β-lactams, TEM-1 (Jeong et al., 2004a, 2005; Kim et al., 2005). In this study, we determined that CTX-M-14 and CTX-M-15 were the most prevalent CTX-M–type ESBLs (Table 4), and that SHV-12 was less frequent than the CTX-M–type ESBLs. Several studies have shown that SHV-12 was also the most prevalent ESBL in isolates of *K. pneumoniae* from Korea (Jeong et al., 2004a; Kim et al., 1998, 2005; Ryoo et al., 2005). In this study, SHV-12 was detected in 16 isolates. In addition to SHV-12, TEM-52 and CTX-M-14 were also detected in several ESBL-producing *K. pneumoniae* isolates.

By way of contrast with *E. coli* and *K. pneumoniae*, ESBL prevalence in other Enterobacteriaceae species was lower than those of the previous studies, with the exception of *E. cloacae* (16.2%). In previous studies, Kim and Lim (2005) estimated that the prevalence of ESBL-producing *C. freundii*, *E. aerogenes*, *E. cloacae*, and *S. marcescens* isolates was 19.0%, 40.0%, 15.9%, and 30.6%, respectively, and Park et al. (2005) reported that the prevalence of ESBL production in *C. freundii*, *E. cloacae*, and *S. marcescens* was 20.6%, 35.4%, and 19.4%, respectively. Although we selected screening-positive isolates and tested them for ESBL production, both previous studies subjected all isolates to double-disk synergy tests. Thus, the ESBL production rates reported herein may have been underestimated to some degree. Schwaber et al. (2004) reported that when the CLSI guidelines were used in studies of non-*E. coli* and non-*Klebsiella* spp. of Enterobacteriaceae, 51.4% of the isolates were identified as screening-positive isolates, but only 2.2% of those were confirmed to generate ESBL, and these figures are consonant with what was determined in the present study. To verify our speculations vis-à-vis the underestimation of ESBL production rates as the result of an inappropriate screening method, we conducted tests of ESBL activity for all *S. marcescens* isolates. However, we did not detect any more *S. marcescens* isolates showing ESBL activity.

In the species of Enterobacteriaceae harboring cephalosporinase genes such as *Citrobacter*, *Enterobacter*, and *Serratia* spp., the isolates become resistant to cefotaxime, ceftazidime, and aztreonam but remain susceptible to cefepime when the chromosomal cephalosporinase is hyperproduced. In this study, 8.3% to 45.3% were resistant to ceftazidime, but 0% to 25.0% was resistant to cefepime (Table 1). This result strongly suggests a great proportion of cephalosporinase-hyperproducing isolates among the isolates resistant to ceftazidime.

We assessed the differences in ESBL production rates in *E. coli* and *K. pneumoniae* isolates among a variety of isolation sources. This was incongruent with data from the Czech Republic, which reported a high prevalence of ESBL-producing *K. pneumoniae* isolates from tracheal secretions, bronchoalveolar lavage, and sputum (41.9%) and a significantly lower rate in blood isolates (8.5%) (Kolar et al., 2006). Hyle et al. (2005) previously reported that *E. coli* and *Klebsiella* isolates from urine were significantly less likely to generate ESBL than isolates from other sources.

It remains unclear as to why the prevalence of ESBL production in *E. coli* isolates from sputum and *K. pneumoniae* isolates from urine was higher than those seen in isolates from other sources. This may be attributable to clonal dissemination of particular ESBL-producing clones, although the confirmation of such a supposition is beyond the scope of this study. Several articles have previously reported that the prevalence of ESBL production was higher in intensive care units (ICUs) than in general wards (Hyle et al., 2005; Kolar et al., 2006; Pena et al., 1998; Villegas et al., 2004; Wu et al., 2006). Higher prevalence of ESBL production in ICUs may be the result of the clonal spreading of ESBL-producing strains (Ko et al., 2008). Besides ICUs, the horizontal transfer of ESBL-producing strains in particular wards may result in an increase in their prevalence rates. However, we collected isolates from several hospitals located throughout Korea. In addition, a total of 28 different combinations of ESBL enzymes were identified among 123 ESBL-producing *K. pneumoniae* isolates, thereby suggesting the presence of diverse clones of ESBL-producing strains of *K. pneumoniae* in Korea. Thus, the higher prevalence of ESBL-producing isolates in particular specimens may not be the result of clonal dissemination within hospitals. The high prevalence of ESBL-producing *E. coli* isolates from sputum and *K. pneumoniae* isolates from urine (particularly, catheterized urine) should then be examined further, with an eye toward the clinical implications of these results.

In this study, we evaluated the prevalence of ESBL production and antimicrobial resistance in Enterobacteriaceae species. Although we could not perform plasmid and genotypic analyses, which are the main defects in this article, we detected some differing results from previous studies, including relatively low prevalence of ESBL production in non-*E. coli* and non-*Klebsiella* spp. isolates. We also found a significantly high prevalence of ESBL production in *E. coli* isolates obtained from sputum and in *K. pneumoniae* isolates from urine.

Acknowledgments

This study was partly supported by a grant from the Korean Food and Drug Administration and the Asian-Pacific Research Foundation for Infectious Diseases.

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