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Epidemiology and Clinical Features of Bloodstream Infections Caused by AmpC-Type-β-Lactamase-Producing *Klebsiella pneumoniae*

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Cases of bacteremia caused by AmpC-type-β-lactamase-producing *Klebsiella pneumoniae* isolates were retrospectively studied to determine the epidemiologic features and clinical outcomes of bloodstream infections. Among 389 blood isolates recovered from 1998 to 2002, 65 isolates (16.7%) were found to be extended-spectrum β-lactamase (ESBL) or AmpC β-lactamase producers. The β-lactamases from 61 of the 65 isolates were characterized; 28 of 61 isolates produced AmpC-type enzymes (14 isolates each produced DHA-1 and CMY-1-like enzymes), 32 isolates produced TEM or SHV-related ESBLs, and 1 isolate produced a CTX-M-14-like enzyme. To compare the clinical features and outcomes of bloodstream infections caused by AmpC producers with those caused by TEM- or SHV-related ESBL producers, 27 patients infected with isolates producing AmpC-type enzymes (AmpC group) and 25 patients infected with isolates producing TEM- or SHV-related enzymes (ESBL group) were analyzed. There was no significant difference between the AmpC and the ESBL groups in terms of risk factors. When the initial response was assessed at 72 h after antimicrobial therapy, the treatment failure rate for the AmpC group was 51.9% (14 of 27 patients) and the 7- and 30-day mortality rates were 14.8 and 29.6%, respectively, which were similar to those for the ESBL group. When the mortality rate for the patients who received extended-spectrum cephalosporins as definitive treatment was assessed, all four patients in the DHA-1 group and one of three patients in the CMY-1-like group died. In summary, the prevalence of AmpC enzyme-producing *K. pneumoniae* was high at the Seoul National University Hospital, and the clinical features and outcomes for the patients infected with AmpC-producing organisms were similar to those for the patients infected with TEM- or SHV-related ESBL producers.

Since the first description of extended-spectrum β-lactamase (ESBL) production by *Klebsiella pneumoniae* in 1983 (11), antibiotic-resistant strains that produce ESBLs have emerged among the members of the family *Enterobacteriaceae*, predominantly in *Escherichia coli* and *K. pneumoniae*, and isolates resistant to broad-spectrum cephalosporins are increasingly being recognized (3). In the past decade, a new problem has emerged in enteric bacteria: plasmid-mediated AmpC enzymes. They are derived from chromosomal AmpC genes of gram-negative organisms, such as *Citrobacter freundii*, *Enterobacter cloacae*, and *Aeromonas* species (21).

Organisms with plasmid-mediated AmpC enzymes are generally resistant to broad-spectrum penicillins, extended-spectrum cephalosporins, monobactam, and cephamycins but are susceptible to cefepime, cefpirome, and carbapenems (21). However, it is difficult to distinguish ESBL-producing organisms from plasmid-mediated AmpC β-lactamase-producing organisms by phenotypic susceptibility testing. Standard guidelines for the detection of AmpC-producing isolates are also lacking.

Although there have been several reports of nosocomial outbreaks caused by organisms which produce plasmid-mediated AmpC enzymes (4, 20, 29), the epidemiology and clinical features associated with infections caused by these organisms have not been well described.

In this report, we describe the epidemiology and microbiological characteristics of AmpC β-lactamase-producing *K. pneumoniae* isolates and analyze the clinical characteristics of the patients infected by AmpC enzyme-producing *K. pneumoniae* isolates. In addition, we compared the clinical features and outcomes of bloodstream infections caused by AmpC β-lactamase-producing *K. pneumoniae* isolates with those caused by TEM- or SHV-related ESBL-producing *K. pneumoniae* isolates.

MATERIALS AND METHODS

Bacterial isolates and patients. The database at the Clinical Microbiology Laboratory of the Seoul National University Hospital was reviewed in order to identify patients with *K. pneumoniae* bacteremia. A total of 480 episodes of *K. pneumoniae* bacteremia among 417 patients were identified during the period from January 1998 to April 2002. Only one isolate from each bacteremic episode and the first bacteremic episode of each patient was included in the analysis. Of the 417 *K. pneumoniae* isolates, 389 were included in this study. Species identification was carried out with VITEK-GNI cards (bioMérieux, Hazelwood, Mo.) by standard methods (7).

Microbiological analyses. (i) Antibiotic susceptibility testing. The MICs of the antibiotics tested were determined by the agar dilution method, as described by the National Committee for Clinical Laboratory Standards (17). *E. coli* ATCC 25922 was used as the reference strain for quality control. The antimicrobials tested were piperacillin and piperacillin-tazobactam (Wyeth Pharmaceuticals, Pearl River, N.Y.); cefoxitin (Choongwae Pharma Co., Seoul, Korea); cefotaxime (Handok Pharmaceuticals Co., Seoul, Korea); ceftazidime (Glaxo Korea

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Co., Seoul, Korea); aztreonam (Dong-A Biotech Co., Seoul, Korea); cefepime, cloxacillin, and amikacin (Yuhan Co., Seoul, Korea); clavulanic acid (Il-Sung Pharmaceuticals, Seoul, Korea); ciprofloxacin (Bayer Korea Co., Seoul, Korea); and gentamicin (Young Jin Pharmaceutical Co, Seoul, Korea).

(ii) Screening and confirmatory tests for ESBL-producing strains. ESBL production was examined by the disk diffusion method, as described previously (6). In brief, the diameters of the inhibition zones on cefotaxime and ceftazidime disks (30 μg each), alone and in combination with clavulanic acid (10 μg), were determined. An increase in the zone diameter of 5 mm or more when either of the antimicrobial agents was combined with clavulanic acid was considered evidence of ESBL production. Isolates that were resistant to cefotaxime, ceftazidime, or cefpodoxime but for which an increase in zone diameter of less than 5 mm was revealed were subjected to the double-disk diffusion test with cefotaxime, ceftazidime, and cefepime disks (27), as described by Thomson and Sanders (26), except that the ceftazidime and amoxicillin-clavulanic acid disks were placed 15 mm apart.

The production of AmpC β-lactamase was phenotypically suspected in isolates that were resistant to either cefotaxime or ceftazidime, did not reveal the enhancement of the inhibitory zone when a clavulanic acid disk was present, and were resistant to both amoxicillin-clavulanic acid and cefoxitin (24). Two control organisms, *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603, were inoculated in each set of tests for quality control.

(iii) Analytical IEF and enzyme inhibition assay. Isoelectric focusing (IEF) was performed with sonicated extracts by the method of Mathew et al. (16) by using a Mini IEF cell system (Bio-Rad, Hercules, Calif.). Enzyme activities were examined by overlaying the gel with 0.5 mM nitrocefin in 0.1 M phosphate buffer (pH 7.0). An inhibition assay was performed by overlaying the gels with 0.5 mM nitrocefin with and without 0.3 mM cloxacillin or 0.3 mM clavulanic acid in 0.1 M phosphate buffer (pH 7.0) (19). Strains carrying plasmids encoding the β-lactamases TEM-1 (R1), TEM-3 (pCFF04), TEM-4 (pUD16), SHV-2 (pMG229), SHV-5 (pAFF2), and CMY-1 (pMVP-1) served as IEF standards (2, 6).

(iv) Transfer of resistance, plasmid analysis, and Southern hybridization. Logarithmic-phase cells of each isolate were mated with similar cultures of *E. coli* J53 Azi^r on Trypticase soy agar plates. Transconjugants were selected on Trypticase soy agar containing 100 μg of sodium azide (Sigma, St. Louis, Mo.) per ml and 64 μg of cefoxitin per ml (19). To confirm the presence of plasmids and to estimate their sizes, plasmids from clinical isolates and transconjugants were extracted, electrophoresed on a 0.7% agarose gel, and subjected to Southern hybridization by the protocol described previously (14, 23). PCR with a bla_{DHA-1}-specific probe generated amplicons labeled with digoxigenin (DIG DNA labeling and detection kit; Boehringer Mannheim, Mannheim, Germany).

(v) PCR and nucleotide sequences of β-lactamase genes. The DHA-1-related genes from clinical isolates were amplified by PCR. The primers used for the amplification were DHA-1U (5′-CACACGGAAGGTTAATTCTGA-3′) and DHA-1L (5′-CGGTTATACGGCTGAACCTG-3′), which correspond to nucleotides −20 to 1 and 961 to 980 of the DHA-1 structural gene, respectively. The PCR conditions were as follows: 5 min at 94°C; 35 cycles of 30 s at 94°C, 45 s at 57°C, and 1 min at 72°C; and finally, 8 min at 72°C. The amplified product from isolate 18 was sequenced with primers DHA-1U, DHA-1L, and DHA-2U (5′-AAGAGATGGCGCTGAATGAT-3′).

CMY-1-, TEM-, SHV-, and CTX-M-14-related genes were amplified as described previously (18, 19).

(vi)Test for induction of AmpC β-lactamases. AmpC β-lactamases were induced with cefoxitin, cefotaxime, and ceftazidime disks on Mueller-Hinton agar (Difco, Detroit, Mich.), as described previously (13).

(vii) PFGE. Pulsed-field gel electrophoresis (PFGE) was performed with a CHEF Mapper XA system (Bio-Rad Laboratories, Inc.), as described previously (9).

Clinical analysis. (i) Definitions. *K. pneumoniae* bacteremia was defined as the detection of *K. pneumoniae* in a blood culture specimen. Clinically significant *K. pneumoniae* bacteremia was defined as at least one positive blood culture, together with clinical features compatible with systemic inflammatory response syndrome.

The bacteremia was categorized as polymicrobial if additional microorganisms were recovered from the blood cultures. Nosocomial infection was defined as an infection that occurred later than 48 h after admission to the hospital, an infection that occurred less than 48 h after admission to the hospital in patients who had been hospitalized within 2 weeks prior to admission, and an infection that occurred less than 48 h after admission to the hospital in patients who had been transferred from another hospital or nursing home. Nosocomial bloodstream infections as well as other nosocomial infections were defined according to the criteria proposed by the Centers for Disease Control and Prevention (5). Neutropenia was defined as an absolute neutrophil count below 500/mm^3.

The antimicrobial therapies were classified into empirical and definitive, with the former defined as the initial therapy provided before the results of blood culture were available and the latter defined as therapy provided after the results of antibiotic susceptibility tests had been reported. The antimicrobial therapy was considered appropriate if the treatment regimen included antibiotics active against *K. pneumoniae* in vitro and the dosage and route of administration were in conformity with present medical standards.

(ii) Review of medical records. We reviewed the medical records of the patients. The data collected included age; sex; underlying disease; site of infection; the severity of illness, as calculated by the Acute Physiology and Chronic Health Evaluation (APACHE) II score (10); the duration of the hospital stay before the onset of bacteremia; the antimicrobial regimen; and any antimicrobial therapy within 30 days prior to the onset of bacteremia. The presence of the following comorbid conditions was also documented: neutropenia, presentation with septic shock, care in an intensive care unit, use of immunosuppressive agents within 30 days prior to the onset of bacteremia, corticosteroid use, postoperative state, and invasive procedures within 72 h prior to the onset of bacteremia. In addition, the patients were assessed for the presence of a central venous catheter, an indwelling urinary catheter, or mechanical ventilation. Since this study was retrospective, the patients' physicians, but not the researchers, had chosen the antimicrobial therapy regimens.

The main outcome measures used were the initial response to treatment and the 7- and 30-day mortality rates. The initial response to treatment was assessed 72 h after the start of antimicrobial therapy and was classified as follows: complete response for patients with resolution of fever, leukocytosis, and all signs of infection; partial response for patients with an abatement but not a complete resolution of fever, leukocytosis, and all signs of infection; failure for patients with no abatement or deterioration of any of the clinical parameters; and death (12).

(iii) Statistical analysis. Student's *t* test was used to compare continuous variables, and the χ^2 or Fisher's exact test was used to compare categorical variables. All *P* values were two tailed, with a *P* value <0.05 considered statistically significant. The SPSS (version 10.0) software package was used for these analyses.

RESULTS

Selection of isolates and patients. Of the 389 blood isolates, 65 (16.7%) were ESBL or AmpC β-lactamase producers. Among those 65 isolates, 61 isolates were further characterized for the presence of β-lactamases. For the clinical analysis, the data for 53 patients whose medical records were available and who had clinically significant bacteremia were analyzed.

Microbiological analyses. (i) IEF and enzyme inhibition assay. Each isolate produced one to three β-lactamases of pI 5.4, 5.9, 7.6, 7.7, 8.0, 8.2, or >8.2 in various combinations. Among the β-lactamases, those with pIs of 7.7 and 8.0 were inhibited by 0.3 mM cloxacillin but not by 0.3 mM clavulanic acid. The β-lactamase production patterns, the number of isolates with each pattern, and the MICs of several antibiotics are summarized in Table 1.

(ii) PCR for DHA-1-, CMY-1-, TEM-, SHV-, or CTX-M-related β-lactamase genes. Fourteen isolates showed inducible β-lactamase production and produced a pI 7.7 β-lactamase which was inhibited by cloxacillin. A DHA-1-specific PCR was performed with these 14 isolates, with positive results for all 14 isolates. The nucleotide sequence of the amplified product from isolate 18 was confirmed to be DHA-1 (1). A CMY-1-specific PCR was performed with the isolates that produced pI 8.0 β-lactamases, which were inhibited by 0.3 mM cloxacillin. All 14 isolates amplified PCR products compatible with the CMY-1 gene and were considered to produce CMY-1-like β-lactamases. An SHV-specific PCR was performed for the isolates that produced a pI 8.2 or a pI 7.6 β-lactamase and the transconjugants of three isolates that produced β-lactamases with pIs of >8.2 which were inhibited by 0.3 mM clavulanic

TABLE 1. Antimicrobial susceptibilities, pIs, and types of β-lactamases for *K. pneumoniae* isolates from patients with bloodstream infections

β-Lactamase pI(s)	No. of isolates	β-Lactamase type(s)	MIC range (μg/ml)[b]									
			FOX	CTX	CAZ	ATM	FEP	PIP	TZP[c]	GEN	AMK	CIP
5.4, 7.7[a]	7	DHA-1, TEM-1-like	64–256	2–64	64–>256	64–>256	<1–16	>256–>256	64–64	64–64	1–4	0.5–1
5.4, 7.6, 7.7	3	DHA-1, SHV, TEM-1-like	128–256	8–8	32–128	8–32	<1–<1	256–>256	4–8	128–>128	128–>128	0.5–0.5
7.7	2	DHA-1	256–256	32–64	>256–>256	64–64	1–2	>256–>256	16–32	64–64	<1–4	0.5–1
5.4, 7.7, 8.2	1	DHA-1, TEM-1-like, SHV-12-like	128	64	>256	>256	4	>256	4	64	4	4
7.6, , 7.7, 8.2	1	DHA-1, SHV, SHV-12-like	>256	64	128	256	16	256	16	>128	>128	128
5.4, 7.6, 8.0	7	CMY-1-like, TEM-1-like, SHV	>256–>256	32–64	64–256	8–256	<1–16	128–>256	16–32	4–4	1–4	8–64
5.4, 8.0	7	CMY-1-like, TEM-1-like	>256–>256	32–128	16–64	8–8	<1–2	128–>256	16–32	4–64	1–4	8–64
7.6, 8.2	7	SHV-12-like, SHV	2–64	4–64	64–>256	128–>256	1–64	64–>256	0.25–16	1–8	<1–2	<0.25–4
5.4, 7.6, 8.2	7	SHV-12-like, TEM-1-like, SHV	4–128	8–128	128–>256	256–>256	2–128	>256–>256	4–32	<1–64	4–8	0.25–4
8.2	4	SHV-12-like	4–32	8–32	128–>256	256–>256	2–16	256–>256	2–8	<1–4	<1–1	8–32
5.4, 8.2	2	SHV-12-like, TEM-1-like	4–256	8–16	128–256	256–>256	2–4	>256–>256	1–4	64–128	<1–4	2–2
5.4, 7.6, >8.2	3	SHV-like, TEM-1-like	8–8	64–64	4–32	32–32	32–128	>256–>256	4–32	64–>128	<1–>128	16–64
7.6	2	SHV	4–32	<1–64	<1–32	<1–64	<1–16	8–256	<0.25–2	<1–2	<1–<1	4–32
5.4, 5.9	4	TEM-52-like, TEM-1-like	2–32	64–128	16–128	8–64	4–32	>256–>256	4–32	32–>128	<1–2	<0.25–0.5
5.4, 5.9, 7.6	2	TEM-52-like, TEM-1-like, SHV	64–64	128–>256	64–64	256–256	32–64	>256–>256	4–16	32–32	<1–1	<0.25–<0.25
5.4	1	TEM	8	<1	1	<1	<1	>256	4	128	16	0.25
5.4, 8.0	1	CTX-M-14-like	4	64	4	16	64	>256	4	<1	<1	4

[a] Underlined values indicate that the β-lactamase with the indicated pI value is inhibited by 0.3 mM cloxacillin.
[b] Abbreviations: FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam; FEP, cefepime; PIP, peperacillin; TZP, piperacillin-tazobactam; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin.
[c] The concentrations of piperacillin and tazobactam were fixed at an 8:1 ratio.

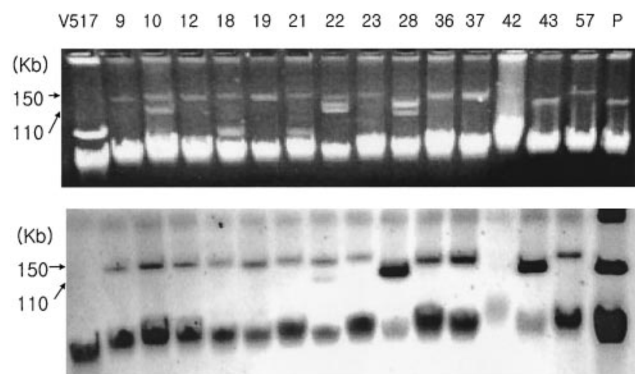

FIG. 1. Plasmid agarose gel electrophoresis and Southern hybridization of DHA-1-producing isolates. Lane V517, plasmid size standards from *E. coli* strain V517 (18); lanes 9 to 57, DHA-1-producing plasmids from the isolates corresponding to the isolate numbers listed in Table 5; lane P, plasmid from *K. pneumoniae* 502321.

acid but not by 0.3 mM cloxacillin. Genes for SHV-related β-lactamases were amplified from all the isolates, which were considered to produce SHV-related β-lactamases. Among those, the pI 8.2 β-lactamase was considered to be an SHV-12-like β-lactamase because seven of seven isolates that produced the pI 8.2 β-lactamase and whose *bla* genes were sequenced were identified to produce SHV-12 in previous studies (8, 9, 19). A TEM-specific PCR was performed with those isolates which produced β-lactamases with a pI of 5.9; and products were amplified from all six isolates, which were considered to produce TEM-52-like ESBLs, as described previously (8, 9, 19).

One isolate produced a β-lactamase with a pI of 8.0 which was inhibited by 0.3 mM clavulanic acid, and a CTX-M-14-specific PCR was performed with this isolate, as described previously (18). The PCR results were positive, and the isolate was considered to produce a CTX-M-14-like enzyme.

(iii) Transfer of resistance and plasmid analysis. To test the transmissibility of cefoxitin resistance, a conjugation experiment was performed with the isolates characterized to have a CMY-1-like or a DHA-1 β-lactamase gene. Of the 14 isolates that produced CMY-1-like β-lactamases, 6 isolates transferred cefoxitin resistance via plasmids of about 130 kb. For DHA-1-producing isolates, cefoxitin resistance was not transferred by conjugation, but plasmid analysis by Southern hybridization showed that 12 of 14 isolates harbored plasmids of about 150 kb containing $bla_{\text{DHA-1}}$ and 2 had plasmids of about 110 kb (Fig. 1).

(iv) Antimicrobial susceptibility. The MIC ranges are listed in Table 1 according to the types of β-lactamases produced by the isolates. The MICs at which 50% of isolates are inhibited (MIC_{50}s) for cefoxitin, cefotaxime, ceftazidime, aztreonam, cefepime, piperacillin, and piperacillin-tazobactam were 256, 8, >256, 16, 1, >256, and 16 μg/ml, respectively, for the DHA-1-producing isolates and >256, 64, 64, 8, 1, >256, and 24 μg/ml, respectively, for the CMY-1-like-producing isolates. For the DHA-1-producing isolates, the distributions of the MICs of cefepime were as follows: <1 μg/ml for seven isolates, 1 μg/ml for two isolates, 2 μg/ml for two isolates, 4 μg/ml for one isolate, and 16 μg/ml for two isolates. For the CMY-1-like β-lactamase-producing isolates, the MICs of cefepime were <1 μg/ml for five isolates, 1 μg/ml for three isolates, 2 μg/ml for three isolates, 4 μg/ml for two isolates, and 16 μg/ml for one isolate.

(v) PFGE. Thirteen DHA-1-producing *K. pneumoniae* isolates and 14 CMY-1-like-producing isolates were included in the PFGE analysis. The DHA-1-producing isolates showed seven PFGE types, and the CMY-1-like-producing isolates revealed two PFGE types (Fig. 2).

Prevalences and annual distributions of ESBLs. During the study period, from January 1998 to April 2002, 16.7% (65 of 389) of the *K. pneumoniae* blood isolates were ESBL or plasmid-mediated AmpC producers. The proportion of ESBL or plasmid-mediated AmpC producers did not increase over the study period: 16% (15 of 94 isolates) in 1998, 14.3% (13 of 91) in 1999, 15.5% (16 of 103) in 2000, and 15.5% (11 of 71) in 2001. During the period from January to April 2002, 10 of 30 isolates produced ESBLs or plasmid-mediated AmpC enzymes.

When the distribution of β-lactamases was assessed by year, DHA-1-producing isolates were consistently isolated from 1998 to 2001, whereas CMY-1-producing isolates first appeared in 2000 and persisted thereafter. SHV-12-like ESBL-producing isolates were isolated in most years, whereas TEM-52-like ESBL-producing isolates were not isolated after 2000 (Fig. 3).

Clinical analyses. DHA-1 or CMY-1-like β-lactamase-producing *K. pneumoniae* isolates were classified as AmpC-type enzyme producers, and other isolates (TEM-, SHV-, or CTX-M-like enzyme producers) were classified as ESBL producers. The demographic and clinical characteristics of the 27 patients infected with AmpC β-lactamase-producing isolates (AmpC group) and the 25 patients infected with TEM- or SHV-related ESBL-producing isolates (ESBL group) were analyzed; 1 patient who was infected with a CTX-M-14-like ESBL-producing isolate was excluded, however (Table 2). There were no significant differences between the two groups by age, sex, APACHE II score, primary site of infection, or underlying disease; but more cases of pancreaticobiliary tract disease was observed in the AmpC group (Table 2).

Among the 27 patients in the AmpC group, 26 (96.3%) had nosocomial infections and 11 (40.7%) had stayed in the hospital for more than 2 weeks. Twenty-one (77.7%) patients had received some antibiotics within 30 days prior to the onset of bacteremia and 20 (74.1%) had received extended-spectrum cephalosporins. Seven (25.9%) patients had an indwelling urinary catheter, and 8 (29.6%) patients had a central venous catheter. No significant difference in risk factors was found when the AmpC group was compared with the ESBL group (Table 3).

When the treatment response was assessed 72 h after antimicrobial therapy, the treatment failure rates were 51.9% (14 of 27 patients) in the AmpC group and 56% (14 of 25 patients) in the ESBL group. The 7- and 30-day mortality rates for the AmpC group were also similar to those for the ESBL group (Table 4).

The clinical details for the 13 patients infected with DHA-1 β-lactamase-producing *K. pneumoniae* isolates are summarized in Table 5. Of these 13 patients, 12 had received extended-spectrum cephalosporins as initial empirical antibiotic therapy

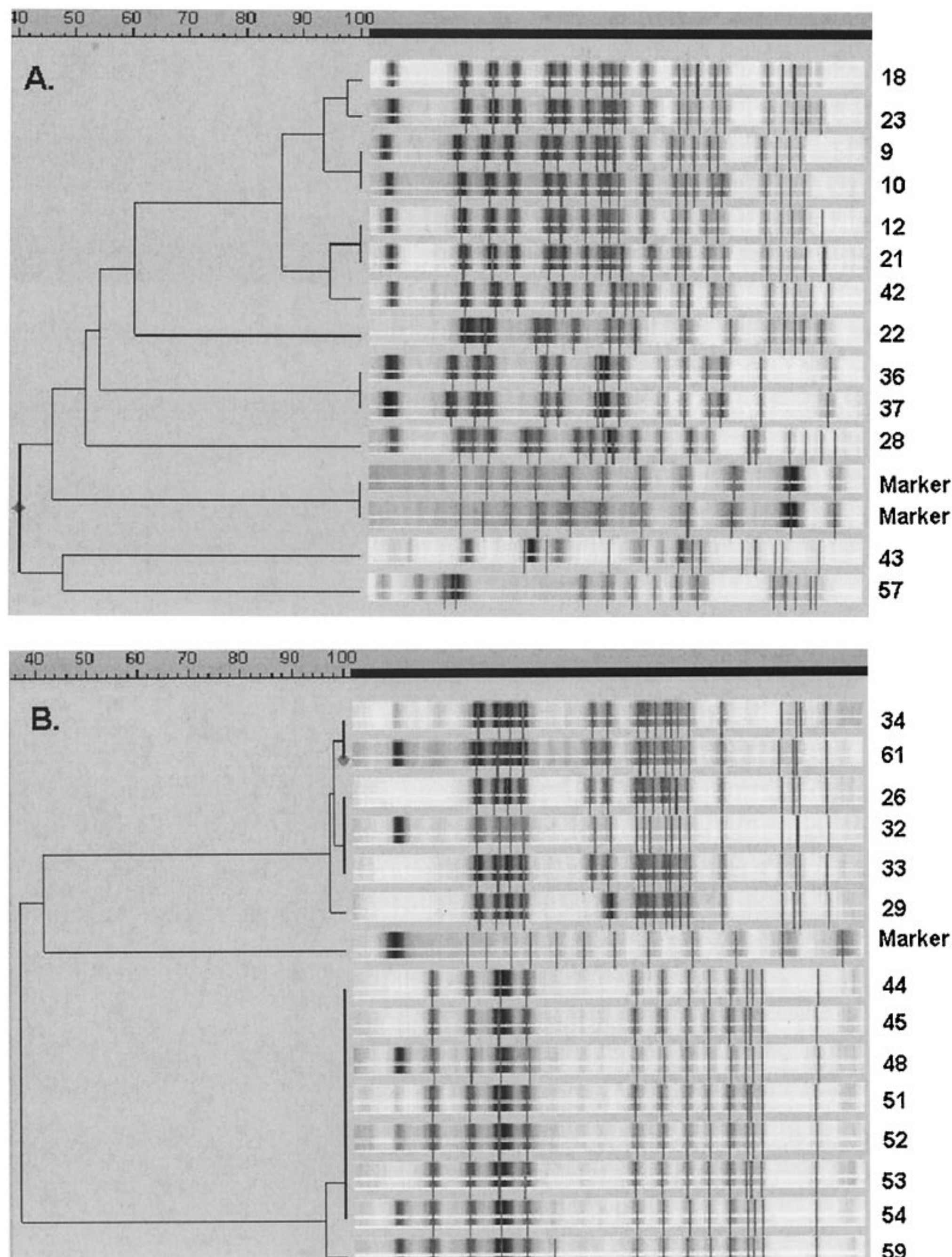

FIG. 2. Dendrograms of 13 DHA-1-producing *K. pneumoniae* isolates (A) and 14 CMY-1-producing *K. pneumoniae* isolates (B). DHA-1 enzyme-producing isolates showed seven PFGE types, and CMY-1-like enzyme-producing isolates revealed two PFGE types. The strains were clustered by the unweighted pair group method with arithmetic averages. The scale indicates the percent genetic similarity. The molecular size marker is a bacteriophage lambda ladder (Bio-Rad). The numbers on the right of each lane correspond to the clinical isolate numbers in Tables 5 and 6.

and the remaining patient had received ciprofloxacin. Nine patients had received imipenem as definitive antimicrobial therapy, and the remaining four patients had received extended-spectrum cephalosporins. All patients who had received extended-spectrum cephalosporins as definitive treatment died, and three of them died before identification of the pathogen. Of nine patients who had received imipenem, seven were cured. The 30-day mortality rate was 46% (6 of 13) for patients with bloodstream infections caused by DHA-1 β-lactamase-producing *K. pneumoniae*.

The clinical details for the 14 patients infected with CMY-related β-lactamase-producing *K. pneumoniae* are summarized in Table 6. Ten of 14 (71.4%) patients had cholangitis. Of these 14 patients, 9 patients had received extended-spectrum ceph-

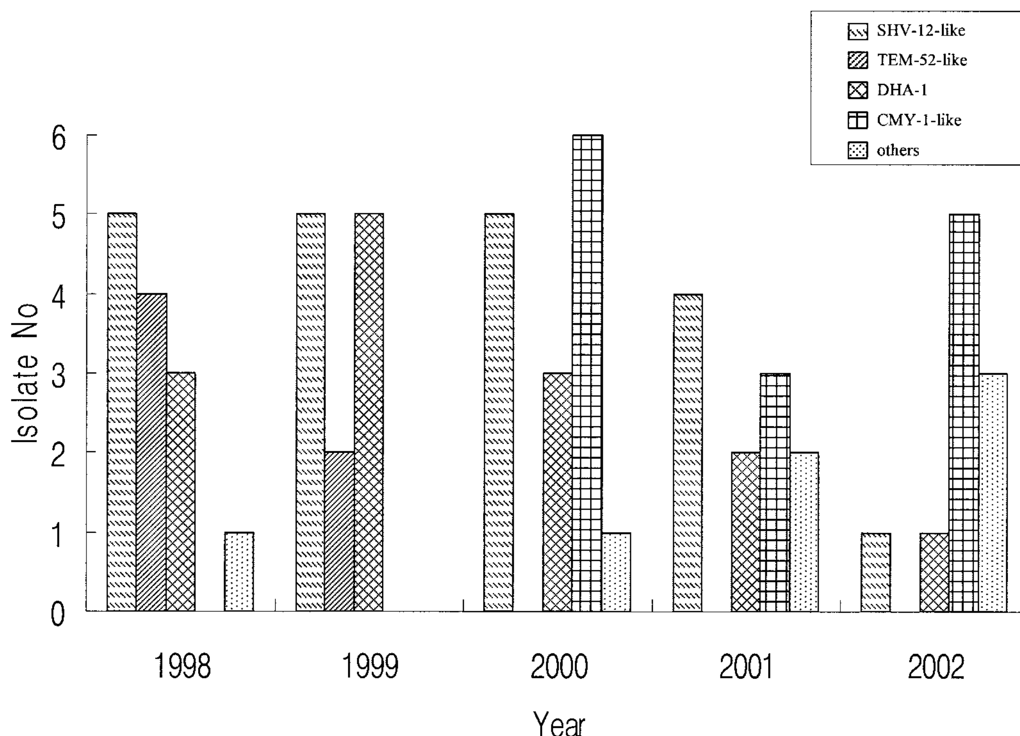

FIG. 3. Distribution of ESBL subtypes from the isolates identified each year. DHA-1-producing isolates were consistently isolated from 1998 to 2001, but CMY-1-producing isolates first appeared in 2000.

alosporins as the initial empirical antibiotic therapy and the remaining 5 patients had received ciprofloxacin or imipenem. For definitive antimicrobial therapy, 11 patients had received ciprofloxacin or imipenem and the remaining 3 patients had received extended-spectrum cephalosporins. Of 3 patients who had received cephalosporins as the definitive treatment, 1 patient died, whereas among the 11 patients who had received ciprofloxacin or imipenem, 1 patient died. The 30-day mortality rate was 14.3% (2 of 14) for patients with bloodstream infections due to CMY-related AmpC β-lactamase-producing *K. pneumoniae*.

TABLE 2. Demographic and clinical characteristics of patients with bloodstream infections due to AmpC β-lactamase-producing *K. pneumoniae* isolates versus those of patients with infections due to TEM- or SHV-related ESBL-producing *K. pneumoniae* isolates

Characteristic	AmpC group (n = 27)	ESBL group (n = 25)	P
Age (yr [mean ± SD])	54 ± 12.6	55 ± 17.7	0.872
No. (%) of male patients	19 (70.4)	14 (56)	0.282
Underlying disease (no. [%] of patients)			
Leukemia	1 (3.7)	4 (16)	0.183
Solid tumor	7 (25.9)	5 (20)	0.612
Liver cirrhosis	9 (33.3)	4 (16)	0.149
Pancreaticobiliary tract disease	6 (22.2)	0 (0)	0.023
Others	5 (18.5)	12 (48)	
Site of infection (no. [%] of patients)			
Cholangitis	13 (48.1)	7 (28)	0.136
Peritonitis	6 (22.2)	4 (16)	0.729
Liver abscess	3 (11.1)	1 (4)	0.611
Pneumonia	1 (3.7)	3 (12)	0.341
Urinary tract infection	1 (3.7)	2 (8)	0.603
Unknown	3 (11.1)	8 (32)	0.065
APACHE II score (mean ± SD)	8.37 ± 4.44	10.68 ± 5.90	0.115

DISCUSSION

Since plasmid-mediated AmpC β-lactamase was first reported from *K. pneumoniae* in 1989 (2), plasmid-mediated AmpC β-lactamases have increasingly been identified worldwide. Plasmid-mediated AmpC β-lactamases have been discovered most frequently in *K. pneumoniae* isolates and also in

TABLE 3. Analysis of risk factors for bloodstream infections caused by AmpC β-lactamase-producing *K. pneumoniae* isolates versus those caused by TEM- or SHV-related ESBL-producing *K. pneumoniae* isolates

Risk factor	No. (%) of patients		P
	AmpC group (n = 27)	ESBL group (n = 25)	
Long hospital stay (>2 wk)	11 (40.7)	16 (64)	0.093
Care in intensive care unit	3 (11.1)	5 (20)	0.458
Central venous catheterization	8 (29.6)	8 (32)	0.853
Indwelling urinary catheter	7 (25.9)	7 (28)	0.866
Polymicrobial infection	9 (33.3)	2 (8)	0.025
Invasive procedure within previous 72 h	10 (37.0)	4 (16)	0.087
Neutropenia	2 (7.4)	5 (20)	0.241
Postsurgical state	4 (14.8)	3 (12)	1.000
Nosocomial infection	26 (96.3)	21 (84)	0.183
Prior use of antibiotics	21 (77.8)	21 (84)	0.729
Broad-spectrum cephalosporins	20 (74.1)	18 (72)	0.866
Penicillins	2 (7.4)	6 (24)	0.134
Fluoroquinolones	4 (14.8)	2 (8)	0.670
Aminoglycosides	15 (55.6)	16 (64)	0.535

TABLE 4. Clinical outcomes for patients with bloodstream infections caused by AmpC β- lactamase-producing *K. pneumoniae* isolates versus those caused by TEM- or SHV-related ESBL-producing *K. pneumoniae* isolates

Outcome	No. (%) of patients		P
	AmpC group (n = 27)	ESBL group (n = 25)	
Initial treatment failure	14 (51.9)	14 (56)	0.764
Death at 7 days	4 (14.8)	6 (24)	0.492
Death at 30 days	8 (29.6)	7 (28)	0.897

other species naturally negative for AmpC, such as *Klebsiella oxytoca*, *Salmonella*, and *Proteus mirabilis* (21).

In our study, on the basis of an analysis of the cases of *K. pneumoniae* bacteremia detected at a single institute in South Korea, DHA-1- and CMY-1-producing isolates were found to be common among the isolates resistant to extended-spectrum cephalosporins. Previous studies showed that CMY-1 is prevalent in Korea (8, 9, 19); however, it should be noted that DHA-1, an inducible AmpC β-lactamase, is prevalent at the Seoul National University Hospital, a 1,500-bed university hospital. Since the first description of DHA-1 from a strain in Saudi Arabia in 1998 (1), DHA-1-producing clinical isolates have been reported in Taiwan (29).

The DHA-1 enzyme, which is mediated by 110-kb plasmid, was first identified from *K. pneumoniae* strain 502321 in Korea in 2000 (unpublished data). We cloned and sequenced nucleotides of the gene and found that the sequence of the *bla* gene of this isolate was identical to that of $bla_{\text{DHA-1}}$. An *E. coli* DH10B isolate containing this clone showed a resistance pattern identical to that of *E. coli* HB101(pSAL-1) (28): resistance to streptomycin and sulfonamides.

It is noteworthy that several geographic clusters of AmpC

TABLE 5. Clinical features and outcomes of bloodstream infections due to DHA-1-related AmpC β-lactamase-producing *K. pneumoniae* isolates[a]

Isolate no.	Age (yr)/sex	Underlying disease	Comorbid condition	Type or site of infection	Empirical therapy	Definitive therapy	Outcome
9	60/M	Colon cancer	Biliary invasion	Cholangitis	CAZ, AMK	IPM	Persistent fever after 3 days; treatment changed to imipenem, with cure
10	50/M	HCC	TACE	Unknown	CTX, AMK	IPM	Persistent fever after 4 days; treatment changed to imipenem, with cure
12	75/M	HCC	ICU, post-op. C-line	Peritonitis	CTX, AMK	IPM	Persistent fever after 4 days; treatment changed to imipenem, with cure
18	43/M	DM, LC	ICU care	Peritonitis	CTX, AMK	CTX, AMK	Death on day 3 of treatment
19	43/F	Brain tumor	Craniotomy, post-op.	Urinary tract infection	CIP	IPM	Persistent fever after 3 days; treatment changed to imipenem, with cure
21	65/M	LC	None	Peritonitis	CAZ	CAZ	Progression to infected right pleural effusion; death on day 16 of treatment
23	58/F	CBD stone	Post-EST	Cholangitis	CAZ	CAZ	Death on day 3 of treatment
28	54/M	Leukemia	Neutropenia, C-line	Unknown	ZOX, AMK	IPM	Persistent fever after 3 days; treatment changed to imipenem, with cure
36	54/F	LC	Foley catheter, C-line	Peritonitis	CTX, AMK	CTX, AMK	Death on day 3 of treatment
37	43/F	MCTD	ICU, Foley catheter, C-line	Pneumonia	CAZ, AMK	IPM, AMK	Persistent fever after 7 days; treatment changed to imipenem, but with death on day 29 of treatment
42	63/M	Pancreas cancer, DM	None	Cholangitis	CTX, AMK	IPM	Persistent fever after 3 days; treatment changed to imipenem, with cure
43	50/M	HCC	TACE	Liver abscess	CTX	IPM	Persistent fever after 5 days; treatment changed to imipenem, with drainage, cured
57	17/M	Aplastic anemia, BMT	Neutropenia, C-line	Unknown	ZOX, AMK	IPM, CIP	Persistent fever after 7 days; treatment changed to imipenem and ciprofloxacin, but with death on day 22 of treatment

[a] Abbreviations: M, male; F, female; DM, diabetes mellitus; LC, liver cirrhosis; ICU, intensive care unit; C-line, central line; Post-op., postoperative state; MCTD, mixed connective tissue disease; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; CBD, common bile duct; EST, endoscopic sphincterotomy; BMT, bone marrow transplantation; CTX, cefotaxime; CAZ, ceftazidime; ZOX, ceftizoxime; AMK, amikacin; CIP, ciprofloxacin; IPM, imipenem.

TABLE 6. Clinical features and outcomes of bloodstream infections due to CMY-1-related AmpC β-lactamase-producing *K. pneumoniae* isolates[a]

Isolate no.	Age (yr)/sex	Underlying disease	Comorbid condition	Type or site of infection	Empirical therapy	Definitive therapy	Outcome
26	73/M	CBD stone	None	Cholangitis	CTX, AMK	CTX, AMK	Cured with biliary decompression and antimicrobial therapy
29	55/M	CBD cancer	Post-ERCP	Cholangitis	CAZ, CIP	CTX, CIP	Death on day 15 of treatment
32	63/M	CBD stone	Post-EST	Cholangitis	CIP, AMK	CIP, AMK	Cured with antimicrobial therapy
33	65/M	CBD stone	Post-EST	Cholangitis	CIP, AMK	CIP, AMK	Cured with biliary decompression
34	50/F	Pancreas cancer	None	Cholangitis	CTX, AMK	CTX, AMK	Cured with antimicrobial therapy
44	57/M	IHD stone, DM	Liver segmentectomy	Abdominal abscess	IPM	IPM	Cured with drainage and antimicrobial therapy
45	54/F	IHD stone	None	Cholangitis	CTX, CIP	CIP	Cured with antimicrobial therapy
48	56/M	GB cancer	None	Cholangitis	CAZ, AMK	IPM	Persistent fever after 3 days; treatment changed to imipenem, with cure
51	28/M	Wilson's disease	Liver transplantation	Liver abscess	CTX	IPM	Cured with drainage and antimicrobial therapy
52	49/M	Liver cirrhosis with biliary obstruction	Post-PTBD	Cholangitis	CTX, AMK	IPM, AMK	Persistent fever after 2 days; treatment changed to imipenem and biliary decompression, cure
53	62/M	CBD cancer	Post-ERCP	Cholangitis	CIP, AMK	IPM	Persistent fever after 3 days; treatment changed to imipenem and biliary decompression, cured
54	45/F	Liver cirrhosis, HCC	Foley catheter	Peritonitis	CTX, AMK	CTX, AMK	Death on day 3 of treatment
59	68/F	Periampullary cancer	Post-ERCP	Cholangitis	CTX, AMK	IPM	Persistent fever after 5 days; treatment changed to imipenem, with cure
61	61/M	Liver cirrhosis, HCC	None	Unknown	CTX, AMK	IPM	Persistent fever after 2 days; treatment changed to imipenem, with cure

[a] Abbreviations: M, male; F, female; GB, gall bladder; IHD, intrahepatic duct; CBD, common bile duct; EST, endoscopic sphincterotomy; ERCP, endoscopic retrograde cholangiopancreaticography; PTBD, percutaneous transhepatic biliary drainage; DM, diabetes mellitus; ESRD, end-stage renal disease; HCC, hepatocellualr carcinoma; CTX, cefotaxime; CAZ, ceftazidime; AMK, amikacin; CIP, ciprofloxacin; IPM, imipenem.

β-lactamase types have been described. These include a North American cluster (MIR-1 and ACT-1), a Central and South American cluster (FOX-1 and FOX-2), an Asian cluster (CMY-1 and MOX-1), and a Mediterranean and Middle Eastern cluster (CMY-2, CMY-2b, LAT-1, and LAT-2) (21). Because few laboratories test for the production of the AmpC β-lactamase and even fewer laboratories test for induction, the occurrence of these enzymes in *K. pneumoniae* and *E. coli* isolates remains uncertain, as do their impacts on therapies and clinical outcomes.

In this study, we evaluated the clinical features and outcomes of bloodstream infections caused by AmpC-type β-lactamase-producing *K. pneumoniae* isolates. In addition, these patients were compared to those infected with ESBL-producing *K. pneumoniae* isolates. The clinical characteristics were similar to those caused by TEM- or SHV-related ESBL producers. Previous studies demonstrated that prior use of antibiotics (9, 12), the presence of a central venous catheter or a urinary catheter (22), and prior hospitalization and the use of extended-spectrum cephalosporins (9) are risk factors for infections caused by ESBL-producing *K. pneumoniae* or *E. coli* isolates.

Analysis of the clinical outcomes demonstrated high rates of failure of the initial antimicrobial therapy, especially cephalosporin treatment, in patients infected with AmpC β-lactamase-producing organisms, as was the case for patients infected with TEM- or SHV-related ESBL producers. Although the number of patients was small and the patients were not controlled for the severity of disease, the 30-day mortality rate was higher in the DHA-1 group than in the CMY-1-like group (46 and 14.3%, respectively). The mortality rate for the patients who received extended-spectrum cephalosporins as definitive treatment was assessed: all four patients in the DHA-1 group died, and one of three patients in the CMY-1-like group died. This result might be partially explained by the fact that β-lactamases had been induced by exposure to β-lactam antimicrobials in DHA-1-producing isolates, thus providing higher levels of resistance.

In the present study, all but three AmpC β-lactamase-producing isolates (one CMY-1 producer and two DHA-1 producers) were susceptible to cefepime. These results suggest that cefepime might be useful for the treatment of infections caused by AmpC β-lactamase-producing organisms (29). However, a report (15) has described the inoculum effect of cefepime or cefpirome in an AmpC producer, which lacked an outer membrane protein. In our study, all the patients treated with extended-spectrum cephalosporins received cefotaxime or ceftazidime, but not cefepime, since cefepime was not available at the Clinical Research Institute of Seoul National University Hospital until recently. Nevertheless, further studies to determine whether cefepime can be used for the treatment of infections caused by plasmid-mediated AmpC β-lactamase producers are needed.

It is difficult to distinguish organisms producing ESBLs from those producing plasmid-mediated AmpC β-lactamases by phenotypic susceptibility testing. Resistance to cefoxitin indicates the possibility of AmpC-mediated resistance but also indicates reduced outer membrane permeability. Some phenotypic tests are available to help distinguish the difference between cefoxitin-resistant non-AmpC producers and cefoxitin-

resistant AmpC producers. These include a three-dimensional test (26) and a new AmpC disk test (J. A. Black, E. S. Moland, and K. S. Thomson, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. D-534, 2002). In addition, the use of β-lactamase inhibitors can help identify possible AmpC-producing organisms (25). However, none of these tests are standardized and they are time-consuming, especially for a clinical microbiology laboratory handling large numbers of isolates.

Reporting of a susceptibility testing result for AmpC β-lactamase producers can be controversial if they show susceptibility to some extended-spectrum cephalosporins in vitro, because no standard method for the detection of these isolates is yet available. Moreover, there are few clinical data on the patients infected with these organisms. Although the number of patients in our study was small, the study has shown that the outcome of cephalosporin treatment for serious infections due to AmpC β-lactamase-producing *K. pneumoniae* isolates was poor, even for infections caused by apparently susceptible organisms. Therefore, a standard test for the detection of the plasmid-mediated AmpC enzyme and new breakpoints for extended-spectrum cephalosporins are urgently necessary.

To the best of our knowledge, this is the first description of the clinical features and outcomes of bloodstream infections caused by AmpC β-lactamase-producing *K. pneumoniae* isolates.

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