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# First Outbreak of Multidrug-Resistant *Klebsiella pneumoniae* Producing both SHV-12-Type Extended-Spectrum β-Lactamase and DHA-1-Type AmpC β-Lactamase at a Korean Hospital

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**Purpose:** Coexistence of different classes of β-lactamases in a single bacterial isolate may pose diagnostic and therapeutic challenges. We investigated a spread of *Klebsiella pneumoniae* isolates co-producing an AmpC β-lactamase and an extended-spectrum β-lactamase (ESBL) in a university hospital. **Materials and Methods:** Over a three-month period, a total of 11 *K. pneumoniae* isolates, which exhibited resistance to cefotaxime, aztreonam, and cefoxitin, were isolated. These isolates showed positive to ESBLs by double disk tests. Minimal inhibitory concentrations (MICs) were determined by broth microdilution testing. All isolates were examined by isoelectric focusing, PCR and sequence analysis to identify $bla_{SHV}$ and $bla_{DHA}$, and molecular typing by pulsed-field gel electrophoresis (PFGE). **Results:** All 11 isolates were highly resistant (MIC, ≥128 μg/ml) to ceftazidime, aztreonam, and cefoxitin, while they were susceptible (MIC, ≤2 μg/ml) to imipenem. The $bla_{SHV-12}$ and $bla_{DHA-1}$ genes were detected by PCR and sequence analysis. PFGE revealed a similar pattern in 10 of the 11 strains tested. **Conclusion:** This is the first outbreak report of *K. pneumoniae* in Korea which co-produced SHV-12 and DHA-1 β-lactamase, and we suggest a clonal spread of multidrug-resistant *K. pneumoniae* at a hospital.

**Key Words:** Outbreak, *Klebsiella pneumoniae*, SHV-12, DHA-1, Extended-spectrum β-lactamase, AmpC β-lactamase

## INTRODUCTION

β-Lactamase production is the predominant mechanism for resistance to β-lactams in Enterobacteriaceae. *Klebsiella pneumoniae* isolates are often multidrug-resistant and are the major hosts for extended-spectrum β-lactamases (ESBLs).[1] In particular, *K. pneumoniae* strains have also acquired plasmid-mediated AmpC enzymes. Unlike ESBLs, AmpCs are poorly inhibited by β-lactamase inhibitors and are less active against cefepime and cefpirome than ESBLs. AmpCs confer resistance to oxyimino- and 7-α-methoxy-cephalosporins.[2] The prevalence of ESBLs and plasmid-mediated AmpC β-lactamases in *K. pneumoniae* has been rising in Korea. Of the ESBLs and the acquired AmpC enzymes, SHV-12 and DHA-1 are the most prevalent and widespread in Korea, respectively.[3,4] Occurrence of ESBL and AmpC β-lactamase co-producing *K. pneumoniae* has been reported,[5,6] but outbreaks of the isolates are extremely rare.

Over a three-month period in 2004, *K. pneumoniae* isolates resistant to cefotaxime, ceftazidime, cefepime, aztreonam, and cefoxitin were isolated from the patients hospitalized at a university hospital in Korea. The aim of our study was to analyze clinical and molecular epidemiology of the multidrug-resistant *K. pneumoniae* isolates.

## MATERIALS AND METHODS

### Patients and bacterial isolates

From May to July 2004, a total of 11 non-duplicated isolates of *K. pneumoniae* that were resistant to cefotaxime, ceftazidime, aztreonam,

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and cefoxitin, were identified by Clinical Laboratory and Standards Institute (CLSI) disk diffusion method.[7] Ten of the 11 isolates were resistant to cefepime. All isolates were also resistant to aminoglycosides (amikacin, gentamicin, and tobramycin), ciprofloxacin, and trimethoprim-sulfamethoxazole. All isolates were suggested to produce ESBL based on the double disk test.[8] Table 1 shows the clinical background of patients for each isolate and their respective treatment outcomes. The first isolate from each source of patient was included in the study.

### Susceptibility testing and β-lactamase phenotype

Minimal inhibitory concentrations (MICs) were determined by the broth microdilution method according to the CLSI guidelines.[9] The antimicrobial agents used were piperacillin (Sigma, Steinheim, Germany), tazobactam (Yuhan, Seoul, Korea), cefoxitin (Sigma), ceftazidime (Glaxo SmithKline, Harlow, UK), aztreonam (Bristol-Myers Squibb, Prinston, NJ, USA), cefepime (Boryung, Seoul, Korea), and imipenem (Merk, West Point, PA, USA). ESBL and AmpC phenotypes were investigated by the CLSI ESBL confirmatory disk diffusion test[7] and the AmpC disk test,[10] respectively.

### Isoelectric focusing (IEF)

Crude β-lactamase preparations were obtained from the isolates by sonication and subjected to analytical IEF as described previously.[11] β-Lactamase activity was detected by overlaying the gels with 0.5 mM nitrocefin in 0.1 M phosphate buffer, pH 7.0.

### PCR amplification and DNA sequencing

Plasmids from the isolates were extracted by a rapid alkaline lysis procedure.[12] PCR with $bla_{DHA}$-[13,14] and $bla_{SHV}$-specific primers and subsequent sequencing of the PCR products were performed. An 1,071-bp fragment of the $bla_{SHV}$ gene was amplified with the primers SHV-F (5′-CGC CGG GTT ATT CTT ATT TG-3′) and SHV-R (5′-CCA CGT TTA TGG CGT TAC CT-3′). Sequence alignment and analysis were performed on-line using the BLAST program of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

### Pulsed-field gel electrophoresis (PFGE)

PFGE was carried out with a CHEF-DRII System (Bio-Rad, Hercules, CA, USA). *Xba*I-digested genomic DNA was prepared according to the instruction of Bio-Rad, and fragments were separated for 20 h at 6 V/cm at 14℃, with initial and final pulse times of 5 and 40 s, respectively. DNA bands were visualized by staining the gel with ethidium bromide and photographed.

## RESULTS

### Bacterial strains and clinical characteristics

*K. pneumoniae* isolates were first found to show resistance to cefotaxime, ceftazidime, cefepime, aztreonam, and cefoxitin, evidenced by disk diffusion test. During a particular time period, a total of 11 isolates showing very similar antibiogram were isolated. These results strongly suggested that there is coexistence of ESBL and AmpC in these isolates. The isolates were obtained from 11 inpatients at a hospital; 43 to 87 years old, 9 males and 2 females, from May to July 2004. Of 11 isolates, 6 isolates were recovered from sputum, 4 isolates from urine, and 1 isolate from a wound. Isepamicin and cefoperazone-sulbactam had most frequently been administered in 10 and 9 patients, respectively. Two of these 11 patients died, and the infection in the remaining 9 patients improved with therapy (Table 1).

### Antimicrobial susceptibility and β-lactamase phenotype

These 11 isolates showed very similar susceptibility profiles, characterized by elevated MICs ($\geq$128 μg/mL) of piperacillin, piperacillin-tazobactam, cefoxitin, ceftazidime, and aztreonam, while MICs of imipenem for all of them were $\leq$ 2 μg/mL. The MICs of cefepime varied among the strains isolated (Table 2). All the isolates yielded positive to AmpC disk tests for AmpCs, double disk tests and CLSI ESBL confirmatory disk for ESBLs.

**Table 1.** Clinical and PFGE Data of the Patients with SHV-12 and DHA-1 Co-Producing *K. pneumoniae* Isolates

| Patient No. | Age/ Sex | Date of | | Underlying disease | Previous medication | Outcome | Isolate No. | Source | Floor (Department) | PFGE type |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Admssion | Isolation | | | | | | | |
| 1 | 45/M | 06/05/04 | 10/05/04 | DM | AMK, CRO, ISP, PTZ | Expired | WJ10 | Sputum | 6th (IM) | Ia |
| 2 | 87/M | 13/05/04 | 10/06/04 | Intracranial hemorrhage | CFS, CFT, ISP | Improved | WJ20 | Sputum | 3rd (RM) | Ib |
| 3 | 64/M | 02/06/04 | 14/06/04 | DM | CFS, CLI, ISP, TEI | Improved | WJ23 | Sputum | 6th (IM) | Ia |
| 4 | 45/M | 10/05/04 | 25/05/04 | Cerebral infarct, COPD, SLE | CFS, ISP | Improved | WJ28 | Urine | 3rd (RM) | Ia |
| 5 | 69/F | 16/05/04 | 16/07/04 | Cerebral infarct, myocardial infarct, Asthma | AMK, CFS, CLI, FEP, FLM, ISP, NET, TEI | Improved | WJ30 | Sputum | MICU (IM) | Ia |
| 6 | 64/M | 29/01/04 | 06/07/04 | Aortic dissection | AMK, CFS, CRO, ISP, MPM, TEI | Expired | WJ31 | Wound | 11th (IM) | Ic |
| 7 | 62/M | 10/05/04 | 25/07/04 | Cerebellar cyst | CIP, CTX, ISP, TEI, VAN | Improved | WJ32 | Urine | 8th (NS) | Ic |
| 8 | 46/F | 16/04/04 | 10/05/04 | Intracranial hemorrhage | CFS, CIP, ISP, NET | Improved | WJ34 | Urine | 3th (RM) | II |
| 9 | 47/M | 06/05/04 | 18/05/04 | Chronic renal failure, COPD, DM | CFS, LEV | Improved | WJ1 | Sputum | 11th (IM) | Ia |
| 10 | 71/M | 18/05/04 | 22/05/04 | Pneumothorax, intracranial hemorrhage | CFS, ISP, IPM | Improved | WJ5 | Sputum | MICU (IM) | Ia |
| 11 | 43/M | 19/04/04 | 30/05/04 | Subdural hematoma, hemothorax | CFT, ISP, TEI | Improved | WJ7 | Urine | 8th (NS) | Id |

MICU, medical intensive care unit; IM, internal medicine; RM, rehabilitation medicine; NS, neurosurgery; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; SLE, systemic lupus erythematosus AMK, amikacin; CFT, cefotiam; CFS, cefoperazone-sulbactam; CIP, ciprofloxacin; CLI, clindamycin; CRO, ceftriaxone; CTX, cefotaxime; FEP, cefepime; FLM, flomoxef; IPM, imipenem; ISP, isepamicin; LEV, levofloxacin; MPM, meropenem; NET, netilmicin; PTZ, piperacillin-tazobactam; TEI, teicoplanin; VAN, vancomycin.

**Table 2.** MIC Distributions of the 11 SHV-12 and DHA-1 Co-Producing *K. pneumoniae* Isolates

| Antimicrobial agent | MIC (μg/mL) distribution (No. of isolates tested) |
|---|---|
| Piperacillin | ≥ 256 (11) |
| Piperacillin-tazobactam | ≥ 256 (11) |
| Cefoxitin | ≥ 128 (11) |
| Ceftazidime | ≥ 128 (11) |
| Cefepime | ≥ 128 (2), 64 (4), 32 (4), 8 (1) |
| Aztreonam | ≥ 128 (11) |
| Imipenem | 2 (2), ≤ 1 (10) |

## β-Lactamase genotype and PFGE profiles

These isolates had β-lactamase bands at isoelectric points of 7.6 and 7.8. The $bla_{SHV}$ and $bla_{DHA}$ alleles were detected by PCR, and the sequences were identical to those of $bla_{SHV-12}$ and $bla_{DHA-1}$. The PFGE analysis identified one major profile with four subtypes (Ia, Ib, Ic, and Id) (Table 1, Fig. 1).

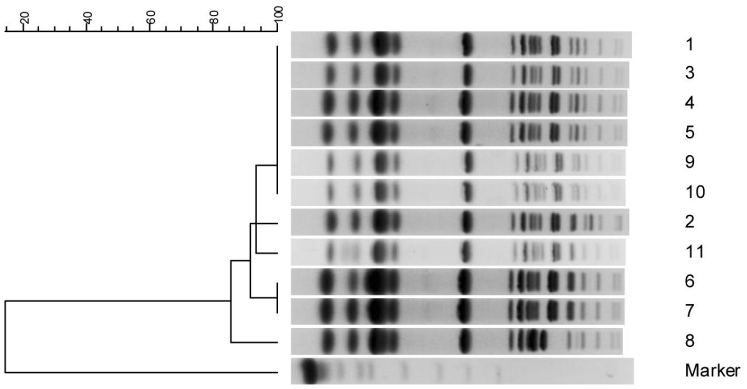


**Fig. 1.** PFGE dendrograms of 11 SHV-12 and DHA-1 co-producing *K. pneumoniae* isolates. The scale indicates the percent of genetic similarity. The molecular size marker includes yeast chromosomes, *Saccharomyces cerevisiae* (Bio-Rad Laboratories, Hercules, CA, USA).

## DISCUSSION

Expanded-spectrum cephalosporin-resistant organisms due to ESBL production have been reported in many countries.[15] ESBL production among *K. pneumoniae* isolates in Korea was 28.4% during 2002 - 2003,[16] which is significantly higher than the United States, Taiwan, or Hong Kong.[1,6] SHV-12, SHV-2a, and TEM-52 were common in the late 1990s in Korea.[17] In 2002, an SHV-12-like enzyme was prevalent, but SHV-2a and TEM-52 were found in a few isolates in Korea.[18] DHA-1, an inducible plasmid-mediated AmpC that was first identified in *Salmonella enteritidis* isolated in Saudi Arabia in 1992,[19] has been reported in several other countries.[5,20] DHA-1 enzyme is the most frequently identified among the AmpC β-lactamase-producing *K. pneumoniae* isolates in Korea.[4] Genes encoding both ESBLs and plasmid-mediated AmpC β-lactamases are usually located on large multidrug resistance plasmids. Therefore, both types of enzymes are typically associated with resistance to multiple antibiotics, thus leaving few therapeutic options.[21]

In our study, the isolates that co-produced SHV-12 and DHA-1 enzymes showed high MICs of ceftazidime, cefepime, aztreonam, and cefoxitin. Four (8.7%) of 46 plasmid-mediated AmpC-producing *K. pneumoniae* isolates also had an ESBL at a Korean hospital.[14] Furthermore, the outbreak of *K. pneumoniae* isolates co-producing SHV-2a ESBL and DHA-1 AmpC has been reported in Korea.[22] The nosocomial infections caused by DHA-1 and SHV-12 β-lactamase producers occurred in Japan.[23] However, this is the first report of its occurrence in Korea.

In this study, *K. pneumoniae* strains were recovered from patients with a mean age of 58 years. These data agree with a previous study showing usually older with ESBL.[24] However, the mean age (55 years old) of the other 164 patients who were infected with broad-spectrum cephalosporins-susceptible *K. pneumoniae* was older in age (data not shown). The extended-spectrum cephalosporins had preferentially been used as first-line drugs at the hospital. Among 11 patients, all of them had received the extended-spectrum cephalosporins (9 had received cefoperazone-sulbactam). The emergence of multi-resistant organisms, characterized by a heavy use of antimicrobial agents, may provide nosocomial transmission.[25] In our present study, the emergence of *K. pneumoniae* isolates co-producing SHV-12 and DHA-1 could be correlated with heavy use of extended-spectrum cephalosporins. PFGE of *Xba*I-digested genomic DNA revealed that 10 out of the 11 isolates in this study, likely corresponded to a single clone, whereas one isolate (WJ34) was not clonally related.

In conclusion, the first outbreak caused by *K. pneumoniae* isolates co-producing SHV-12 ESBL and DHA-1 AmpC β-lactamase occurred at a university hospital in Korea. The spreads was caused by an endemic clone of the resistance gene.

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