원 저

Failure of Cephalosporin Treatment for Bloodstream Infection Caused by Apparently Susceptible *Klebsiella pneumoniae* which Produced DHA-1 β-Lactamase Induced by Clavulanic Acid

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유도성 DHA-1 β-Lactamase를 생성하는 폐렴 간균에 의한 균혈증이 발생한 환자에게 투여된 감수성 있는 Cephalosporin의 치료실패 경험

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목 적 : AmpC beta-lactamase를 생성하는 *Klebsiella pneumoniae* 균주가 보고되고 있으나 이에 대한 적절한 치료 항균제 선택에 제한이 있다. Cephalosporin 내성이 유도되는 형질을 지닌 *K. pneumoniae*에 의한 균혈증이 2례 발생하였다. 혈액에서 분리된 이 균주들의 내성 기전을 확인하고 항균제 감수성의 특성을 알아보고자 하였다.
방 법 : NCCLS 지침에 따라 항균제 감수성 검사와 ESBL 확인 검사를 시행하였다. Isoelectric focusing과 PCR을 이용하여 beta-lactamase characterization을 시행하였다.
결 과 : 균주들은 DHA-1 AmpC beta-lactamase를 생성하고 있었고 clavulanic acid에 의해 cephalosporin 내성이 유도되었다. 항균제 감수성 검사 결과 내성이 아닌 광범위 cephalosporin을 투여받았으나 환자들은 치료실패를 경험하였다.
결 론 : 이러한 결과들은 DHA-1을 생성하는 *K. pneumoniae*에 의한 중증 감염증에 광범위 cephalosporin을 투여하는 것은 감수성이 있는 항균제라 하더라도 적절한 항균제 선택이 아닐 수 있음을 시사한다

Key Words : Klebsiella, β-lactamase, Cephalosporin resistance

INTRODUCTION

Many plasmid-mediated AmpC enzymes, such as CMY-type β-lactamases, have been found in bacterial species, such as *Klebsiella pneumoniae*, which naturally lack chromosomal AmpC β-lactamases (1). Unlike chromosome-mediated AmpC, plasmid-mediated AmpC enzymes are almost always expressed constitutively (1). Plasmid-mediated inducible β-lactamases are extremely rare. DHA-1 from a clinical isolate of Salmonella enteritica serovar Enteritidis from Saudi Arabia was the first identified plasmid-encoded inducible cephalosporinase (2). Inducible DHA-1 β-lactamase producing *K. pneumoniae* isolates have also been

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reported in Taiwan (3). Studies on determining the therapeutic success or failure of third-generation cephalosporins in treating infections with plasmid-mediated inducible AmpC producers, such as DHA-1-producing *K. pneumoniae* isolates, are lacking. Therefore, whether such *K. pneumoniae* strains, like gram-negative organisms that naturally produce inducible AmpC enzymes, should also be reported as resistant to all third-generation cephalosporins deserves further investigation. Recently, we experienced two cases of bloodstream infections cause by *K. pneumoniae* strains, which showed an unusual cefotaxime and ceftazidime resistance phenotype by standard confirmatory testing for the detection of extended-spectrum β-lactamases (ESBL). Thus, a retrospective analysis was carried out to characterize these isolates and their clinical features.

CASE REPORTS

Case 1

A 54-year-old woman with advanced liver cirrhosis developed spontaneous bacterial peritonitis in hospital. She had indwelling urinary catheter and was undergoing hemodialysis for acute renal failure. Ascitic fluid contained >1,000 WBCs/mm^3, of which 79% were neutrophils. A blood culture yielded *K. pneumoniae* (designated as isolate A1) (Table 1). The patient was treated empirically with intravenous cefotaxime, but despite this therapy she developed intractable septic shock and died 3 days after the initiation of therapy.

Table 1. Antimicrobial susceptibilities of *Klebsiella pneumoniae* strains isolated

Antibiotics	A1	A2
Cefotaxime	I	S
Ceftizoxime	I	S
Ceftriaxone	S	S
Ceftazidime	R	I
Cefpodoxime	R	R
Cefepime	S	S
Cefoxitin	R	R
Cefotetan	R	R
Aztreonam	S	S
Imipenem	S	S
Ciprofloxacin	I	S
Amikacin	R	R
Gentamicin	R	R
Tobramycin	R	R
Amoxicillin/clavulanic acid	R	R
Piperacillin/tazobactam	S	S

Disk-diffusion method by NCCLS performance standards

Case 2

A 17-year-old man with aplastic anemia developed neutropenic fever in hospital. He had undergone allogenic bone marrow transplantation for aplastic anemia. His absolute neutrophil count was zero, and a blood culture yielded *K. pneumoniae* (designated as isolate A2) (Table 1). The patient was treated empirically with intravenous ceftizoxime and amikacin, but despite this therapy he had persistent fever. His antimicrobial therapy was changed to imipenem and ciprofloxacin after 7 days, but he died on the 22nd day of treatment.

MATERIALS AND METHODS

1. Bacterial strains and Antibiotic susceptibility testing

Species identification was carried out using VITEK-GNI CARDS by standard methods (4). The antibiotic susceptibility of each isolate was determined by the disk diffusion method, employing the criteria of the National Committee for Clinical Laboratory Standards (5). Minimal inhibitory concentrations (MICs) of antibiotics were determined by using the broth microdilution method with inocula that differed 100-fold in density. The inocula were comprised of approximately 10^5 (standard inoculum) and 10^7 CFU/ml suspended in Mueller-Hinton broth (Becton Dickinson, Sparks, USA). The standard-inoculum tests were based on NCCLS methodology (6). An inoculum effect was defined as an eightfold or greater increase in MIC on testing with the higher inoculum (7).

2. ESBL confirmatory tests and induction testing of AmpC β-lactamases

ESBL production was determined by the disk diffusion method and the broth microdilution method according to the NCCLS performance standards (5). The inducibility of AmpC β-lactamases was examined as previously described with cefoxitin, cefotaxime, and ceftazidime disks on Mueller-Hinton agar (Becton

Dickinson, Sparks, USA) (8). Clavulanic acid and cefoxitin were used as inducing agents. Two control organisms, *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603, were inoculated into each set of tests for quality control purposes.

3. Analytical isoelectric focusing

Isoelectric focusing (IEF) was performed using sonicated extracts, using the method of Mathew et al. and a Mini IEF cell system (Bio-Rad, Hercules, CA) (9). Enzyme activities were detected by overlaying the gel with 0.5 mM nitrocefin in 0.1 M phosphate buffer, pH 7.0.

4. PCR for the β-lactamase gene

The DHA-1-related gene from clinical isolates was amplified by PCR. The primers used for the amplification were; DHA-1U (5′-CACACGGAAGGTTAATTCTGA-3′) and DHA-1L (5′-CGGTTATACGGCTGAACCTG-3′), corresponding 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℃; 35 cycles of 30 sec at 94℃, 45 sec at 57℃, and 1 min at 72℃; and a final extension of 8 min at 72℃. The amplified product was sequenced using the primers; DHA-1U, DHA-1L, and DHA-2U (5′-AAGAGATGGCGCTGAATGAT-3′).

RESULTS

The results of the susceptibility tests are shown in Table 1. Both strains (A1, A2) exhibited resistance to cefoxitin and amoxicillin-clavulanic acid. In the standard ESBL confirmatory test, the zone diameters for cefotaxime and ceftazidime in combination with clavulanic acid decreased, compared with those obtained when cefotaxime and ceftazidime were tested alone (Table 2). In the presence of clavulanic acid, increases in the MICs of cefotaxime and ceftazidime were also noted, suggesting the presence of β-lactamases induced by clavulanic acid (Table 2). Inducibility of the β-lactamases was further recognized by the disk antagonism test, which demonstrated blunting of the cephalosporin disks adjacent to the cefoxitin and clavulanic acid disks.

A1 and A2 strains were analyzed for ESBL characterization. IEF demonstrated that both strains produced β-lactamases suggestive of DHA-1 (isoelectric point [pI], 7.7), TEM-1 (pI, 5.4), and SHV-1 (pI, 7.6). PCR amplifications for bla_{DHA-1} were performed for two strains, and both strains were found to be positive by DHA-1-specific PCR. The nucleotide sequence of the amplified product was confirmed as DHA-1.

In broth microdilution antimicrobial susceptibility testing, the MICs for ceftazidime were 256 and 128 μg/ml, respectively. In addition, the MICs for cefotaxime were 16 and 8 μg/ml, respectively. However, both strains showed an inoculum effect in the MIC testing for cefotaxime, that is, the MIC on testing with the higher inoculum increased more than eight-fold (Table 2).

DISCUSSION

In this report, we have showed that a suboptimal clinical outcome occurs when cephalosporin treatment is used for bacteremia caused by inducible DHA-1 β-lactamase-producing *K. pneumoniae*, which may not

Table 2. Cephalosporin resistance induced by clavulanic acid in the ESBL confirmatory test and inoculum effect in MIC testing

Isolate	ESBL confirmatory test (inhibition zone, mm)				ESBL confirmatory test (MIC, g/ml)				MIC (g/ml) of agent at inocula of 10^5 and 10^7 CFU/ml			
									CTX		CAZ	
	CTX	CTX+CLA	CAZ	CAZ+CLA	CTX	CTX+CLA	CAZ	CAZ+CLA	10^5	10^7	10^5	10^7
A1	20	12	12	8	16	>256	256	>256	16	>256	256	>256
A2	23	10	15	10	8	>256	128	>256	8	>256	128	>256

ESBL confirmatory test using NCCLS performance standards
Abbreviations : CTX, cefotaxime; CAZ, ceftazidime; CLA, clavulanic acid

appear to be resistant on the basis of cephalosporin MICs of 8 to 16 μg/ml (the conventional MIC cut-off value). Both patients in this report had received extended-spectrum cephalosporin (susceptible in vitro) and experienced treatment failure.

For serious infections caused by ESBL-producing organisms, treatment failure has been documented in patients treated with cephalosporins, to which were organisms were found to be "susceptible" or "intermediate" in vitro (10). The outcome of cephalosporin treatment for serious infections due to ESBL-producing *K. pneumoniae* was poor, even in apparently susceptible organisms (10). The notion, that ESBL-producing organisms with cephalosporin MICs in the susceptible range may not be truly susceptible (when serious infections are considered) arose from in vitro studies of the inoculum effect and from animal studies (7, 10).

Similarly, DHA-1 β-lactamase-producing *K. pneumoniae* isolates would not have been reported as being resistant to all third-generation cephalosporins, as is the case for ESBL-producing organisms (3). However, after induction by clavulanic acid, these isolates showed reduced susceptibilities to these agents. Thus, third-generation cephalosporins might not provide an optimal therapeutic option in inducible DHA-1 β-lactamase-producing *K. pneumoniae* infections, even in apparently susceptible strains. Furthermore, the "inoculum effect" with cephalosporins was also demonstrated in these strains.

The drugs of choice for the treatment of infections with such organisms are also undetermined. All these isolates, which produced DHA-1 enzyme, remained susceptible to cefepime and imipenem even in the presence of clavulanic acid (3). Thus, fourth-generation cephalosporins and carbapenems might be better choices for the treatment of infections caused by DHA-1 producers; however, no clinical data is available.

In conclusion, sporadic bloodstream infections caused by DHA-1 β-lactamase-producing *K. pneumoniae* strains were found in a University Hospital setting in South Korea. The patients concerned experienced treatment failure when treated with extended-spectrum cephalosporin, and this cephalosporin resistance was induced by clavulanic acid. Thus extended-spectrum cephalosporins might not provide an optimal therapeutic option in inducible DHA-1-producing *K. pneumoniae* infection, even in apparently susceptible strains.

ABSTRACT

Backgroud : The therapeutic option is limited for the infections caused by organisms producing plasmid-mediated AmpC beta-lactamases, increasingly identified worldwide. Two sporadic patients with bacteremia caused by *K. pneumoniae* possessing an unusual inducible β-lactam resistant phenotype were found in a university hospital.

Materials & Methods : We conducted antibiotic susceptibility test according to NCCLS guideline. Also, we characterized β-lactamase by isoelectric focusing.

Results : DHA-1 gene conferred the resistant phenotype. The patients had experienced treatment failure when treated with extended-spectrum cephalosporin. For the isolates the cephalosporin resistance was induced by clavulanic acid (and cefoxitin).

Conclusion : Theses results suggest that the extended-spectrum cephalosporins might not provide optimal therapeutic option for inducible DHA-1-producing *K. pneumoniae* infection, even when the pathogens are susceptible in vitro.

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