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Extended-Spectrum β-lactamase Genes Acquired Multidrug-Resistant *Klebsiella pneumoniae* in a Dog and Its Owner

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Abstract : A 2-year-old female Pomeranian dog was referred with multiple pelvic fractures. The surgical correction was performed for the fractures. However, after the surgery, purulent exudation was occurred in the surgical site. Antibiotic susceptibility test revealed that the isolated bacteria are resistant to penicillins, cephalosporins, aminoglycosides, quinolones, and trimethoprim/sulfamethoxazole. Bacterial identification and extended-spectrum β-lactamase (ESBL) confirming test indicated that the isolated bacteriae is ESBL-producing *Klebsiella pneumoniae*. Minimum inhibitory concentration (MIC) and maximum bactericidal concentration (MBC) tests revealed that meropenem, one of carbapenems, is the only effective antibiotic. The patient was treated with meropenem for 5 days. After 10 days, the exudation was disappeared and the infection was cured. The molecular typing of the ESBL revealed that TEM-1 ESBL is present in the bacteria isolated from the patient. The bacteria isolated from the owner's palm also revealed that TEM-1 and SHV-1 ESBLs are present.

Key words **:** *Klebsiella pneuminiae*, extended-spectrum β-lactamase, TEM, SHV, meropenem.

Introduction

According to the chemical structure, β-lactam antibiotics can be divided into four different groups, consisting of penicillins, cephalosporins, carbapenems, and monobactams (7). The antibiotics have the β-lactam ring in common, but secondary ring structures are different among each group (30). β-lactamase hydrolyzes the β-lactam ring, so the enzyme protects the microorganisms against the lethal effects of β-lactam antibiotics (11). Based on the amino acid sequences, β-lactamase can be divided into four different molecular groups consisting of the Ambler classes A, B, C, and D (3). Among them, class A β-lactamases, which are represented by the plasmid-encoded TEM and SHV families, are the most common molecular group of β-lactamases produced by the *Enterobacteriaceae*, such as *Escherichia coli* and *Klebsiella pneumoniae* (30).

In the early 1980s, extended-spectrum cephalosporins were approved to use in clinic and bacteria resistant to these cephalosporins began to appear (30). The β-lactamases produced by plasmid-encoded genes confer the resistance to these cephalosporins (5), so it called as extended-spectrum β-lactamase (ESBL). The enzymes also confer the resistance to broad-spectrum penicillins, narrow-spectrum cephalosporins, and monobactams (2,16). Because of rapid transmission of the resistant gene, the human infections by ESBL-producing bacteria have been increasing worldwide including Korea (4,10).

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This report describes a case of the infection by ESBL-producing *K. pneumoniae* in a dog. We also found that same type of β-lactamase is also present on the owner.

Case

A 2-year-old female Pomeranian dog was referred to Veterinary Medical Center of Chungbuk National University after car accident (Fig 1). After the accident, the patient was bright, alert and responsive, but couldn't stand up. On physical examination, external hemorrhage and subcutaneous bleeding were

Fig 1. The photograph of the patient.

found around anus and external genitalia. Inguinal hernia, loss of anal reflex and deep pain of left hindlimb, and involuntary defecation were also found. On palpation, the patient was nervous, especially when the hip was palpated.

On radiography, multiple pelvic fractures were shown. Ultrasonography revealed that the inguinal hernia is caused by the translocation of intestinal segments. CBC and serum biochemistry showed neutropenia, elevation of liver enzyme (AST, ALT) and BUN. Thus, the patient was diagnosed as multiple pelvic fractures by car accident. The patient was hospitalized and was treated with crystalloid fluid and prophylactic antibiotics (ampicillin and sulbactam, Bacillin®, Samsung Pharm, Korea).

Surgical correction for the inguinal hernia and pelvic fracture was performed after 3 days and 7 days, respectively. For correction of pelvic fracture, surgical approach was performed through the skin over the iliac crest. After the surgery, the patient was treated with broad-spectrum antibiotics (Bacillin®, Korea) and analgesics (tramadol HCl, Tamadoll®, Dongkwang Pharm, Korea). However, after 13 days, purulent exudation was found in the subcutaneous areas, which were incised for correction of the pelvic fracture. On microscopy, many rod-shaped bacteria and degenerative neutrophils were found. The exudation was collected using sterile swab and antibiotic susceptibility test was performed by disc diffusion method using the discs of amikacin, ampicillin, amoxicillin/clavulanic acid, ampicilin/sulbactam, cefaclor, cefazolin, cefotaxime, ceftriaxone, cephalothin, chloramphenicol, enrofloxacin, erythromycin, gentamicin, tetracycline, and trimethoprim/sulfamethoxazole. However, all antibiotics were resistant. Bacterial identification using remel Rapid^{TM} one kit (remel, Lenexa, USA) showed that the infection was caused by *K. pneumoniae*.

According to the guideline of Current Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards (CLSI/NCCLS) (19), the confirming test for the ESBL-producing bacteria was performed using the discs of ceftazidime, ceftazidime/clavulanic acid, cefotaxime, and cefotaxime/clavulanic acid. The test showed that the discs that include the clavulanic acid make an inhibition zone larger than 5 mm (Fig 2). In PCR and direct sequencing for identifying the presence of β-lactamase genes (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}) using the primer pairs reported previously (28), bla_{TEM-1} gene was identified (Fig 3). Thus, the causative bacteria were identified as ESBL-producing *K. pneumoniae*.

Minimum inhibitory concentration (MIC) and maximum bactericidal concentration (MBC) for carbapenems (imipenem, ertapenem, and meropenem) and monobactam (aztreonam) were examined. The tests revealed that meropenem is an only effective antibiotic (Fig 4). The drainage and debridement of the surgical site were performed and the patient was retreated with meropenem for 5 days (12 mg/kg, SC, TID). Consequently, after 10 days, the purulent exudation was disappeared.

To clarify the transmission of the ESBL gene between the patient and its owner, the samples were collected from two owners' palms and nostrils using sterile swabs. Bacterial identification and DNA analyses for ESBL genes revealed that *K. pneumoniae*, that have two ESBL genes (bla_{TEM-1} and bla_{SHV-1}), was isolated from one owner's palm.

Discussion

The resistance to the extended-spectrum β-lactam antibiotics due to β-lactamase has been emerged quickly. Today, over 150 different ESBLs have been described (2). These β-lactamases have been found worldwide in many different genera of *Enterobacteriaceae* and *Pseudomonas aeruginosa* (2). The confirming test for ESBL-producing bacteria is important to select a proper antibiotic, however its laboratory diagnosis is not easy. Currently, CLSI/NCCLS has recommended that more than one of the 5 indicators (cefpodoxime, ceftazidime, cefotaxime, aztreonam, and ceftriaxone) should be used to con-

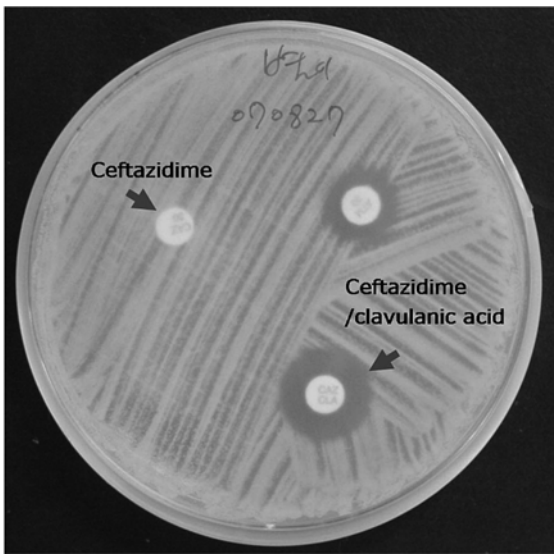

Fig 2. ESBL confirming test using ceftazidime with or without clavulanic acid. In presence of clavulanic acid, ceftazidime made an inhibition zone more than 5 mm.

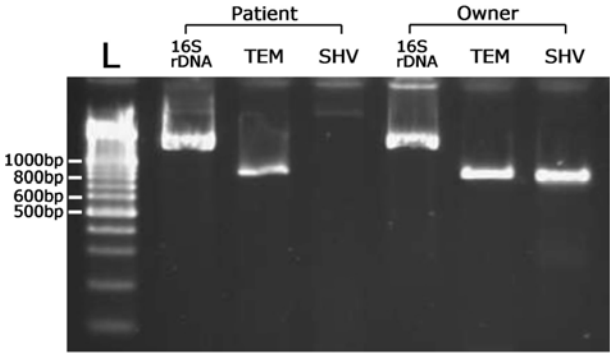

Fig 3. Molecular typing of the ESBL of the bacteria isolated from the patient and its owner's palm. 16S ribosomal RNA gene was used to demonstrate the presence of the bacterial DNA (lane 2 and 5).

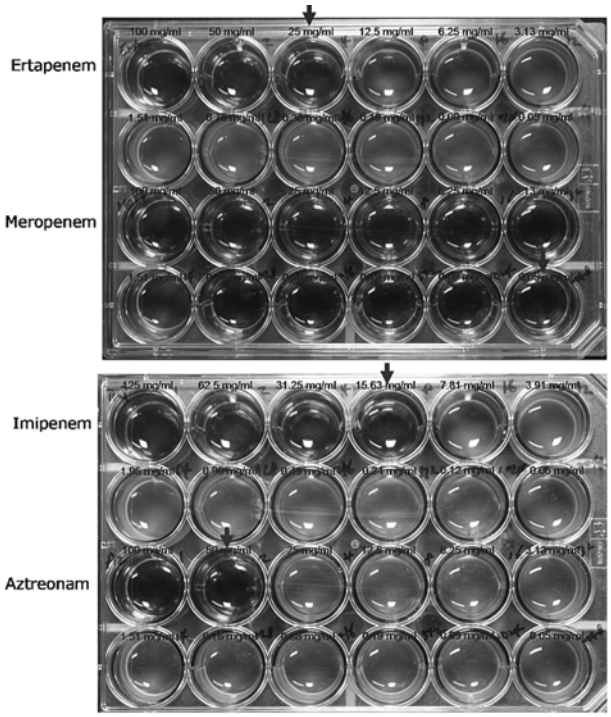

Fig 4. MIC concentrations of ertapenem, meropenem, imipenem, and aztreonam. Arrows indicate the well showing the MIC concentration.

firm for expression of ESBL (19). Also, CLSI/NCCLS suggests the use of cefotaxime or ceftazidime discs with and without clavulanic acid for phenotypic confirmation of the presence of ESBL. If an inhibition zone is increased more than 5 mm by cefotaxime or ceftazidime in the presence of clavulanic acid, the bacteria can be considered as ESBL-producing bacteria. In our report, we used the CLSI/NCCLS guideline for confirming whether the bacteria isolated from the patient have the ESBL or not. The isolated bacteria showed the resistance to ceftriaxone, cefotaxime, ceftazidime, and aztreonam, however an inhibition zone by ceftazidime was increased more than 5 mm in the presence of clavulanic acid. Thus, we confirmed that the isolated bacteria produce the ESBL.

Interestingly, the isolated bacteria from the patient also revealed the resistance to aminoglycosides, quinolone, and trimethoprim/sulfamethoxazole besides penicillins and cephalosporins. Although the cause is unknown, several reports indicate the high rates of co-resistance to aminoglycosides, quinolones, trimethoprime/sulfamethoxazole among ESBL-producing bacteria (6,15,29).

Due to the stability against the ESBL and consistently low MIC, carbapenems have been recommended as the drugs of choice for ESBL-producing bacteria (6,21-25,27). However, carbapenems can cause several adverse effects (1,18,20), its therapeutic dosage and applications have been strictly limited. In addition, in our report, we examined that meropenem is the only susceptible antibiotic. This finding indicates that antibiotic susceptibility test should be needed to perform, even in carbapenems for the treatment of ESBL-producing bacteria.

Most ESBLs can be divided into three groups based on the molecular structure; TEM, SHV, and CTX-M type (26). TEM-1 and SHV-1 is most commonly encountered β-lactamases in gram-negative bacteria (2). In Korea, TEM-52 and SHV-12 ESBLs have been reported as most common type in human (12). Currently, TEM-52 is considered as a variety of TEM-1 by amino acid substitution of the enzyme (8). In our report, the bacteria isolated from the patient and its owner revealed the TEM-1 type ESBLs in common. In addition, the bacteria isolated from the owner revealed the SHV-1 type ESBL. This finding indicates that the resistance gene may be transmitted from one to another. In order to confirm the cross-transmission of the resistance genes cassettes between the patient and owner, we cloned an integron in the bacteria isolated from the patient and owner. Integron is placed in plasmids or transposon. It possess two conserved segment separated by a variable region which includes integrated antibiotic resistance genes or cassettes (14). Multidrug resistance in *Enterobacteriaceae* is strongly associated with integrons (9,13,17). However, unfortunately, we failed to clone integron in the bacteria isolated from the owner.

In conclusion, ESBL-producing bacteria is increasing worldwide including Korea. Contacts or several instruments transmit the ESBL genes rapidly. Therefore, in cases that represent refractory bacterial infections, antibiotic susceptibility test should be performed, and veterinarians should caution its transmission.

Acknowledgements

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개와 보호자에서 Extended-Spectrum β-lactamase 유전자를 획득한 다약제내성 *Klebsiella pneumoniae*

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요 약 : 2년령 암컷 포메라니안이 교통사고로 인한 골반골절로 내원하여 골절 교정수술을 받았다. 그러나 수술 후 술부에서 감염으로 인한 화농성 삼출물이 발생하였고, 분리한 세균의 항생제 감수성 검사 결과 penicillins, cephalosporins, aminoglycosides, quinolones, 그리고 trimethoprim/sulfamethoxazole에 내성이 관찰되었다. 분리한 세균의 동정 및 extended-spectrum β-lactamase (ESBL) 확진시험을 통해 ESBL 생성 *Klebsiella pneumoniae*임을 확인하였다. 치료를 위한 Carbapenem계 항생제의 감수성 시험 결과에 따라 meropenem을 선택하여 치료에 이용하였다. 분리된 세균에서 ESBL 유전자의 분자생물학적 검사 결과 TEM-1 ESBL 유전자가 있음을 확인하였으며, 보호자의 손바닥에서 분리된 세균에서도 TEM-1, SHV-1 ESBL 유전자가 검출되었다.

주요어 : *Klebsiella pneuminiae*, extended-spectrum β-lactamase, TEM, SHV, meropenem.