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CTX-M-15 Extended-Spectrum β-Lactamase 생성 *Salmonella enterica* Serotype Enteritidis에 의한 소아 위장염 증례보고

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Case Report of Pediatric Gastroenteritis Due to CTX-M-15 Extended-Spectrum β-Lactamase-Producing *Salmonella enterica* Serotype Enteritidis

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A clinical isolate of *Salmonella enterica* serotype Enteritidis in Korea was found to produce the extended-spectrum β-lactamase CTX-M-15. The isolate was recovered in 2008 from the stool of a 3-yr-old boy with gastroenteritis. This isolate was found to be resistant to multiple drugs, including ampicillin, piperacillin, cefotaxime, ceftazidime, cefepime, and aztreonam. The resistance to cefotaxime was transferred by conjugation to recipient *Escherichia coli* J53. The patient was eventually successfully treated with trimethoprim-sulfamethoxazole. This is the first report of the $bla_{CTX\text{-}M\text{-}15}$ gene in *S. enterica* serotype Enteritidis in Korea. *(Korean J Lab Med 2009;29:461-4)*

Key Words : *Salmonella enterica, CTX-M-15, Korea*

INTRODUCTION

Nontyphoidal *Salmonella* are among the most important enteric pathogens, even in developed countries. However, although *Salmonella* spp. are usually susceptible to many antimicrobial agents, a recent increase in resistance has become a cause for concern [1]. A recent study of multidrug-resistant *Salmonella* spp. in Kuwait and the United Arab Emirates reported a 5-fold increase in the rate of resistance to ceftriaxone and cefotaxime [2]. Extended-spectrum β-lactamases (ESBLs) are rare in *Salmonella enterica* strains compared with other *Enterobacteriaceae*, such as *Escherichia coli* and *Klebsiella pneumoniae*. However, there have been an increasing number of reports on ESBL-containing *Salmonella* strains throughout the world [3]. The ESBLs reported in *Salmonella* spp. include derivatives of the TEM, SHV, and CTX-M families [4]. CTX-M-type β-lactamases form a growing family that comprises at least 40 enzymes [5].

We report here for the first time the identification of a CTX-M-15-ESBL-producing *S. enterica* serotype Enteritidis isolate associated with pediatric gastroenteritis in Korea.

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CASE REPORT

In September 2008, a 3-yr-old boy with fever and watery diarrhea was admitted to the pediatric ward of a university hospital in Seoul, Korea. His medical history was unremarkable. Physical examination revealed a pulse rate of 130/min, a respiratory rate of 24/min, and a body temperature of 39℃. His bowel sounds were normal. The initial laboratory findings revealed a WBC of 11,110/μL (neutrophils, 81%), a CRP of 54.7 mg/L, an AST of 64 IU/L, and an ALT of 22 IU/L. The patient was initially treated with a combination of ampicillin-sulbactam and netilmicin. A stool culture performed during the first admission period isolated *S. enterica* serotype Enteritidis. The isolate was serotyped using anti-O, -Vi, and -H sera at the Seoul Metropolitan Government Research Institute of Public Health and Environment. Although no blood cultures were performed initially, this practice was subsequently changed after isolation of *S. enterica* from the stool specimen. The patient was cured after administration of trimethoprim-sulfamethoxazole.

Antimicrobial susceptibility testing was performed using the Vitek 2 system (bioMerieux, Inc., Hazelwood, MO, USA). The isolate was found to be resistant to ampicillin, piperacillin, cefotaxime, ceftazidime, cefepime, aztreonam, and tetracycline, but remained susceptible to amoxicillin-clavulanate, piperacillin-tazobactam, imipenem, meropenem, ciprofloxacin, and trimethoprim-sulfamethoxazole. E-test strips (AB BIODISK, Solna, Sweden) were used to determine the minimal inhibitory concentrations (MICs) of cefotaxime, ceftazidime, and aztreonam, which were >256 μg/mL, 64 μg/mL, and >256 μg/mL, respectively. Confirmation of the presence of a *bla*$_{CTX-M}$ β-lactamase gene was obtained by PCR using the primers CTX-M universal F and R. Specific primers were then designed for CTX-M-1 and CTX-M-9 in order to amplify the complete genes [6]. Sequencing of the entire coding region of *bla*$_{CTX-M}$ revealed the presence of CTX-M-15.

Conjugation experiments were performed as described previously using sodium azide-resistant *E. coli* J53 as a recipient [7]. Mueller-Hinton agar plates supplemented with 2 μg/mL cefotaxime and 150 μg/mL sodium azide were used to select transconjugants. We accordingly established that cefotaxime resistance was transferred by conjugation from the clinical isolate to the recipient strain.

After the administration of trimethoprim-sulfamethoxazole for 3 days, a follow-up stool culture was negative for *Salmonella*, and the patient was discharged with outpatient follow-up.

DISCUSSION

A majority of the ESBL-producing *Salmonella* isolates have been either *S. enterica* serotype Enteritidis [8] or *S. enterica* serotype Typhimurium [9]. *S. enterica* serotype Enteritidis is the most common serotype in Korea. Of the 3,108 *Salmonella* isolates serotyped in Korea between 1995 and 1997, 1,386 were Enteritidis [10]. A few isolates of TEM-25 and CTX-M-14 ESBL-producing *Salmonella* spp. have been reported in Korea [11, 12]; however, prior to the present case, there had been no reports of ESBL production in *S. enterica* serotype Enteritidis.

Among the six groups of CTX-M enzymes, the CTX-M-1, -2, and -9 groups have been reported in serotypes Typhimurium, Enteritidis, Virchow, Kentucky, and Kedougou [5, 13-15]. CTX-M-15 ESBL was first reported in *E. coli*, *K. pneumoniae*, and *Enterobacter aerogenes* isolates in 2000-2001 in India [16]. CTX-M-15 has been detected in *Salmonella* isolates from various countries, including France, Kuwait, the United Arab Emirates, South Africa, Senegal, and Algeria [2, 13, 15, 17]. In this report, we describe a pediatric *S. enterica* serotype Enteritidis isolate that harbored the ESBL CTX-M-15, which is the first time this enzyme has been found in Korea.

CTX-M-type β-lactamases are generally much more active against cefotaxime than against ceftazidime and aztreonam [18]. However, there has been a recent report of CTX-M-15 exhibiting high enzyme activity against ceftazidime as well as cefotaxime [19]. The isolate described in this study similarly exhibited a high-level resistance to cefotaxime (MIC, >256 μg/mL), ceftazidime (MIC, 64 μg/mL), and aztreonam (MIC, >256 μg/mL), as determined by E tests.

Since ESBL genes reside on plasmids or integrons, they have the potential to spread to other organisms [13, 14]. And indeed, in this study, we successfully demonstrated the transfer of resistance by conjugation, suggesting that *bla*$_{CTX-M-15}$ is located on a self-transferable plasmid.

Antimicrobial treatment is generally not required in cases of nontyphoidal *Salmonella* gastroenteritis; however, treatment with trimethoprim-sulfamethoxazole or ceftriaxone is recommended if a patient is under 6-months or over 50-yr-old, or has an underlying disease [20].

In conclusion, the CTX-M-15-type ESBL was detected for the first time in an *S. enterica* serotype Enteritidis strain, which was isolated from a pediatric patient with gastroenteritis. The appearance of the CTX-M-15-producing *S. enterica* serotype Enteritidis in Korea suggests a gradual spread of this resistance to various serotypes of *Salmonella* spp.

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