**Case Report:**

**Bacteremia Due to *Salmonella enterica* Serotype Montevideo Producing Plasmid-Mediated AmpC β-Lactamase (DHA-1)**

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**Abstract.** A *Salmonella enterica* Serotype Montevideo is described that harbors DHA-1, a plasmid-mediated AmpC β-lactamase. The organism was isolated from blood and stool specimens of a 3-yr-old girl. The isolate was multi-drug resistant, including cefoxitin, gentamicin, piperacillin, cefuroxime, ceftazidime, and cefotaxime, and an antagonism was observed between cefoxitin and oxyiminocephalosporins. The resistance to ceftazidime and cefotaxime was transferred by conjugation to the recipient *E. coli* J53. To our knowledge, this is the first description of *Salmonella enterica* Serotype Montevideo harboring DHA-1. *(received 2 January 2004; accepted 8 March 2004)*

**Keywords:** *Salmonella enterica* Serotype Montevideo, DHA-1, multi-drug resistance

**Introduction**

The principal mechanism of resistance to broad-spectrum cephalosporins, such as cefotaxime and ceftazidime in *Salmonella* strains, is the production of Class A extended-spectrum β-lactamases such as SHV-2, TEM-3, TEM-25, TEM-27, CTX-M-2, and PER-2 [1-4]. These enzymes confer resistance to most β-lactam antibiotics, including oxyiminocephalosporins such as cefotaxime, ceftazidime, and aztreonam, but are not active against cephemycins and can be inactivated by clavulanic acid. β-lactam resistance due to plasmid-mediated AmpC β-lactamases has been observed in *Salmonella* species [5-7]. AmpC β-lactamases are primarily chromosomal cephalosporinases. Overexpression of AmpC β-lactamases usually confers resistance to all the β-lactams, with the exception of dipolar ionic methoxyimino-cephalosporins, such as cefepime and cefpirome, and the carbapenems [8]. Plasmid-mediated AmpC β-lactamases are derived from the chromosomally encoded enzymes of Gram-negative bacteria such as *Enterobacter cloacae*, *Morganella morganii*, and others. A plasmid-mediated AmpC β-lactamase, DHA-1, which is the first plasmid-encoded AmpC β-lactamase found to be inducible, has been identified in a few clinical isolates of *Salmonella enteritidis* [5,6].

*Salmonella enterica* Serotype Montevideo belongs to group C1, and is a cause of ovine abortion and other diseases in domestic animals and humans [9-12]. To our knowledge, this is the first report on the presence of DHA-1 in *Salmonella montevideo*.

**Case Report**

A 3-yr-old girl with diarrhea and fever was admitted to the hospital in October 2002. Despite empirical antibiotic therapy with ampicillin and gentamicin,
her symptoms did not improve. The child had no underlying disease. Blood and stool cultures yielded \textit{S. enterica} Serotype Montevideo (7:g,m,p,s:1,2,7), which appeared unusual in its resistance to cefoxitin and ceftazidime. An antibiotic susceptibility test using the disk diffusion and phenotypic confirmation tests for ESBL was performed using a double disk synergy test, according to NCCLS guidelines [13]. Crude \(\beta\)-lactamase preparations, derived from sonicated bacterial cultures of the \textit{S. montevideo} isolate, were assessed for \(\beta\)-lactamase pIs and a general inhibitor profile by isoelectric focusing (IEF).

IEF was performed at room temperature using a mini isoelectric focusing III apparatus (Bio-Rad, Richmond, CA, USA). The enzymes were visualized by staining the gel with a 0.5 mM solution of nitrocefin (BBL, Cockeysville, MD, USA). The isoelectric points of the enzymes from the \textit{Salmonella} isolate were estimated by comparison with TEM-1, TEM-10, SHV-1, SHV-5, and CMY-1.

The isolate was resistant to multiple antibiotics, including cefoxitin, gentamicin, piperacillin, cefuroxime, and ceftazidime, but was susceptible to imipenem, meropenem, ciprofloxacin, amikacin, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole in the disk diffusion test. A double-disk synergy test using ceftazidime, cefotaxime, and aztreonam was negative and showed blunted ends, suggestive of AmpC \(\beta\)-lactamase. Antagonism was observed between cefoxitin and oxyiminocephalosporins, which was suggestive of inducibility of AmpC \(\beta\)-lactamase (Fig. 1). IEF showed two lactamase bands at pIs 5.4 and 7.6. The \(\beta\)-lactamase with a pI value of 5.4 was inhibited by clavulanate (0.5 mM), but not cloxacillin (0.5 mM); the \(\beta\)-lactamase with a pI value of 7.6 was inhibited by cloxacillin, but not clavulanate.

Conjugal transfer of the resistance to \(\beta\)-lactam antibiotics was performed in mixed broth cultures as described by Tassios et al [14] with modification. A single colony of the isolate was inoculated from a blood agar plate, into 5 ml of Luria-Bertani (LB) broth, (Difco., Detroit, MI, USA) and incubated for 20 hr at 37°C with shaking. Conjugation was performed in LB broth for 3 hr at 37°C using sodium-azide resistant \textit{Escherichia coli} J53 as the recipient. Trans-conjugants were selected on a nutrient agar containing sodium azide (150 mg/ml) and ceftazidime (4 mg/ml), or sodium azide (150 mg/ml) and cefoxitin (10 mg/ml). By conjugation, resistance to both ceftazidime and cefoxitin were transferred to the recipient \textit{E. coli} J53.

TEM and SHV PCR were performed using multiplex PCR, as described by Chong et al [15]. Plasmid-mediated AmpC \(\beta\)-lactamase genes were investigated using multiplex PCR, as described by Perez-Perez and Hanson [16]. The primers for the PCR amplification are listed in Table 1. The PCR

<table>
<thead>
<tr>
<th>Primers</th>
<th>5' → 3' nucleotide sequence</th>
<th>Expected amplicon size</th>
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</thead>
<tbody>
<tr>
<td>TEM-S</td>
<td>AAG CCA TAC CAA ACG ACG AG</td>
<td>107 bp</td>
</tr>
<tr>
<td>TEM-AS</td>
<td>ATT GTT GCC GGG AAG CTA GA</td>
<td></td>
</tr>
<tr>
<td>SHV-S</td>
<td>TCT CCC TGT AAG CCA CCC TG</td>
<td>598 bp</td>
</tr>
<tr>
<td>SHV-AS</td>
<td>CCA CTG CAG CAG CTG C(A/C)G TT</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Primers used in multiplex PCR for TEM and SHV.

Fig. 1. Double disk synergy test. (A) ESBL test showing blunted end between clavulanic acid and 3rd generation cephalosporins suggestive of AmpC \(\beta\)-lactamase; (B) an antagonism between cefoxitin and oxyiminocephalosporins suggestive of inducibility of AmpC \(\beta\)-lactamase. Disks: 1, ceftazidime; 2, cefotaxime; 3, clavulanic acid; 4, cefoxitin; and 5, aztreonam.
amplification results suggested the presence of blaDHA1-like and blaTEM-like genes. After PCR screening, sequencing of DHA-1 and TEM was performed using the primers: DHA-1S, 5’-GCCCATAAAGCAATTATGG-3’, DHA-1AS, 5’-AACGTCTGACCATAATCCAC-3’ and T1, 5’-AGAGTATGAGTATTCAACATT-3’, T2, 5’-ATCTCACGGATCTG-TCTAT-3’. Sequencing of each amplicon identified the genes as blaDHA-1 and blaTEM-1b.

Discussion

Non-typhoid salmonellae (NTS) infections are a frequent cause of self-limited diarrheal illness in healthy children. However, NTS can be invasive under certain conditions, causing bacteremia or localized infections in various organs. Incidences of Salmonella bacteremia have varied from 5.2-13.7% in different studies [17]. In NTS bacteremia, Salmonella group B were the most common isolates, followed by group D, group C2, and group C1 [18]. Salmonella enterica Serotype Montevideo has caused food poisoning and human infection on rare occasions [10,11]. In recent reports, Salmonella montevideo is one of the serovars that frequently cause diarrhea [10], including outbreaks associated with a supermarket hot food outlet [14]. The incidence rate of those strains is likely to increase owing to expanded international travel and trade, modifications of food production systems, and changing dietary habits [19].

This report describes a pediatric Salmonella montevideo isolate that harbored the plasmid-mediated inducible AmpC β-lactamase, DHA-1, which was first found in a Salmonella enterica serovar Enteritidis strain [5]. Transfer of resistance by a conjugation experiment was successful in this study, suggesting that blaDHA-1 was on a self-transferable plasmid. The blaDHA-1 gene is closely related to the chromosomal ampC of Morganella morganii, and is associated on the same plasmid with the regulator ampR gene, which is responsible for inducibility [6].

Antimicrobial resistances of NTS to ampicillin (62%), chloramphenicol (67%), and trimethoprim/ sulfamethoxazole (37%) are high, and the rate of antimicrobial resistance to the newer generation, cephalosporin and ciprofloxacin, is gradually increasing [20]. Indeed, the most antimicrobial-resistant Salmonella has multiple drug resistance [21]. Multi-drug resistant, cephalosporin-resistant Salmonella spp. present significant therapeutic problems in animal and human health care, and raise questions about associations among antimicrobial resistance, antibiotic use in animals, and the transfer of multi-drug-resistant Salmonella spp. between animals and humans.

There is geographic variation in the resistance mechanisms of Salmonella species. In the United States, blaCMY-2 was the most prevalent β-lactamase gene causing extended-spectrum cephalosporin resistance in Salmonella species [22]. In contrast, only PER-1 was recognized in multiple-antibiotic-resistant S. enterica serovar Typhimurium strains isolated over a 28-mo period from a nosocomial environment in Turkey [23]. In Korea, the blaDHA gene is harbored in 2% and 13% of cefoxitin non-susceptible E. coli and K. pneumoniae [24], respectively. Transfer of the plasmids from E. coli or Klebsiella to Salmonella montevideo is known to occur in vivo [9], and the presence of blaCMY-containing plasmid, with an identical restriction pattern in Salmonella, E. coli, and K. pneumoniae isolates has been identified, suggestive of interspecies and horizontal transfer of the resistance determinant [7].

This report of bacteremia due to DHA-1-producing Salmonella montevideo in Korea, where the blaDHA gene was found in E. coli and K. pneumonia, supports the above findings [7,9]. The intergenus exchange of plasmids may play an important role in the evolution of multiresistant nontyphoid Salmonella species [25]. This has significant public health implications.

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References

Salmonella montivideo carrying β-lactamase (DHA-I)