Pediatric Infection Due to Multiresistant Salmonella enterica Serotype Infantis in Honduras

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We report the case of a pediatric patient with a Salmonella enterica serotype Infantis infection. Detailed microbiological investigation revealed that this isolate carries four β-lactamase genes (blaTEM-1b variant, blaqSV-8, blacroX-M-15, and blaqCMV-2) conferring resistance to all β-lactams but imipenem. This is the first report of a Salmonella isolate with CTX-M and AmpC enzymes on the American continent, the first report of blaqCMV-2 in Salmonella serotype Infantis, and the first report of blacroX-M-15 in the genus Salmonella.

CASE REPORT

In August 2002, an 18-month-old male infant with a clinical syndrome indicating an enteritis-like infection was admitted to the Santa Teresa Hospital in Comagüaya (Honduras). The patient, whose symptoms began 3 days before admission, presented with a fever up to 40°C, mucous bloody diarrhea, nausea, and vomiting. His urine was cloudy, with a pH of 5, and laboratory analysis showed elevated numbers of red blood cells (4/field), white blood cells (6/field), and granular casts (6/field). Laboratory analysis of fecal samples revealed the presence of erythrocytes (4/field) and neutrophils (15/field). Because of limited resources available at the hospital, no microbiological techniques were performed on these samples. An empirical treatment without a confirmatory microbiological diagnosis was immediately initiated with intravenous amikacin (50 mg every 12 h) and intravenous ceftriaxone (350 mg every 12 h) and continued for 9 days. Oral anti-inflammatory drugs (acetaminophen) to control pain and fever were also administered, and the patient was rehydrated intravenously as required. The antibiogram determined that these isolates were sensitive to ciprofloxacin. Unfortunately, these results were not available before the patient was discharged for the second time. After the second empirical treatment, the patient showed an improvement in the clinical condition and was discharged. However, microbiological eradication of the Salmonella infection was not confirmed before discharge, and attempts to locate the patient after his second stay in the hospital have failed.

Microbiology and genetic characterization. The clinical isolate was obtained by the Veterinary Laboratories Agency in the United Kingdom as part of a project focusing on the genetic characterization of β-lactam resistance. An international collaborative effort with groups in the Universidad de La Rioja (La Rioja, Spain) and Creighton University (Omaha, Nebr.) was adopted to complete this study. The serotype of the isolate (UCM267) was identified as Infantis by following standard methods. It was screened for susceptibility to a panel of 12 β-lactams plus 12 other antibiotics by using a disk diffusion method (10) and was found to be resistant to ampicillin, amoxicillin plus clavulanic acid, cefotiofur, cefuroxime, cefazidime, cefotaxime, ceftriaxone, cefoperazone, cefoxitin, cefpodoxime, cefotaxime.
aztreonam, amikacin, chloramphenicol, colistin sulfate, gentamicin, sulfamethoxazole-trimethoprim, and a triple-sulfonamide solution and sensitive to imipenem, nalidixic acid, neomycin, tetracycline, furazolidone, and streptomycin. MICs of cefoxitin were 128 mg/liter and determined as previously described (6). The resistance phenotype suggested the presence of an extended-spectrum β-lactamase (ESBL) enzyme and/or the presence of an AmpC β-lactamase.

The presence of β-lactamases was assessed by isoelectric focusing (6). Four bands with approximate pI values of 5.2, 8.2, 8.6, and 9 were detected, indicating the production of four enzymes.

The isolate was analyzed by an AmpC multiplex PCR (6) and produced a product consistent with the CIT group (comprises LAT-1 to LAT-4, CMY-2 to CMY-7, and BIL-1). Subsequently, the full-length bla_CMY PCR amplicon was sequenced as described before (6) and identified as bla_CMY-2. Identification of TEM-, SHV-, and CTX-M-type β-lactamases was carried out by amplification and sequencing of the respective genes by using methods previously described (1, 11). The amplicons were sequenced on both strands in an Applied Biosystems ABI 310 sequencer, and analysis revealed that the amplicons were sequenced on both strands in an Applied Biosystems ABI 310 sequencer, and analysis revealed that the isolate carried a blaTEM-1B variant gene with a silent mutation at base 739 (C to T), a blaSHV-5 gene, and a blaCTX-M-15 gene. In addition, the isolate was positive for class 1 integrons by PCR with primers L2 and L3 located at the conserved region (5). Furthermore, a single amplicon of approximately 1,300 bp was generated by using primers L2 and R1 (7), revealing that the integron contained a single gene cassette encoding the blaSHV-5 determinant.

Preparation of DNA for pulsed-field gel electrophoresis (PFGE) was as described by the U.S. Centers for Disease Control and Prevention (2). Figure 1 shows the XbaI-PFGE fingerprint for the isolate. Plasmid extraction was performed as described previously (6); two plasmids with approximate sizes of 86 and 59.6 MDa were identified.

**Assessment of the transferability of resistance.** Conjugation experiments were performed with the Salmonella serotype Infantis isolate and a rifampin-resistant recipient Escherichia coli K-12 20R764, by use of in-broth and filter-mating methods (6). Conjugation mixtures were plated on CHROMagar ECC (M-Tech Diagnostics) containing rifampin (100 mg/liter) and cefotaxime (1 mg/liter) or CHROMagar ECC containing rifampin (100 mg/liter) and cefoxitin (32 mg/liter) and then incubated for 24 and 48 h at 37°C. Transconjugants were obtained only on the cefotaxime plates. Attempts to transfer resistance to cefoxitin from the Salmonella serotype Infantis isolate by conjugation consistently failed. Transformation experiments were conducted as follows: plasmid DNA was prepared with a QIA-GEN high-speed MIDI kit. Electrocompetent E. coli cells (ElectroMAX DH10B; Invitrogen) were transformed by electroporation with a Bio-Rad GenePulsor II electroporator, under standard conditions (2 kV, 200 Ω, and 25 μF). Transformants were selected on nutrient agar containing 32 mg of cefoxitin/liter after 16 h of incubation at 37°C. Plasmid analysis demonstrated the acquisition of plasmids of 59.6 and 86 MDa by the transconjugants and the transformants, respectively. Antimicrobial susceptibility testing of transconjugants and transformants showed that both plasmids in isolation were able to confer resistance to the same β-lactams. However, the 86-MDa plasmid also conferred resistance to cefoxitin and aztreonam.

PCR analysis of the transformants carrying the 86-MDa plasmid indicated that the genetic determinants for bla_CMY-2 and the class 1 integron with the blaSHV-5 cassette were colocalized on the same plasmid. Also, PCR analysis of the transconjugants carrying the 59.6-MDa plasmid indicated that the genetic determinants for blaTEM-1B and blaCTX-M-15 were colocalized on the same plasmid. Figure 2 illustrates the transfer (by transformation or conjugation) of plasmids of approximately 86 and 59.6 MDa from the Salmonella serotype Infantis isolate to E. coli recipients.

**Conclusion.** Salmonella serotype Infantis has previously been reported to occur in children’s infections in hospitals. The first outbreak due to multiresistant Salmonella serotype Infantis (including resistance to several ESBL) affecting hospitalized children was reported in 1996 in Brazil (9). These isolates had a single conjugative plasmid and were sensitive to cefoxitin. There is a second report, from Brazil in 1999, of a nosocomial outbreak in a neonatal unit caused by ESBL-producing Salmonella serotype Infantis (12). Salmonella serotype Infantis has been the second-most-common serotype in Argentina in recent years, being isolated mostly from hospitalized pediatric patients (8). Data presented in our study document the first description of a Salmonella isolate from Central America producing four β-lactamases. This strain originated from a pediatric patient that received two empirical treatments with cephalosporins. It is impossible to know if the patient was infected with a resistant strain carrying those genes or if the emergence of resistance was due to clinical treatment or in vivo transfer of the genetic determinants from resident flora to the Salmonella serotype Infantis isolates. All the genetic determinants responsible for the β-lactam resistance were located on potentially transferable plasmids. Treatment failure of a patient infected with a Salmonella serotype Anatum isolate resistant to ceftriaxone has been observed due to the in vivo acquisition of a plasmid containing the blaCTX-M-3 gene (15).
AmpC enzymes confer resistance to a wide spectrum of β-lactams. In *Salmonella* serotypes, the majority of AmpC-like enzymes have been reported to be CMY-2. The first report (*Salmonella* serotype Senftenberg) came from Algeria in 1997 (3), and since then, CMY-2 has been found in the following *S. enterica* serotypes: Heidelberg, Newport, Typhimurium, Bre- deney, Mikawasima, and Montevideo. Also, *S. enterica* sero- type Wien has been found to carry bla\textsubscript{CMY-4}. This case report represents the first incident of a bla\textsubscript{CMY-2} gene in serotype Infantis. The fact that plasmid-mediated AmpC enzymes have been reported by many countries suggests that a global problem has developed.

CTX-M β-lactamases efficiently hydrolyze many newer broad-spectrum oximino-β-lactams. CTX-M-producing enter- obacteria are endemic in Latin America and in some areas of northeastern Europe. CTX-M enzymes have increasingly been found in *Salmonella* organisms over recent years. Reports exist for *Salmonella* serotype Wien with CTX-M-3; *Salmonella* se- rotype Virchow with CTX-M-9; *Salmonella* serotype Infantis with CTX-M-2; *Salmonella* serotype Typhimurium with CTX- M-4, -5, and -6; *Salmonella* serotype Anatum with CTX-M-3; *Salmonella* serotype Oranienburg with CTX-M-3; and Salmo- nella serotype Enteritidis with CTX-M-3. To the best of our knowledge, we are presenting the first report of CTX-M-15 within the genus *Salmonella*. Unlike the majority of CTX-M enzymes, CTX-M-15 confers resistance to ceftazidime (13). This enzyme has recently been found in *Enterobacteriaceae* in India, Poland, Russia, and Turkey, but it had never before been detected on the American continent.

The ESBL SHV-5 was first described to occur in *Salmonella* serotype Senftenberg in India (14) and has recently been de- scribed to occur in *Salmonella* serotype Typhimurium in Ro- mania. However, it has never been reported to occur in Sal- monella serotypes on the American continent.

To date, a total of seven bla\textsubscript{TEM-1} molecular variants have been reported due to the pattern of silent mutations in the bla\textsubscript{TEM-1} structural gene and in its promoter (from bla\textsubscript{TEM-1A} to bla\textsubscript{TEM-1G}) (4). The molecular variant bla\textsubscript{TEM-1B} has fre- quently been found in the family *Enterobacteriaceae*, including in the genus *Salmonella* (1). The new molecular variant found in our study, with a silent mutation with respect to bla\textsubscript{TEM-1B}, at nucleotide position 739, could be named bla\textsubscript{TEM-1H}.

In conclusion, this report provides a good example of the emergence of β-lactamase genes among bacterial species and especially *Salmonella* serotypes. In addition, this emergence of β-lactam resistance is not limited to any one country or con- tinent; it is worldwide. This situation is of particular concern to public health, as these mechanisms of resistance now threaten the value of cephalosporin treatment against pathogenic en- terobacteria.

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REFERENCES


