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A Case of Multidrug-Resistant *Salmonella enterica* Serovar Typhi Treated with a Bench to Bedside Approach

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We report a relapsed case of a 25 year-old man with multi-drug resistant *Salmonella* serovar Typhi (MDRST) bacteremia who had recently returned from travel in India. Due to unresponsiveness to ciprofloxacin and ceftriaxone, we examined the strain's resistance to quinolones and extended-spectrum β-lactamases (ESBLs). The strain had a single *gyrA* mutation at codon 83 (Ser83Phe), which explains its decreased susceptibility to fluoroquinolone and resistance to nalidixic acid. In the screening tests of ESBLs, TEM-1 was positive, which is beta-lactamase but not ESBL. The patient was finally successfully treated with meropenem and aztreonam. In the presence of clinical unresponsiveness despite favorable sensitivity tests, further laboratory evaluations are needed, which should include studies of genes related to antibiotic resistance and ESBLs. In addition, further prospective trials should be done about the possible inclusion of antibiotics not yet mentioned in the current guidelines. With MDRST on the rise worldwide, the most optimal and effective line of antibiotic defense needs to be devised.

Key Words: *Salmonella enterica* serovar Typhi, multi-drug resistance, treatment

INTRODUCTION

Typhoid fever, a systemic infectious disease caused by *Salmonella enterica* serovar Typhi, affected an estimated 16 million people in the 1990s worldwide,[1] and the increasing incidence of multi-drug-resistant strains poses a risk to public health.[2] Investigators from the U.S. Center for Disease Control and Prevention estimate that there are 21.6 million typhoid cases annually, with the yearly incidence varying from 100 to 1,000 cases per 100,000 people.[3] An estimated 600,000 deaths from enteric fever occur annually throughout the world.[1]

Antimicrobial-resistant *Salmonella enterica* serovar Typhi isolates emerged in the 1970s in Latin America[4] and Asia.[5] Meanwhile, *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi A are the predominant types of *Salmonella* responsible for enteric fever in India. In Korea, no resistant strain was documented until 1992, when a chloramphenicol, ampicillin, and co-trimoxazole resistant strain was isolated from a patient who returned from a Southeast Asian country.[6] Lee et al. isolated two additional multi-drug-resistant strains in 1995,[7] and subsequently, Shin et al. reported a chloramphenicol resistance rate of 15% among isolates in Korea in 1997.[8] The emergence of multi-drug-resistant *Salmonella enterica* serovar Typhi (MDRST) in Korea could be attributed to acquisition of resistance by endemic strains from other resistant gram-negative bacilli or the spread of resistant strains brought back from other countries to which there have been recent increases in international travel.[7]

Here we present a case of a patient who previously returned from travels in India. He received treatment for MDRST bacteremia that was based on a bench to bedside approach that included studies of genes related to antibiotic resistance and extended-spectrum β-lactamases (ESBLs).

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CASE REPORT

A 25-year-old male patient was admitted to our hospital with an ongoing fever that began ten days previous. He had just returned from traveling in India 20 days before admittance. His past medical history was unremarkable and he showed no abdominal symptoms or anything else significant except for a sore throat. The initial laboratory findings showed WBC 6,410/uL, ESR 19 mm/hr, CRP 5.31 mg/dL, and AST/ALT 67/87 IU/L. Malaria smear showed negative findings. Under the initial impression that he might have acute pharyngotonsillitis, antibiotic therapy was administered with ampicillin 4,000 mg and sulbactam 2,000 mg per day for four days while he was instituted. The patient became afebrile and was discharged to the outpatient department for follow up.

Eight days later, the patient returned to the emergency room with a fever and abundant watery diarrhea. Physical examination upon second admission revealed a blood pressure of 110/70 mmHg, pulse rate 110/min, respiratory rate of 20/min, and body temperature of 38.5°C. Increased bowel sounds and diffuse abdominal tenderness were found. The laboratory examination showed WBC 3740/uL, ESR 27 mm/hr and CRP 6.15 mg/dL. The AST/ALT were 79/97 IU/L, gamma GTP 140 IU/L, and LDH 800 IU/L. The Widal agglutination test at the first visit showed values of O/A/H 1:20/1:20/1:20, respectively but increased to 1:20/20/320 at second visit. The blood culture done during the first admission period isolated *Salmonella* serovar Typhi sensitive to antibiotics including amikacin, ampicillin, ampicillin/sulbactam, aztreonam, cefazolin, cefepime, cefoxitin, ceftriaxone, ciprofloxacin, gentamicin, imipenem, piperacillin/tazobactam, and tobramycin except for trimethoprim/sulfamethoxazole (TMP-SMX) (MIC≥16/304 μg/mL) in only one pair of blood cultures. The sensitivity was assessed by the bioMerieux Vitek II susceptibility test system (Hazelwood, MO, USA). However, upon the second admission, the blood culture showed *Salmonella* serovar Typhi resistant to ampicillin (MIC ≥ 32 μg/mL), ampicillin-sulbactam (MIC ≥ 32/16 μg/mL), and TMP/SMX (MIC ≥ 16/304 μg/mL) in three paired blood cultures showing MDR pattern. The MIC results for ceftriaxone and ciprofloxacin were ≤ 8 and 1 μg/mL, respectively. Stool and urine cultures were all negatives. Abdominal computed tomography showed hepatomegaly and splenomegaly. The transthoracic echocardiography showed no vegetations on the cardiac valves. The patient had a relapse from MDRST bacteremia, which we treated with ciprofloxacin 400 mg and ceftriaxone 2 g per day for 4 days; however, the patient continued in poor clinical condition. As a result, we empirically changed the antibiotics regimen to meropenem 2 g and aztreonam 2 g per day and the patient showed progressive improvement of clinical and laboratorial conditions. We reexamined the isolate with the disk diffusion method, which showed resistance to nalidixic acid and decreased sensitivity to ciprofloxacin (inhibition diameter of 28 mm). The considerations were done for *Salmonella* serovar Typhi, which has quinolone resistance. We assayed the isolated strains for *gyrA* mutations at codons 81, 83, and 87, using allele-specific polymerase chain reaction and restriction fragment length polymorphism (AS-PCR-RFLP) as previously described.[2] The single *gyrA* mutation at codon 83 was found, explaining the decreased susceptibility to fluoroquinolone and resistance to nalidixic acid. Sequencing of the quinolone resistance-determining region (QRDR) of the *gyrA* gene of this isolate revealed a Ser83 Phe substitution. We also examined whether the isolates expressed ESBLs. Isolates were subjected to molecular screening for β-lactamases using PCR tests as previously described for TEM,[9] SHV,[10] family specific CTX-M[11] and plasmid-borne *AmpC* genes.[12] The results showed TEM-1 positive finding, which is β-lactamase but not ESBL. After the administration of meropenem and aztreonam for 14 days, follow-up blood and stool cultures were negative for *Salmonella* serovar Typhi and the patient was discharged for outpatient department follow up.

DISCUSSION

In India, reports of *Salmonella* serovar Typhi drug resistance began from 1960, with the first outbreak of MDRST occurring in Calicut.[13] MDRST is defined as strain resistance, which acquired on the same plasmid type,[14] to the three first-line

antibiotics used to treat typhoid fever, namely chloramphenicol, ampicillin, and co-trimoxazole. A study of the sensitivity pattern of *Salmonella* in northern India found an increase in MDRST from 53.6% to 63.9% from 1997 to 2001. Out of these MDR isolates, 75% were even resistant to amikacin, cefotaxime, ciprofloxacin, ceftizoxime, and ceftriaxone.[14] Initially our patient responded to ampicillin, however, upon switching to oral antibiotics, he relapsed and his blood culture upon second visit showed MDR pattern that can probably be attributed to secondary acquisition of resistance. We then treated him with ciprofloxacin and ceftriaxone, but his clinical state continued to deteriorate. The evolution in India of MDR *Salmonella* possessing ESBLs threatens to compromise the clinical utility of third-generation cephalosporins.[15] Most of the MDR *S. enterica* showed reduced susceptibility to ampicillin, chloramphenicol, TMP-SMX, ofloxacin, and ciprofloxacin, but had good susceptibility to extended-spectrum cephalosporins and carbapenems.[16] In addition, Jean et al.[17] suggested that a carbapenem might be considered the drug of choice for the treatment of infections caused by cefotaxime- and ciprofloxacin-resistant bacteria. Jones et al.[15] also showed complete susceptibility of endemic *Salmonella* spp. to meropenem, which has potential value as an alternate therapy. Imipenem, a carbapenem, was the only antimicrobial agent tested with 100% activity against *Salmonella* spp.,[18] with no association between ciprofloxacin and imipenem resistance.[19] As a result, we changed the regimen to meropenem and aztreonam. Although there have not been any reports on intracellular activity of aztreonam against *Salmonella* spp., intracellular activity against *E. coli* showed a dose dependent 32 to 90% decrease of number for up to 16 × MBC concentration.[20] Pruul et al. reported that exposure of *S. typhimurium* to aztreonam enhances phagocytic killing through modification of cell surface structures, which is mediated through an increase in surface hydrophobicity which enhances bacterial association with leukocyte membranes with subsequent phagocytosis and intracellular killing.[21] In the mouse typhoid model, aztreonam is not only very effective but can completely eradicate the *Salmonella* from the reticuloendothelial system compared to ampicillin and ceftazidime when given early in the infection[22] and Gotuzzo E et al.[23] reported that aztreonam is more effective in the elimination of the infecting *Salmonella* organisms from the bloodstream. Aztreonam exhibited superior antimicrobial activity compared to other antibiotics, including ceftazidime, with inhibition of 90% of the strains by 0.8 micrograms/mL (MIC 0.05 to 1.56 micrograms/mL).[24] Girglis et al. reported that safety and efficacy were comparable for ceftriaxone and aztreonam, although ceftriaxone was the most cost-effective on an inpatient basis due to a more rapid clinical cure.[25] After changing the regimen, he showed progressive improvement in his condition.

Few cases of treatment failure due to fluoroquinolone resistance in *Salmonella* strains, including *Salmonella* serovar Typhi, have been reported. However, there is evidence of an increasing number of strains that are resistant to nalidixic acid and exhibit decreased susceptibility to the most recent fluoroquinolones used in human therapeutics.[2] With regard to the mechanisms of quinolone resistance, there are alterations in target enzymes (DNA gyrase [*gyrA* and *gyrB*] and/or topoisomerase IV [*parE* and *parC*]) or impaired access to the target enzymes, due to changes in porin expression or as a result of efflux mechanisms.[26,27] These principal means of resistance are a result of chromosomal mutations. *gyrA* gene coding for the A subunit of gyrase, whose complex with DNA is the primary target of quinolones, has a major role in the resistance of quinolone and nalidixic acid.[2] The contribution of *gyrB* mutation to quinolone resistance is still unclear. Topoisomerase IV, whose *ParC* and *ParE* subunits are homologous to *gyrA* and *gyrB*, respectively, is a secondary target. Alterations in the *ParC* and *ParE* genes at positions equivalent to those identified in *gyrA* and *gyrB* participate in strains exhibiting high resistance levels to quinolones.[2] With AS-PCR-RFLP, the single *gyrA* mutation at codon 83 (Ser83Phe) was found, explaining its decreased susceptibility to fluoroquinolone and resistance to nalidixic acid.

ESBLs derive mainly from TEM and SHV β-lactamases. These enzymes confer resistance to all oxyimino cephalosporins and monobactams except for cephamycins and carbapenems. ESBL gene studies were done in our patient with PCR method showing negative results for SHV, CTX-M, OXA, DHA and CMY. A positive result for

TEM was found, but it was TEM-1 type, which is a broad-spectrum β-lactamase but not ESBL. TEM-1 is known to confer resistance to ampicillin, amoxicillin, and other penicillins as well as to early- but not later-generation cephalosporins.[27] It is hypothesized that the use of ampicillin-sulbactam at first visit may have acted as a selective pressure. The consequent acquisition of TEM-1 by *Salmonella* serovar Typhi may have resulted in resistance to ampicillin with the second blood culture susceptibility test.

Careful attention should be taken in the clinical field with regard to the lack of an exact correlation between the commonly done *in vitro* and *in vivo* resistance studies. In the presence of clinical unresponsiveness despite favorable sensitivity tests, further evaluations like studies of genes related to antibiotics resistance and ESBLs should be considered. MDRST is on the rise worldwide and with increasing levels of international travel, even further spread of MDRST can be anticipated. In addition, strains resistant to ceftriaxone and ciprofloxacin are starting to appear nowadays. So, further prospective trials need to be done to determine possible use of antibiotics that are not yet included in the current treatment guidelines.[3]

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