Salmonella enterica Serotype Gambia with CTX-M-3 and *armA* Resistance Markers: Nosocomial Infections with a Fatal Outcome[▽]

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We report two cases of bacteremia caused by the *Salmonella enterica* serotype Gambia in our children's hospital, with one fatal outcome. The isolates showed indistinguishable genotypes and infrequent resistance markers: CTX-M-3 extended-spectrum β -lactamase and *armA* methyltransferase. This is the first report of *S. Gambia* exhibiting CTX-M-3 and *armA* markers involved in serious infections.

CASE REPORT

From 8 to 10 August 2005, two babies in the intensive care unit (ICU) of the gastroenterology-nutrition ward of Trousseau Children Hospital, Paris, France, developed bacteremia with a *Salmonella* isolate. The first baby, a male (age 9 months) born in France to Senegalese parents, had been hospitalized at Trousseau Hospital for 7 months. He was immunodeficient and exhibited intestinal epithelial dysplasia (mitochondriopathy), with chronic diarrhea (six stools per day). On 8 August, during a febrile episode, a *Salmonella enterica* isolate (05-5547) was identified in a blood culture, whereas no *S. enterica* isolate had been detected in the stools of the baby or a close member of its family (mother). The blood isolate was resistant to cefotaxime, ceftazidime, gentamicin, tobramycin, amikacin, and trimethoprim-sulfamethoxazole but susceptible to ciprofloxacin. The baby recovered rapidly with a treatment combining imipenem and ciprofloxacin. At the same time, a second baby, age 8 months, was present in the ward for cystic lymphangiomatosis of the pancreas with ascitic bouts. On 10 August, the baby's condition suddenly deteriorated with the occurrence of fever, vomiting, tachycardia, and unstable blood pressure, leading to the initiation of broad-spectrum antibiotic therapy combining imipenem, gentamicin, metronidazole, and vancomycin. Aggravation of sepsis with failure of blood flow dynamics, disorders of consciousness, and tachypnea with pulmonary base crepitations were observed, making it necessary to drain 300 ml of ascitic fluid. Gram stain examination of the centrifuged deposit showed Gram-negative rods. Both ascitic fluid and blood cultures showed *S. enterica* (05-5546), with death occurring in less than 24 h. No autopsy was performed.

The two blood isolates (05-5446 and 05-5447) recovered from both babies were studied: identification with the rapid

32E system (bioMérieux, Marcy-L'Etoile, France) and serotype determination at the French National Reference Centre for *Salmonella* (FNRC-Salm), Institut Pasteur, by agglutination with antiserum (Bio-Rad, Marnes-la-Coquette, France, and the WHO Collaborative Centre for Reference and Research on *Salmonella*, Institut Pasteur) as specified by the White-Kauffmann-Le Minor scheme (11). Susceptibility testing was performed with the disk diffusion method according to the Comité de l'Antibiogramme, Société Française de Microbiologie (<http://sfm.asso.fr/>). Extended-spectrum β -lactamase (ESBL)-producing isolates were screened by using the double-disk synergy test between cefotaxime or ceftazidime and amoxicillin-clavulanate. MICs were determined by the Etest technique. To assess the genetic diversity of the *S. enterica* isolates, a randomly amplified polymorphic DNA analysis (RAPD) technique was performed as described elsewhere (15). Isolates 05-5446 and 05-5447, the *S. enterica* serotype Gambia reference strain 543K (isolated from a pig with gastroenteritis in 1953 in Gambia), and six unrelated collection strains from the FNRC-Salm (1996 to 2003) were also studied by standard pulsed-field gel electrophoresis (PFGE) of XbaI-digested chromosomal DNA (13, 23) (Table 1). A type (e.g., type XGAM-1) was assigned to each PFGE pattern differing by one or more additional bands >50 kb in size. PCR assays to detect *bla*_{TEM}, *bla*_{CTX-M}, and *bla*_{SHV} were performed on isolates 05-5446 and 05-5447, as described previously (7, 8). PCR products were subjected to direct sequencing with an Applied Biosystems sequencer (model ABI 377), and nucleotide sequences were analyzed with the BLAST and ClustalW programs. The primers used for PCR detection of the genes encoding 16S rRNA methyltransferase *armA* were metF and metR, as described by Galimand et al. (9). A resistance transfer experiment was carried out by using isolate 05-5447 grown on liquid and solid media, mated with *Escherichia coli* C1a resistant to nalidixic acid as the recipient strain (8). Transconjugants were selected on Drigalski agar (Bio-Rad, France) supplemented with cefotaxime (4 mg/liter) and nalidixic acid

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TABLE 1. Characteristics of *S. enterica* serotype Gambia strains^a

Isolate	Antimicrobial resistance phenotype	MICs (mg/liter)	PFGE type
05-5446	A Ctx S Sp K To G Ak Is Sul Tmp	Ctx, >32; Caz, 3; Ipm, 0.25; G, To, Ak, >256; Cip, 0.012	XGAM-1
05-5447	A Ctx S Sp K To G Ak Is Sul Tmp	Ctx, >32; Caz, 3; Ipm, 0.25; G, To, Ak, >256; Cip, 0.012	XGAM-1
03-1573	Pan susceptible		XGAM-2
03-5649	Pan susceptible		XGAM-4
03-6880	Pan susceptible		XGAM-5
01-9145	Pan susceptible		XGAM-6
97-8049	Pan susceptible		XGAM-7
96-2611	Pan susceptible		XGAM-7
543K	Pan susceptible		XGAM-3

^a A, amoxicillin; Ctx, cefotaxime; Caz, ceftazidime; Imp, imipenem; S, streptomycin; Sp, spectinomycin; K, kanamycin; To, tobramycin; G, gentamicin; Ak, amikacin; Is, isepamicin; Sul, sulfonamides; Tmp, trimethoprim; Cip, ciprofloxacin.

(64 mg/liter). Three *E. coli* transconjugants were arbitrarily selected for each experiment. Plasmid DNA was analyzed by S1 nuclease treatment and PFGE as described previously (8), and PCR-based replicon typing analysis was performed as described by Carattoli et al. (3). The 18 primer pairs targeting FIA, FIB, FIC, HI1, HI2, I1-Ig, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FII replicons were used in separate PCRs.

Identifying the *S. Gambia* serotype (antigenic formula 35:i:e, n, z₁₅) for isolates 05-5446 and 05-5447 proved difficult in routine hospital laboratories, thus making it necessary to call for the assistance of FNRC-Salm. The *S. Gambia* serotype isolates were resistant to all β -lactam agents (cefotaxime MIC, >32 mg/liter; ceftazidime MIC, 3 mg/liter) except imipenem (MIC, 0.25 mg/liter), all aminoglycosides (gentamicin MIC, >256 mg/liter; tobramycin MIC, >256 mg/liter; amikacin MIC, >256 mg/liter), and cotrimoxazole but susceptible to nalidixic acid and ciprofloxacin (ciprofloxacin MIC, 0.012 mg/liter). The RAPD patterns of isolates 05-5446 and 05-5447 were indistinguishable (data not shown). The *S. Gambia* serotype isolates 05-5446 and 05-5447 exhibited indistinguishable PFGE patterns, i.e., XGAM-1 (Table 1 and Fig. 1). This pattern was close to those of isolate 03-1573 (XGAM-2) and reference strain 543K (XGAM-3). XGAM-1 differed from XGAM-2 only by the presence of a single additional band of 80 kb and differed from XGAM-3 by the presence of two additional bands of 310 and 80 kb. The 80-kb band most likely corresponds to the plasmid carrying the antimicrobial resistance determinants (see below). The PFGE patterns of the other isolates were different from XGAM-1. The ESBL *bla*_{CTX-M-3} gene and the methyltransferase *armA* gene were found in *S. Gambia* serotype isolates. Both genes were located on an $\approx$ 80-kb conjugative plasmid of replicon IncL/IncM. The determinants of resistance to sulfonamides and trimethoprim were also located on this plasmid.

Nontyphoidal *Salmonella* (NTS) infections in healthy children are usually confined to the gastrointestinal tract and are characterized by acute diarrhea and fever that is generally self-limited (25). However, the clinical course of these infections may be affected by factors such as age, underlying diseases, and immunodeficiency, which may predispose to bloodstream invasion and focal complications. Antimicrobial treatment for gastroenteritis in healthy children is generally not required, but treatment with ceftriaxone or trimethoprim-sulfamethoxazole is recommended if the patient is under 6 months or over 50 years of age or has underlying diseases (12).

Fluoroquinolone is not recommended for children, but a short treatment with ciprofloxacin is considered safe and allows recovery (16). The use of ciprofloxacin might have contributed to curing the first of the two babies, while the second was not saved, despite the use of imipenem combined with gentamicin. Unfortunately, in the latter case, the first-line treatment was not documented by the strain susceptibility pattern, which was obtained only 24 h later and exhibited gentamicin resistance. The extreme rarity of the *S. Gambia* serotype (eight isolates out of the 164,932 *Salmonella* isolates received at FNRC-Salm from 1996 to 2008) associated with PFGE results and antimicrobial resistance characteristics led to the assumption that the same strain was responsible for both infections. Although the possibility that the second baby had acquired an infection independently with an *S. Gambia* serotype strain exhibiting similar PFGE (observed in only one of the seven unrelated isolates: 03-1573) and this very rare resistance pattern seems very unlikely, it cannot be excluded. Nevertheless, the hypothesis of cross-transmission was made and control measures aimed at

FIG. 1. XbaI pulsed-field gel electrophoresis patterns of *S. enterica* serotype Gambia isolates. Lanes: M, XbaI-digested DNA of *S. enterica* serotype Braenderup H9812, used as a molecular size marker; 1, Trousseau isolate 05-5446 (case 2); 2, Trousseau isolate 05-5447 (case 1) (similar pattern to that shown in lane 1). Unrelated isolates of *S. enterica* serotype Gambia (year of isolation, country of isolation): lane 3, 03-1573 (2003, unknown); lane 4, 03-5649 (2003, Senegal); lane 5, 03-6880 (2003, unknown); lane 6, 01-9145 (2001, unknown); lane 7, 97-8049 (1997, unknown); lane 8, 96-2611 (1996, unknown); and lane 9, serotype Gambia reference strain 543K (1953, Gambia).

preventing dissemination through hand carriage in particular were implemented immediately: all the staff members were informed of the cluster, hand hygiene protocols were emphasized, and environment hygiene procedures (quaternary ammonium disinfection of room surfaces, equipments, materials, etc.) were intensified. Entry into the rooms was limited to staff members directly treating the babies, thereby excluding all the students. An educational session was organized for the staff and visitors, the latter being restricted to the nearest family members (mother and father). Fortunately, no other new case of *S. enterica* infection was observed within the ICU.

The majority of ESBL-producing *Salmonella* isolates belong to two serotypes: *S. enterica* serotype Typhimurium (1, 6) and *S. Enteritidis* (1, 20). Nosocomial infections caused by ESBL-producing NTS are not frequently reported (14, 18, 19, 22, 24). In France, at FNRC-Salm during the period 2003 to 2006, 77 of the 96 ESBL-producing NTS isolates belonged to the CTX-M group, which included 60 CTX-M-15, 10 CTX-M-2, 4 CTX-M-1, and 3 CTX-M-3 (including isolates 05-5446 and 05-5447). CTX-M-3 has been described in both Eastern Europe and in North Africa (2, 17). The ESBL CTX-M-3 gene was identified for the first time in clinical isolates of *Citrobacter freundii* and *Escherichia coli* in a Polish hospital in 1996. It was associated with a large conjugative IncL/IncM plasmid that was fully sequenced (pCTX-M3; GenBank accession no. AF550415). Since then it has been detected in various species of the *Enterobacteriaceae* family (including rare *Salmonella* isolates from Europe, Africa, and Asia) worldwide (10). Plasmids of various groups (IncL/IncM, IncI1, IncA/IncC, and IncN) were found to contain this CTX-M-3 gene. However, only IncL/IncM plasmids were shown to carry both CTX-M-3 and *armA* genes. The production of 16S rRNA methyltransferase has recently attracted attention, as it is a novel aminoglycoside resistance mechanism in pathogenic Gram-negative bacteria (21). Furthermore, high-level resistance to aminoglycosides mediated by the production of 16S rRNA methyltransferase in different Gram-negative pathogens has been increasingly reported (21). Six of the 16S rRNA methyltransferase enzymes have been identified, namely, *rmtA* to *rmtD*, *armA*, and *npmA* (4, 21), and a nomenclature of these enzymes has recently been given (5). The *armA* aminoglycoside methyltransferase gene now in the process of global dissemination was initially identified as the *nbrB* gene, similar to *Streptoalloteichus hindustanus* 16S rRNA methyltransferase on the pCTX-M3 plasmid. It was later characterized on the IncL/IncM plasmid pIPI204, which also encodes the CTX-M-3 gene in *Klebsiella pneumoniae* isolated in France in 2003 (9) (GenBank accession no. AY220558). Combined CTX-M-3 and *armA* resistance markers have rarely been reported in *Salmonella*, being found in only three serotypes: *S. Virchow* in Russia (7), *S. Enteritidis* in Bulgaria, and *S. Oranienburg* in Poland (4). Our *S. enterica* serotype Gambia strain showed that both CTX-M-3 and *armA* genes cotransferred on an 80-kb IncL/IncM plasmid, probably close to pCTX-M3. The mobility of plasmids in *Salmonella* serotypes is not an unusual phenomenon and can be expected.

To our knowledge, this is the first report of *S. enterica* serotype Gambia exhibiting CTX-M-3 and *armA* resistance markers, highlighting the continuous spread of the pCTX-M3-like plasmid in *Salmonella* populations.

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