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ORIGINAL ARTICLE

Development of ceftriaxone resistance in *Salmonella enterica* serotype Oranienburg during therapy for bacteremia

Wei-Chiun Yang [a,f], Oi-Wa Chan [a,f], Tsu-Lan Wu [b], Chyi-Liang Chen [c], Lin-Hui Su [b,c,d,*], Cheng-Hsun Chiu [c,e,]**

[a] *Division of Pediatric Gastroenterology, Department of Pediatrics, Chang Gung Children's Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan*
[b] *Department of Laboratory Medicine, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan*
[c] *Molecular Infectious Disease Research Centre, Chang Gung Memorial Hospital, Taoyuan, Taiwan*
[d] *Department of Medical Biotechnology and Laboratory Science, Chang Gung University College of Medicine, Taoyuan, Taiwan*
[e] *Division of Pediatric Infectious Diseases, Department of Pediatrics, Chang Gung Children's Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan*

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Background: The majority of nontyphoid *Salmonella* infection is identified in children. When an invasive or severe *Salmonella* infection is encountered, ceftriaxone is recommended for such patients. A 2-year-old girl was hospitalized for the treatment of *Salmonella* bacteremia and discharged with standard ceftriaxone treatment. She was readmitted to the hospital after 2 days due to the recurrence of the *Salmonella* bacteremia. The study aimed to unveil the mechanism for the relapse.
Methods: Six isolates (4 blood and 2 stool) were recovered from the patient, with the last two blood isolates being ceftriaxone-resistant. Pulsed-field gel electrophoresis was used for genotyping. Ceftriaxone resistance genes and transferability of the resistance plasmid were examined by molecular methods.
Results: All isolates were identified as *Salmonella enterica* serotype Oranienburg. Five isolates demonstrated almost identical electrophoresis patterns, except that in the two ceftriaxone-resistant isolates an extra band (>100 kb) was noted. A bla_{CMY-2} gene, carried by a 120-kb

* Corresponding author. Department of Medical Biotechnology and Laboratory Science, Chang Gung University College of Medicine, 259, Wen-Hwa First Road, Kweishan 333, Taoyuan, Taiwan.
** Corresponding author. Division of Pediatric Infectious Diseases, Department of Pediatrics, Chang Gung Children's Hospital, 5 Fu-Hsin Street, Kweishan 333, Taoyuan, Taiwan.
E-mail addresses: sulinhui@gmail.com (L.-H. Su), chchiu@adm.cgmh.org.tw (C.-H. Chiu).
[f] These authors contributed equally to this work.

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conjugative IncI1 plasmid of the sequence type 53, was identified in the two ceftriaxone-resistant isolates. Transfer of the resistance plasmid from one blood isolate to *Escherichia coli* J53 resulted in the increase of ceftriaxone minimum inhibitory concentration from 0.125 μg/mL to 32 μg/mL in the recipient.

Conclusion: Ceftriaxone is the standard therapeutic choice for invasive or serious *Salmonella* infections in children. Pediatricians should be aware of the possibility of resistance development during therapy, especially in areas with a widespread of ceftriaxone resistance genes that are carried by a self-transferrable plasmid, such as the bla_{CMY-2}-carrying IncI1 plasmid identified herein.

Introduction

Salmonella infection is an important public health problem worldwide, and children younger than 5 years constitute the largest population among patients with *Salmonella* infection in both developed or developing countries.[1,2] Nontyphoid *Salmonella* cause self-limited gastroenteritidis as well as systemic infections that require antibiotic treatment.[1,3] Appropriate antimicrobial options may include ampicillin, chloramphenicol, trimethoprim–sulfamethoxazole, fluoroquinolones, or expanded-spectrum cephalosporins (ESCs).[1,4] However, resistance of *Salmonella* to conventional antibiotics has been high in recent years, leaving fluoroquinolones and ESCs as the only effective agents against nontyphoid *Salmonella* infection.[5] However, resistance to ESCs among *Salmonella* has been noted since the late 1980s.[4] Spread of plasmid-mediated AmpC or extended-spectrum β-lactamase genes further lead to the increase of ceftriaxone resistance in nontyphoid *Salmonella*.[4] Because fluoroquinolones are generally not recommended in children,[6] ceftriaxone resistance in pediatric *Salmonella* infection therefore represents a serious clinical problem. Here, we report a case of relapse bacteremia caused by *Salmonella enterica* serotype Oranienburg. Laboratory investigation revealed that ceftriaxone resistance developed in the *Salmonella* during therapy, leading to the relapse of bacteremia caused by the same strain.

Materials and methods

The patient

On September 1, 2011, a 2-year-old girl presented to the outpatient department with a 1-week history of intermittent fever, fatigue, diarrhea, and abdominal pain. She had received regular vaccination and did not have any significant diseases in the past. The physical examination was otherwise unremarkable except that she appeared mildly dehydrated. Laboratory studies revealed a leukocyte count of 7.2×10^9 cells/L with 52% neutrophils; hematocrit, 34.4%; platelet count, 212×10^9/L; and elevated C-reactive protein, 135.81 mg/L. She was hospitalized on the same day and her blood, urine, and stool were collected for bacterial culture.

On admission, her temperature was 39.4°C, pulse rate 122 beats/minute, respiratory rate 26 breaths/minute, and blood pressure 118/43 mmHg. The patient was fluid resuscitated and started to receive intravenous ampicillin (100 mg/kg/day). The blood culture yielded Gram-negative bacilli on the next day, and the antibiotic was shifted to ceftriaxone. Both blood and stool cultures grew ceftriaxone-susceptible *S. enterica* serogroup C1 (SC834 and SC773, respectively). A second blood culture drawn on Day 3 still yielded the same organism (SC835).

The patient's fever subsided on Day 4. Ceftriaxone was applied for 5 days and then shifted to oral antibiotic therapy with cefixime (8 mg/kg/day). A third blood culture was drawn on Day 6. The patient was discharged on Day 9 with an apparently well condition, despite a preliminary laboratory report indicating the growth of *S. enterica* serogroup C1 (SC836) from the third blood culture. For follow-up, a fourth blood culture, later grew *S. enterica* serogroup C1 (SC837), was drawn just prior to the patient's discharge. Both SC836 and SC837 were subsequently reported to be resistant to ceftriaxone.

Two days later, the patient presented to the emergency department with an intermittent fever for 1 day. Laboratory studies at the time revealed a leukocyte count of 6.4×10^9/L with 66% neutrophils; hematocrit, 35.2%; platelet count, 290×10^9/L; and C-reactive protein, 17.17 mg/L. A fifth blood culture was drawn but later found to be sterile. Because the blood culture taken prior to the discharge of the first hospitalization grew ceftriaxone-resistant *S. enterica* serogroup C1, intravenous imipenem (80 mg/kg/day) was prescribed. A gallium-67 scan was arranged and showed negative findings. The fever subsided on the next day. Imipenem was used for 6 days and the patient was discharged without any sequelae.

Bacteria and antimicrobial susceptibility

A total of six isolates, four from blood and two from stool cultures, of *S. enterica* serogroup C1 were identified from the patient with standard methods (Table 1). Serogroups and serotypes of the isolates were analyzed with O and H antisera using standard methods. Antimicrobial susceptibility of the isolates was examined by a standard disk diffusion method, and minimum inhibitory concentrations (MICs) of ceftriaxone were assessed with E-test strips (AB Biodisk, Solna, Sweden). The results were interpreted

Table 1 Laboratory characterization of the six *Salmonella enterica* serotype Oranienburg isolates

Isolate	Specimen	Antimicrobial susceptibility								MIC_{CRO} (μg/mL)	*bla* gene	Plasmid profile (kb)
		AMP	CHL	SXT	CIP	CFM	CRO	ETP	IPM			
Salmonella Oranienburg												
SC834	Blood	S	S	S	S	S	S	S	S	0.064	—	—
SC773	Stool	S	S	S	S	S	S	S	S	0.032	—	120,190
SC773B	Stool	S	S	S	S	S	S	S	S	0.125	—	—
SC835	Blood	S	S	S	S	S	S	S	S	0.064	—	—
SC836	Blood	R	R	S	S	R	R	S	S	32	CMY-2	120[a]
SC837	Blood	R	R	S	S	R	R	S	S	32	CMY-2	120[a]
Escherichia coli												
J53		S	S	S	S	S	S	S	S	0.125	—	—
J53/pSC837		R	R	S	S	R	R	S	S	32	CMY-2	120[a]

[a] bla_{CMY-2}-carrying IncI1 plasmid.
AM = ampicillin; C = chloramphenicol; CFM = cefixime; CIP = ciprofloxacin; CRO = ceftriaxone; ETP = ertapenem; IPM = imipenem; SXT = sulfamethoxazole–trimethoprim.

according to the suggestions of the Clinical Laboratory Standards Institute.[7]

Ceftriaxone resistance mechanisms and genetic relatedness of the isolates

Genetic relatedness of the isolates was investigated by pulsed-field gel electrophoresis (PFGE) and interpreted as described earlier.[8] Ceftriaxone resistance was investigated by using polymerase chain reaction and sequencing as reported previously.[9] Plasmid profiles of the isolates were analyzed by an alkaline lysis method.[10] DNA–DNA hybridization was used to locate the resistance genes.[11] Conjugation experiments using azide-resistant *Escherichia coli* J53 as the recipient were performed with a filter mating method.[12] Replicon typing and plasmid multilocus sequence typing were used to characterize the resistance plasmids with published methods.[13,14]

Results

The six isolates of *S. enterica* serogroup C1 were all confirmed to be *S. enterica* serotype Oranienburg. Five of the isolates were found to have almost identical PFGE patterns, with a minor difference demonstrated by an extra band of slightly larger than 100 kb in SC836 and SC837 (Fig. 1A, upper panel, arrowhead). SC773 was the only isolate showing a completely different PFGE pattern (Fig. 1A, upper panel). Actually, SC773 and SC773B were both isolated from the only stool specimen subjected for microbial culture examination. During the preliminary PFGE analysis, SC773 was found to have a different PFGE pattern compared to those demonstrated by the other blood isolates. SC773B was subsequently identified with an extra effort trying to find other isolates from the original stool culture media that may have the same PFGE patterns as those of the blood isolates.

A bla_{CMY-2} gene was identified in SC836 and SC837, the last two blood isolates that showed ceftriaxone resistance. No other genes for extended-spectrum β-lactamases or plasmid-mediated AmpCs were further identified. Plasmid profile analysis also revealed an additional 120-kb plasmid in SC836 and SC837 (Table 1; Fig. 1B, upper panel). The bla_{CMY-2} gene was found to be carried by this plasmid, which was later proved to be a self-transferrable IncI1 plasmid with a sequence type 53 (Fig. 1B, lower panels). The extra band identified by PFGE analysis in SC836 and SC837 was also due to the additional bla_{CMY-2}-carrying IncI1 plasmid as revealed by DNA–DNA hybridization (Fig. 1A, lower panel). Transfer of the bla_{CMY-2}-carrying IncI1 plasmid from SC837 to *E. coli* J53 resulted in the increase of ceftriaxone MICs from 0.125 μg/mL to 32 μg/mL in the recipient (Table 1).

Discussion

The standard therapeutic choice for serious *Salmonella* infections in children is ceftriaxone. In the present study, standard antimicrobial therapy had been given and the clinical presentation had been improved when the patient was allowed to be discharged during the first hospitalization. Unfortunately, the patient had to be re-admit to the hospital due to a relapse of the infection. Recurrent invasive nontyphoid *Salmonella* infection is common among immunocompromised patients, despite appropriate antimicrobial therapy.[15,16] Our patient does not have other significant disease history, nor did the image study reveal any focal infection. The young age, which includes her in the most susceptible population for suffering from *Salmonella* infection,[1] may be one of the key host factors leading to the relapse of the infection by the same organism while with additional ceftriaxone resistance. However, although the change in the ceftriaxone susceptibility had not been finally reported at the discharge of the patient, a preliminary report did show that there were remaining *Salmonella* organisms in the patient's blood. This might be a strong hint that the patient should stay for a longer period in hospital until all the bacteria have been cleared. The case also suggests that for an invasive *Salmonella* infection, particularly in young children, eradication of the infection as evidenced by microbial culture results may be one of the

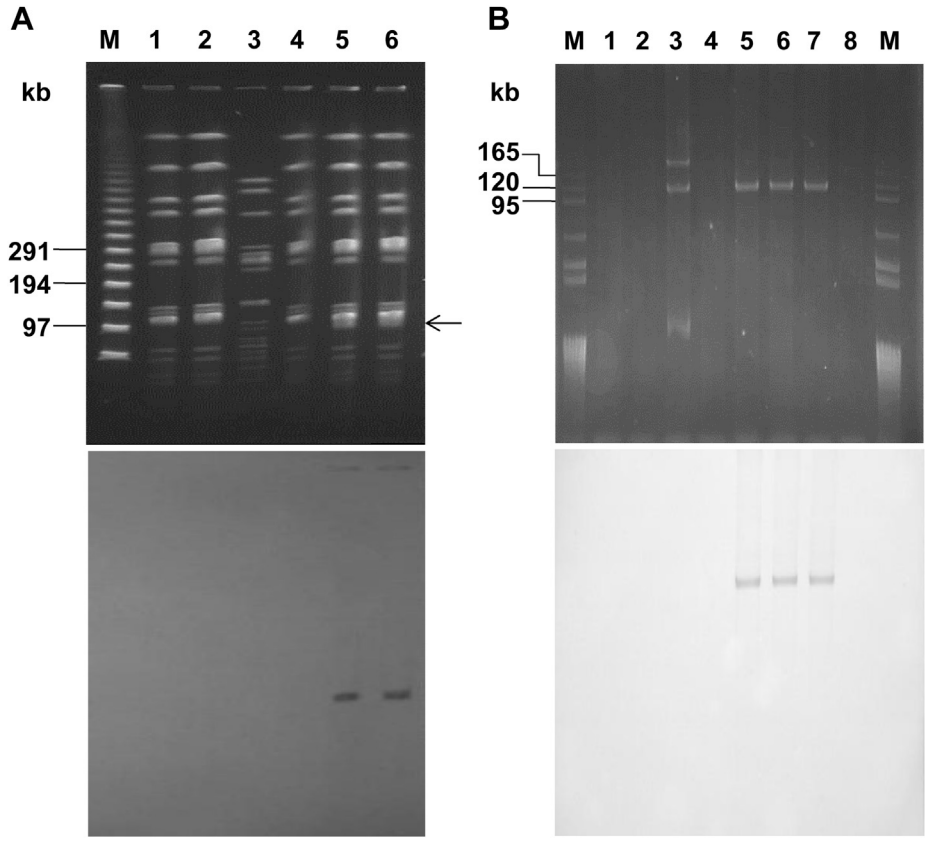

Figure 1. (A) Pulsed-field gel electrophoresis patterns and (B) plasmid profiles of the isolates studied. DNA-DNA hybridization results are shown in the corresponding lower panel. Hybridization with CMY-2 or IncI1 probes demonstrated the same results. M = λ DNA concatemer standard (A) or plasmid size markers (B); Lanes 1—6, *Salmonella enterica* serotype Oranienburg SC834, SC773B, SC773, SC835, SC836, and SC837; Lanes 7 and 8, *Escherichia coli* J53/pSC837, and *E. coli* J53 (B). The extra band presented in SC836 and SC837 is indicated by the arrowhead (A).

most important prerequisite factors, other than the remission of clinical symptoms and signs, to discharge the patient.

Recurrence of invasive nontyphoid *Salmonella* infection after antimicrobial treatment may be due to a relapse infection with the same organism that developed antimicrobial resistance during therapy, and/or reinfection with a different strain that had been resistant to the antibiotics used.[15] Recurrence of *Salmonella* infection after ciprofloxacin or ceftriaxone treatment has been reported.[17,18] With PFGE analysis, we were able to show that the secondary infection was actually a relapse infection of the prior bacteremia. By acquiring the bla_{CMY-2}-carrying IncI1 plasmid, the organism became resistant to both ceftriaxone and cefixime. Fortunately, the patient readmitted to this hospital, with the documentation of previous culture results, the antimicrobial agent was changed to imipenem, and the illness was soon controlled. The successful treatment of the relapse infection with imipenem provides further evidence that carbapenems can be used for the treatment of severe *Salmonella* infection with ceftriaxone resistance, especially in children or when the causative agent also demonstrated ciprofloxacin resistance.[18] However, development of carbapenem resistance during therapy for nontyphoid *Salmonella* infection has been reported.[19] Physicians are advised to use with caution and follow-up microbial examinations are necessary to monitor the therapeutic effect.

Infection caused by *S. enterica* serotype Oranienburg can progress to become severe infections, including sepsis, and the subsequent focal infections such as aneurysm, cholecystitis, osteomyelitis, and abscesses in various organs or tissues.[20—25] Timely use of appropriate antimicrobial therapy is therefore required for a satisfactory recovery. However, resistance to the antimicrobial agent used could occur during therapy as described herein. Ceftriaxone resistance in *S. enterica* serotype Oranienburg has been linked to the production of CTX-M-2, CTX-M-3, and CTX-M-14.[26—28] In the present study, the acquisition of the bla_{CMY-2}-carrying IncI1 plasmid in the *S. enterica* serotype Oranienburg appears to be the major reason for the ceftriaxone resistance, leading to the subsequent relapse infection in the patient.

Previously we have demonstrated the prevalence of the bla_{CMY-2}-carrying IncI1 plasmid among several *Salmonella* serotypes that belonged to the serogroups B and D.[29] In the present report, the resistance plasmid was found to have been transmitted into a serogroup C serotype, Oranienburg. Compared to adult patients, children appear to be more vulnerable to nontyphoid *Salmonella* infection.[1] Although

ceftriaxone is the most frequently used antimicrobial agent in treating pediatric patients with invasive or severe *Salmonella* infection, cautions should be taken regarding the possibility of resistance development during therapy. It is especially important in areas with a widespread of ceftriaxone resistance genes that are carried by self-transferrable plasmids, such as the bla_{CMY-2}-carrying IncI1 plasmid identified herein.

Conflicts of interest

All authors have no conflicts of interest.

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