

A School Outbreak of *Shigella sonnei* Infection in China: Clinical Features, Antibiotic Susceptibility and Molecular Epidemiology

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Objective: To describe the clinical features of infection, and the antibiotic susceptibility of epidemic strains, and investigate plasmid maps and integrons of the isolates from an outbreak of *Shigella sonnei* infection at an elementary school in southwest China.

Study design: Cross-sectional study.

Setting: An elementary school and five hospitals in Chengdu in southwest China.

Results: There were 1,134 students in the school. 937 (82.6%) students had signs and symptoms. Of the 568 (60.6%, 568/937) hospitalized students, 93.3%, 86.8%, 72.4%, and 28.9% of the hospitalized patients had diarrhea, fever, abdominal pain, and

vomiting, respectively. *S. sonnei* strains were isolated from the stool samples of 36.0% (337/937) students. All of the outbreak isolates had the same high-level antimicrobial resistance and plasmid profiles, which were different from that of sporadic strains. All the outbreak *S. sonnei* isolates were positive for the *integrin* gene and contained class 2 integron; however, two outbreak isolates contained class 1 and class 2 integrons.

Conclusions: Diarrhea, fever, and abdominal pain were the three most common clinical manifestations observed in patients infected with *S. sonnei*. High-level antibiotic resistance was observed among *Shigella* species.

Key words: Antibiotic susceptibility, China, Outbreak, *Shigella sonnei*.

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Shigella infection remains a serious public health problem in developing countries where sanitation is poor [1,2]. In China, the incidence of shigellosis was 32.1 cases per 100000 population in 2006 [3]. In southwest China, during the period from 1 September 2006 to 10 September 2006, many students from the same school presented to the hospital with diarrhea, abdominal pain, vomiting, and fever. Stool cultures of these patients were positive for *Shigella sonnei*. This study was performed to investigate the clinical characteristics, antibiotic susceptibility and molecular epidemiology of this outbreak of *S. sonnei* infections in an elementary school in southwest China.

METHODS

All students in the concerned school were subjected to physical examination. Children with signs and symptoms were separated and given oral antibiotics. All food and beverage supplied by the school canteen were sampled, and every food handler was screened by interview and

stool culture. Drinking water and surface of cookers were also sampled for biochemical detection. An investigation of food consumed by these patients recently and the procedures of food processing and storage of the school canteen was carried out under the direction of epidemiologists to find out the origin of the epidemic.

Case definition and case ascertainment: A suspected case was defined as follows: after 1 September, 2006, any student at the school who had at least two of the following clinical manifestations: acute onset of diarrhea, >3 loose stools/day; blood and/or mucus in stool; fever; and abdominal pain. A confirmed case was a suspected case whose stool specimen was cultured positive for *S. sonnei*.

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Bacterial strains: Stool specimens of all who had signs and symptoms were screened for cholera by dark-field microscopy, and were cultured on xylose lysine deoxycholate agar (Oxoid, UK) and MacConkey agar (Oxoid, UK), respectively for *Shigella*, enteropathogenic

Escherichia coli, and *Salmonella*. *S. sonnei* strains were identified by conventional biochemical tests and serological tests with monovalent antisera (Denka Seiken, Japan). Antibiotic susceptibility tests were performed using the standard disk diffusion method according to the National Committee for Clinical Laboratory Standards [4]. A total of thirteen *S. sonnei* strains obtained from sporadic clinical cases in this region between 2004 and 2006 were studied, for comparison of the antibiotic resistance and plasmid profiles between the outbreak and sporadic strains.

Plasmid analysis: 20 randomly selected isolates of *S. sonnei* from this outbreak were analyzed. Plasmid DNA was extracted by the Biospin Plasmid DNA Extraction Kit (Hangzhou Biotech Co, Ltd, China) according to manufacturer's instructions, and was separated by horizontal agarose gel electrophoresis. After the electrophoresis, the gel was stained with ethidium bromide and video images were performed by a gel documentation system. The molecular mass of the unknown plasmid DNA was assessed by comparing its mobility with that of a super-coiled DNA ladder with known molecular mass.

Integron analysis: DNA was extracted from each strain. And integrons were detected by PCR with the degenerate primers hep35 (5'-TGCGGGTYAARGATBTKGATTT-3') and hep36 (5'-CARCATGCGTRTARAT-3'). The reaction conditions of PCR amplification were as follows: 1 cycle of 95°C for 5 min, 30 cycles of 94°C for 50 s, 55°C for 90 s and 72°C for 60 s, and 1 cycle of 72°C for 5 min. The amplified DNA products were analyzed by conventional 1.5% (wt/vol) agarose gel electro-phoresis in 1×TBE buffer and stained with ethidium bromide. Cassette PCR products were restricted with *HinfI*. The sequences of amplicons were confirmed by restriction analysis with the restriction endonucleases *HinfI* in a water bath at 37°C for 4 hours. Sizes of the digestion products were analyzed by electrophoresis on a 2.0% agarose gel. The digestion fragments were classified as per White Classification [5].

RESULTS

In the elementary school where the outbreak took place, there were 1,134 students and 140 faculty and staff members. The first reported case had abdominal pain and diarrhea 7 hours after common lunch at the school canteen. The number of reported cases peaked 2-3 days after the first case. 937 students had different manifestations (**Table I**). Among students with manifestations, 568 were hospitalized, including 306 boys and 262 girls. The mean age of the 568 cases was 8.4 years (range 5 to 13 years). Mean incubation period of

these hospitalized patients was 2 days (ranging from 7 hours to 9 days). The first laboratory-confirmed case occurred on 3 September, and the last on 13 September. Four teachers had mild abdominal pain and diarrhea. None of them was hospitalized and they recovered well in two to three days. In the samples of food and general environments from the school canteen, the cold meat made of cold cooked pork was positive for *S. sonnei*. However, no evidence of origin of contamination of the meat was found.

S. sonnei strains were isolated from 337 out of 937 stool samples. All the 337 *S. sonnei* strains had the same antimicrobial susceptibility. They were resistant to amoxicillin, trimethoprim/sulfametho-xazole (TMP-SMX), gentamicin, piperacillin, ticarcillin, cefuroxime, and cephalothin, and were susceptible to cefoxitin, imipenem, ciprofloxacin, amoxicillin/clavulanic acid, cefotaxime, cefopera-zone, fosfomycin, netilmicin, tobramycin and amikacin. The earlier isolated 13 sporadic strains of *S. sonnei* had different antimicrobial susceptibility as compared to the outbreak strain.

At the early stage of the outbreak, empirical antibiotic treatment was given to the patients at the discretion of the attending physicians. As soon as the *S. sonnei* strains were isolated and antimicrobial susceptibility was obtained, a 7-day intravenous antibiotic treatment plan of ceftazidime (50 mg/kg every 12 h) or cefotaxime (50 mg/kg every 12 h) was administered to the patients, and the follow-up stool cultures were carried out on the fifth day after treatment. The next course of treatment was given if the follow-up stool cultures were still positive. Hospitalized patients whose two consecutive stool cultures were negative for *S. sonnei* were allowed to go home. Patients who were not hospitalized received oral

TABLE I CLINICAL MANIFESTATIONS AND LABORATORY EVALUATION IN THE STUDY SUBJECTS

<i>Clinical/laboratory finding</i>	<i>n (%)</i>
Fever	493 (86.8)
Abdominal pain	411 (72.4)
Diarrhea 530 (93.3)	
Bloody diarrhea 18 (3.8)	
Watery diarrhea 319 (34.0)	
Mucoid diarrhea 193 (56.2)	
Vomiting	164 (28.9)
Shakes	75 (13.2)
Tenesmus	21 (3.7)
Convulsions	2 (0.4)
Leukocytosis	348 (61.3)
Hypokalemia	230 (40.5)

WHAT IS ALREADY KNOWN?

- Antibiotic resistance in *Shigella* organisms is an emerging problem.

WHAT THIS STUDY ADDS?

- High-level antibiotic resistance was observed among *S. sonnei* isolated during a school outbreak in southwest China.

antibiotic treatment with cefixime (3 mg/kg every 12 h for 3 days). After treatment, manifestations had disappeared within 2-3 days in 58.8% children. No mortality was reported and there were no serious complications.

Plasmid analysis revealed that all the 20 *S. sonnei* strains had the same plasmid profile. Plasmid profiles of the outbreak strains were different from those of the sporadic strains. All *S. sonnei* isolates except one sporadic strain were positive for the integrons, and class 1 and class 2 integrons were detected. The results of gel electrophoresis showed that 3 strains (including 1 sporadic strain and 2 outbreak strains) contained class 1 integrons, and class 2 integrons were found in 31 strains (including 11 sporadic strains and 20 outbreak strains). One sporadic isolate which was resistant to ciprofloxacin contained only class 1 integron. No class 3 integron was detected.

DISCUSSION

S. sonnei is an important cause of acute gastroenteritis in both developing and developed countries. It is the second most common serotype in China where *S. flexneri* is the most common serotype [3]. In this outbreak, 82.6% of all students who had lunch at school had manifestations. To our knowledge, this high attack rate has been rarely reported before.

Diarrhea, fever, and abdominal pain were the three most common clinical manifestations observed in the patients infected with *S. sonnei* in this study. Compared with a previous report about outbreak of *S. flexneri* infections [6], the incidence of diarrhea, fever, abdominal pain and vomiting of *S. sonnei* infections is much lower.

High-level antibiotic resistance to many antibiotics recommended in the treatment of shigellosis was observed among the epidemic and sporadic *S. sonnei* strains. This is in accordance with other studies [7-9]. Antimicrobial resistance among *Shigella* organisms is an emerging problem. Resistance to ampicillin and TMP-SMX is common [10]. *S. flexneri* strains are much more resistant to most antibiotics presently used in the treatment of shigellosis than *S. sonnei* strains. Multi-resistance of *Shigella* strains is also striking [7, 8, 11].

In this outbreak, children with severe shigellosis showed good clinical response to the third generation cephalosporin such as cefotaxime and ceftazidime. However, resistance of *S. sonnei* to the third generation cephalosporin has also been reported [7,9]. Although all outbreak *S. sonnei* strains were sensitive to ciprofloxacin in our study, fluoroquinolones are not recommended in children in China because there remains doubt about the potential damage of fluoroquinolones to growing cartilage [2,10].

In our study, plasmid profile analysis indicated that these outbreak isolates came from the same source. However, because it relies on the detection of extra-chromosomal genetic elements which can be lost [12], plasmid profile analysis technique has its limitations, which are not seen with Pulsed-Field Gel Electrophoresis (PFGE) [13]. PFGE could not be done due to limited resources.

High prevalence of class 2 integrons in *S. sonnei* isolates has been observed in our study, which is comparable with previous reports [8,14]. Resistance of *S. sonnei* strains to ciprofloxacin has also been observed in other reports [15, 16]. Not all the isolates were examined for plasmid profiles and integrons, which may limit the strength of our conclusion in this regard.

In conclusion, high rate of antimicrobial resistance as well as high prevalence of class 2 integrons among *S. sonnei* species was observed in this study. It is mandatory to continuously monitor the local antibiotic resistant patterns of *Shigella* species to help to formulate appropriate treatment plans.

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