A Shigella Infection in Chushan, Nantou County

Abstract

The purpose of the study is to describe the process of investigation, laboratory testing, and control of a Shigella infection occurred in Chushan of Nantou County in late October 1998. The study also presents laboratory findings of the drug sensitivity test, plasmid profile, and pulsed field gel electrophoresis typing of Shigella strains for the future reference of molecular epidemiology in evidence collection and diagnosis of similar incidents. This Shigella infection had seven confirmed cases of *Shigella sonnei* in two families and one school. Findings of molecular typing in laboratory of specimens collected from Shigella positive cases and Shigella strains collected from controls showed that the nine strains isolated from Chushan and the one strain isolated from a patient in Puli in May 1998 shared the same drug sensitivity, plasmid, and pulsed field gel electrophoresis profiles. This seemed to indicate that both the Chushan and the Puli cases could have been infected by a common strain. The dates of onset, and the fact that both incidents could have been induced by a common strain indicated that the mode of transmission of the present Shigella infection was through person-to-person contact.

Introduction

Bacillary dysentery is a major gastrointestinal infection. Epidemics are still common in areas of poor sanitary conditions. Usual symptoms are vomiting, fever, diarrhea, or bloody stool\(^{(1)}\). Patients may develop sever hemolytic hematuria if infected by shiga toxin strain, leading to renal failure. Fatality can be
as high as 40% \(^{(2)}\). The pathogenic agent of bacillary dysentery is dysentery bacillus (Shigella), which comes in four groups of *Shigella dysenteriae* (group A), *S. flexneri* (group B), *S. boydii* (group C), and *S. sonnei* (group D). Each strain can be further grouped by serology into one to 18 serotypes \(^{(1)}\). Dysentery bacillus is highly infectious, 10-100 pathogens are sufficient to cause infection \(^{(3)}\). Direct person-to-person contact is the most important mode of transmission. Personal contact is the major cause of small-scale infection among family members. In overcrowded places such as jails, kindergartens, and schools, personal contact can lead to large-scale outbreaks \(^{(4-6)}\). Contaminated drinking water or food can also cause serious outbreaks \(^{(7-10)}\). In endemic areas, houseflies are a common medium of transmission \(^{(11)}\). Control of houseflies is an effective measure in the control of bacillary dysentery \(^{(12)}\).

On 23 October 1998, the Tzushan Hospital of Chushan, Nantou County, reported to the local health station one case of bacillary dysentery, a 10-year old, fourth-grade boy of a primary school in Chushan. He became ill on 19 October and was the index case. Subsequent surveys and laboratory testing of family members, neighbors, schoolmates, and contacts identified seven cases (see Table 1). This report describes the process of investigation, laboratory testing, and disease control, and presents laboratory findings of drug sensitivity test, plasmid profile, and pulsed field gel electrophoresis typing for the future reference of molecular epidemiology in evidence collection and diagnosis of similar incidents.

**Materials and Method**

Detection and Follow-up of Cases:

Rectal swab specimens were collected from contacts of the index case such as family members, neighbors, and schoolmates for case detection. Medical records from mid-October and on of all students of the primary school were reviewed. Any students with diarrhea or fever during this period were considered suspects and rectal swabs taken for laboratory testing.

Definition of Case:

A confirmed case was defined as one who had developed, since mid-October, diarrhea of at least twice a day, and had been isolated dysentery bacillus. A diarrhea case of at least twice a day during the same period without the isolation of dysentery bacillus was considered a suspect.

Survey of the Source of Infection:

Water sources of the households and school of the positive cases were investigated and tested for residual chlorine. Samples of well water and spring water bought from elsewhere were also collected from households of the positive
cases for laboratory testing by the then Central Branch Laboratory of the National Institute of Preventive Medicine (now the Third Branch Bureau of the Center for Disease Control).

Bacteriological Testing and Analysis of Strains:

1. Bacteriological testing: Rectal swabs were cultured with Hektoen Enteric (HE) and Shigella-Salmonella (SS) media at 35°C overnight. Suspected non-fermented colonies were further bio-chemically screened with identification media of Triple Sugar Iron (TSI), Lysine Iron (LI), and Sulfite-Indole-Motility (SIM). Colonies showing TSI red/yellow, H$_2$S negative, lysine fermentation of LI negative, and SIM mobility negative were further serologically tested with Seiken’s antiserum (Denka Seiken, Japan) for agglutination test and typing assessment, and bio-chemically assessed with AP120E (bioMérieux, France).

2. Drug Sensitivity Test, Plasmid Profile and Pulsed Field Gel Electrophoresis Typing

1) Source of Strains: Sources of the 14 S. sonnei strains used for drug sensitivity test, plasmid profile, and pulsed field gel electrophoresis typing are shown in Table 2. Of them, nine were isolated from the present Chushan incident; the rest five were SH10657 and SH11620 strains of the Puli incident of 1998, SH47209 and SH47127 from the east coast, and SH37 from the southern area.

2) Drug Sensitivity Test: Test was conducted with the disk diffusion method$^{(13)}$. Antibiotics tested were ampicillin, cefixime, cephalothin, malidixic acid, tetracycline, amickacin, and gentamicin.

3) Plasmid Profile: Kado and Liu$^{(14)}$ method was used for the detection and isolation of plasmids.

4) Pulsed Field Gel Electrophoresis Analysis: Soldati and Piffaretti$^{(15)}$ method was used for the handling of bacilli. DNA were bisected with Not I and Xba I, and analyzed with Biometra’s Rotaphor Type V electrophoresis (Biometra, Germany).

Control Measures

After the drug sensitivity test, disease control authorities took immediate action for the mandatory medication and isolation of positive cases on one hand, and spot disinfection of door knobs, telephones, tables, chairs and other items in the houses and school that could have been touched by the positive cases. Houses and school of cases were visited and placed under surveillance by the local health station. Suspected cases were taken specimens immediately for laboratory testing. Cases were told to use the same toilet facilities. Soaps were available for hand
washing after toilet use.  Health education on hand washing before meals and after toilet use was given to cases, family members, and students in general.

**Results**

Investigation, Laboratory Testing, and Follow-up of Cases:

Both the younger brother and sister of the index case, surnamed Chang, had developed symptoms of diarrhea and fever. The younger brother (Case No. 3) had also been treated at the Tzushan Hospital of Chushan. Infection within family was evident. On 5 November, 74 rectal swabs were collected for laboratory testing from likely contacts of the index case such as family members, and classmates of the younger sister. *S. sonnei* was isolated in two of them, the younger sister (Case No. 2) of the index case and a student surnamed Liu in a neighboring house across the street (Case No. 4) (Table 1). On 8 November, family members of student Liu were tested to find two more confirmed cases, grandfather (Case No. 5) and younger sister (Cases No. 6) of the Liu student.

As the younger brother of Case No. 4 (the Liu student) had developed fever and sever diarrhea, 24 contacts in his kindergarten and 33 children with diarrhea after 19 October were also tested, though no new cases were identified. In the later surveillance, a classmate surnamed Lee of Case No. 2 (the younger sister) was found to develop fever and diarrhea on 12 November, though was negative on the 5 November screening. His laboratory testing confirmed positive (Case No. 7). His house was not in the neighborhood of the index case; he could have been infected by Case No. 2 in the school. No *S. sonnei* was isolated from Lee’s four family members; no suspected symptoms had developed either. It seemed that no infection had taken place in the Lee family. Subsequently, the then Disease Surveillance Center of the National Quarantine Service (now the Third Branch Bureau of the Center for Disease Control) asked the local health bureau and the school to enforce medication of positive cases and related surveillance. In the meantime, 14 more rectal swabs from classmates of student Lee had been collected for testing. No *S. sonnei* was isolated in any of them. Surveillance and follow-up of positive cases had continued till 7 December when two consecutive laboratory tests showed negative.

Source and Mode of Transmission:

The Chang’s and the Liu’s where cases of bacillary dysentery were detected, bought spring water from elsewhere for drinking. Water from the well around the houses was used for cleansing. The well though was located close to the septic tank, water specimens collected from it were negative of *S sonnei*. The dates of onset in the two families differed by six days, exceeding the incubation period of bacillary dysentery⁽¹⁾. Chances of getting the infection from a common source of
drinking water or the well water were slight. Tap water was used in the school of the index case. The residual chloride of water specimens collected from four washing basins and the kitchen was 0.4 ppm. Contamination of the water source in the school leading to the dysentery infection was unlikely.

All seven cases were infected with the same strain type, S. sonnei. By their dates of onset (Table 1), the mode of transmission could have been person-to-person contact. The index case could have passed the infection to his younger brother and sister. They were classmates of the Liu student across the street, they also visited each other quite often. The infection could have been then transmitted to the Liu family across the street to induce in-family cross infection. The Lee student, being classmate of Case No. 2, could have been infected through contact.

Analysis of Strains:

Findings of the drug sensitivity test, plasmid profile, and pulsed field gel electrophoresis typing of the nine strains isolated from six cases (strains of Case No. 3 not available, the pathogens were confirmed by the Tzushan Hospital) and the five control strains (two from Puli of Nantou County, two from the east coast, and one from the southern part) are given in Table 2. Table 2 shows that the nine strains of Chushan demonstrated similar sensitivity profiles to seven antibiotics as the SH10657 strain of Puli. Their plasmid and electrophoresis profiles were also similar. The Chushan strains and the SH47127 strain from Taitung County though were similar in their drug sensitivity, they were different in plasmid and electrophoresis profiles. They were, however, quite different from the SH11620 strain of Puli, SH47209 strain of Hualien County, and SH37 strain of Pingtung County in their drug sensitivity, plasmid, and electrophoresis profiles. The SH11620 strain of Puli and the SH47209 of Hualien though were similar in drug sensitivity and plasmid profiles, were different in their electrophoresis profile. The SH37 strain of Pingtung was different from other strains in drug sensitivity, plasmid profile, and electrophoresis profile.

Control Measures and Effectiveness:

Control measures such as medication and isolation of positive cases, health education of family members and students, surveillance of the health conditions of students, use of active iodine for disinfection had succeeded in confining the infection to two families and a school. No new cases other than the seven confirmed ones had been detected during the surveillance period.

Discussion

The effective reporting, investigation, laboratory testing, and control measures of the disease control system had successfully contained the incident of Chushan
without developing into an epidemic. The physician who reported the first case to the local health authority was the sentinel physician of the National Quarantine Service. For his prompt reporting, the disease control authority was able to monitor the case. Once the case was confirmed bacillary dysentery, immediate action was taken for field investigation and screening of contacts to decide on the scope of the infection. At the same time, health education was given to family members and school children, cases were isolated, and areas disinfected. These measures had successfully contained the spread of the infection.

Instead of general prophylactic medication, strict surveillance of the school and isolation of cases were enforced. The school was asked to strictly follow-up the health conditions of the children. Antibiotics were given to only those who could have been infected to minimize the use of drugs and to avoid the development of drug resistant strains. When the effect of prophylactic medication was in doubt, the measures taken in the present incident, strict surveillance to understand the development of the infection, isolation of positive cases, disinfection of the surroundings, and health education stressing hand washing, proved to be effective. The main purpose of screening was to detect potential cases and to understand the scope of the infection. After the initial screening of contacts of the index case such as family members and classmates, it was decided that the infection was confined to the family and the next family across the street. A general screening of all school children became unnecessary. Manpower and resources were saved.

Laboratory analysis of pathogens was an effective support to disease control. The drug sensitivity test helped to decide on the effective drugs to be used. Ampicillin, nalidixic acid, and cefixime are commonly used drugs in disease control. Ampicillin is the first priority; nalidixic acid the next; and cefixime, being the third generation of cephalosporin, is the last. Lin and Chang in their 1992 study report\(^{(16)}\) pointed out that 52% and 25% of the dysentery bacillus strains in the southern part of Taiwan were drug resistant to ampicillin and nalidixic acid respectively (Table 2). The present drug sensitivity test showed that the nine Chushan strains were already resistant to ampicillin, but were susceptible to nalidixic acid and cefixime. Drugs will selectively impose certain pressure on microbes, making some pathogens develop resistance to certain drugs. Therefore, laboratories should conduct, at the very early stage of outbreaks, drug sensitivity test for the already isolated strains to help decide on the drugs to be used. As long as the front-line drugs are still effective, they should be used with priority to reduce costs and also to minimize chances of strains developing resistance to second-line drugs.

A correct judgement of the mode of transmission at an outbreak can minimize the costs of prevention and control. Molecular typing of strains can help in the
judgement of the mode of transmission. The molecular typing of strains of the present incident (Table 2) showed that the Chushan strains were similar in their drug sensitivity, plasmid, and electrophoresis profiles to those of the strain isolated from a Puli patient by the Puli Christian Hospital on 18 May 1998. Chances of different strains having similar results on three different analyses (particularly the pulsed field gel electrophoresis profile of strains bisected by two restriction enzymes) are low, chances of the two groups of strains coming from a common source were high. The Puli case is a 20-year-old woman. She visited Penghu two days before the onset. She rarely leaves Puli, and has never been to Chushan. Though there was a five-month interval between this case in question and the Chushan incident, Puli and Chushan are geographically close, dysentery patients often have a fairly long carrier state\(^{(17)}\), and a high proportion of those infected are inapparent\(^{(18,19)}\) and go around unnoticed, chances of the Chushan strains originating from Puli were there. The infection, however, could not have been direct. It could have been spread by a third person as the medium.

Distribution of strains and serotypes is associated with the development stage of an area. General speaking, \textit{S. sonnei} is more prevalent in developed areas\(^{(20,21)}\), whereas \textit{S. flexneri} and \textit{S. dysenteriae} are more prevalent in developing areas\(^{(22,23)}\). Reports of recent years\(^{(24,25)}\) show that Taiwan is somewhere between the two in the distribution of strains. Bacillary dysentery infection is more often induced by \textit{S. flexneri} (especially serotype 2a); outbreaks are by \textit{S. sonnei}, and \textit{S. dysenteriae} is rarely seen. The Central Branch Laboratory of the National Institute of Preventive Medicine (now the Third Branch Bureau of the Center for Disease Control), in their statistical analysis of 50 bacillary dysentery cases in Nantou County in 1998, reported that, of them, 40 were infected by \textit{S. flexneri} 2a, and 10 by \textit{S. sonnei}, including the seven cases of Chushan, two of Puli, and one of Yuchi. The indigenous strain of Nantou County should be \textit{S. flexneri} 2a. \textit{S. sonnei} strain probably is imported.

Typing of strains can provide information for the epidemiological follow-up of the source and mode of transmission. Evidence from the drug sensitivity test, plasmid profile, and pulsed field gel electrophoresis typing suggested that the mode of transmission of the present incident was direct contact by a common strain. The follow-up also suggested that the strain came from the same strain isolated five months ago in Puli. As \textit{S. sonnei} has only one serotype, and the distribution of strain types is localized, \textit{S. flexneri} 2a for instance is more prevalent in Jenai Township of Nantou County, the conventional serological typing method will not apply to the typing of dysentery bacilli. The usefulness of drug sensitivity test and plasmid profile analysis for typing is also limited\(^{(26)}\), they alone cannot be a method of typing. Currently, the pulsed field gel electrophoresis is the most effective method of typing\(^{(27)}\). However, this method, being difficult to operate and quite
time-consuming, cannot be easily performed in a laboratory, and thus cannot meet the urgent demand of disease control. A quick and highly effective typing method based on Polymerase Chain Reaction (PCR) should be developed.

Acknowledgment

The authors wish to thank the Eastern Branch Laboratory and the Southern Branch Laboratory of the former National Institute of Preventive Medicine (now the Sixth and the Fourth Branch Bureaus of the Center for Disease Control, DOH) for providing dysentery bacilli strains as controls for analysis. Contributions of Ms YM Shen and LC Yang of the Central Branch Laboratory of the former National Institute of Preventive Medicine (now the Third Branch Bureau of the Center for Disease Control, DOH) in the isolation and assessment of strains are highly appreciated. Our thanks also go to the staffs of the Nantou County Health Bureau and the Chushan Township Health Station for their help in specimen collection.

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References:


Table 1. Confirmed Cases of Bacillary Dysentery, Chushan, 1998

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
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<td>19 Oct 1998</td>
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</tr>
<tr>
<td>2</td>
<td>8</td>
<td>21 Oct 1998</td>
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<td>4</td>
<td>24 Oct 1998</td>
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<td>4</td>
<td>9</td>
<td>28 Oct 1998</td>
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<td>5</td>
<td>56</td>
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<td>7</td>
<td>8</td>
<td>12 Nov 1998</td>
<td>Lee, classmate of Case No. 2</td>
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Table 2. Sources of Strains and their Drug Sensitivity Test, Plasmid Profile, and Pulsed Field Gel Electrophoresis Analysis

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<tr>
<th>Strains</th>
<th>Source</th>
<th>Location</th>
<th>Date of Isolation</th>
<th>Ap/Cfm/Kf/Na/</th>
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*Ap: ampicillin; Cfm: cefixime; Kf: cephalothin; Na: nalidixic acid; T: tetracycline; Gm: gentamicin; An: amikacin.

**R: resistance; I: intermediate; S: susceptible