
Confirmation of Homologous Strains as the Cause of
Bacillary Dysentery Outbreaks in Nanjhuang Township of
Miaoli County and Heping Township of Taichung County
by Molecular Epidemiology

Shao-Hui Tsai¹, An-Ju Chen¹, You-Wun Wang², Pei-Tsan Liao¹, Tsuey-Fong Lee¹,
Chih-Tsung Tu¹, Li-Shu Tsai¹, Hsiao-Lun Wei², Chien-Shun Chiou²

¹ Third Branch of Center for Disease Control, Department of Health

² Central Taiwan Laboratory of Center for Laboratory Research

Abstract

Between Nov. 18 and Dec. 5 in 2005, an outbreak of bacillary dysentery caused by *Shigella sonnei* occurred in Nanjhuang Township of Miaoli County. Twelve cases were identified (asymptomatic positive rate was 8.3%). Cases included the index case, his/her family members, his/her neighbors and relatives of the neighbors, with an infection rate of 14.5% (12/83). Between Jan. 1 and 23 in 2006, an outbreak caused by *S. sonnei* also occurred in Heping Township of Taichung County. Five positive cases were identified, including the index case, his/her classmates and their family members, and the director of the class, with an

infection rate of 14.7% (5/34). The average infection rate of the two outbreaks was 14.5%, and the positive rate of water specimens was 0% (0/82). Since the two outbreaks had close temporal and spatial relationships, pulsed-field gel electrophoresis was used to analyze the DNA fingerprint maps of isolated strains, the results of which suggested that the strains in the two outbreaks were homologous. Subsequent disease surveillance also confirmed the relationship between these two outbreaks: that is, the outbreak occurred in Heping Township was related to two infected cases in Nanjhuang Township. We suspected that the two cases brought the bacteria to Heping Township, leading to subsequent transmissions by contact. Comparing with the *Shigella* DNA fingerprint map database of the CDC, the strains in the two outbreaks were most closely related to strains from Indonesia.

Introduction

Shigellosis is a major cause of diarrhea in developing countries [1]. According to WHO, 164 million people are infected annually, leading to the death of 675 thousand to one million children under the age of 5 [2]. *Shigella*, including *Shigella dysenteriae* (group A), *S. flexneri* (group B), *S. boydii* (group C), and *S. sonnei* (group D), is the pathogen of shigellosis. *Shigella* is highly infectious, causing disease by only 10 – 100 bacteria [3, 4]. Shigellosis is transmitted by fecal-oral transmission through human-to-human contact or contaminated food and water [5]. In crowded and unhygienic areas, *Shigella* can cause large-scale outbreaks. Children and elders are risk groups of the disease [6, 7]. Taiwan is not a *Shigella* prevalent area. Strains isolated are mainly *S. flexneri* and *S. sonnei*, with the former prevalent in aboriginal tribes in mountainous areas [8], and the later frequently causing outbreaks in lowland areas, schools, nursing homes and military camps [9]. *S. dysenteriae* and *S. boydii* are rare in Taiwan, and cases are often imported from foreign countries.

On Nov. 26, 2005, Wei-Gong Hospital in Miaoli County reported a case of bacillary dysentery, and after investigation by health authorities, new cases were

discovered. Twelve additional cases were identified, establishing a cluster infection. On Jan. 14, 2006, health authority of the Taichung County was also notified of a bacillary dysentery case from the Fong Yuan Hospital. Ensuing investigation revealed a cluster of five positive cases. Since the strains isolated from the two clusters were both *S. sonnei*, and the outbreaks had close temporal and spatial relationships, we suspected the cluster of bacillary dysentery in Heping Township might be related to the infection in Nanjhuang Township.

Pulsed-field gel electrophoresis (PFGE) has recently been applied to bacterial molecular typing. PFGE has high typing power and reproducibility, and results of PFGE could be applied to tracking transmission of bacterial strains and investigation of outbreaks of transmissible diseases. The CDC established a PFGE protocol in 2002 to standardize routine typing of several pathogenic bacteria and has constructed a PFGE DNA fingerprint database that includes several types of bacteria strains. In several of CDC's previous research projects, PFGE has also been shown to have advantages in the investigation of bacillary dysentery infection [12, 13]. In this study, we used PFGE typing and drug sensitivity tests to analyze *S. sonnei* strains isolated in order to investigate the relationship between these two outbreaks. The PFGE map was also compared with the Shigella DNA fingerprint database of the CDC to trace the origin of the bacteria. Our results showed that the two outbreaks were related and that the strains isolated were from Indonesia. Our study also demonstrated the value of PFGE typing and DNA fingerprint database in investigating the epidemiology of bacillary dysentery.

Materials and Methods

Disease surveillance and specimen examination

Contacts of the index cases, including their family members, close relatives, neighbors and classmates, were interviewed by health authorities. Rectal swabs were also taken and examined by the CDC laboratory to identify possible cases of bacillary dysentery. Health status of students in the schools of the index cases was

also examined, and rectal swabs were taken from students having diarrhea. Since there were only 77 teachers and students in the school of the index case in Heping Township, rectal swabs were taken from all of them by the Health Bureau of Taichung County. The surveillance and rectal swab examination were repeated once a new case was identified. A total of 66 and 113 specimens were collected by the health bureaus of Miaoli County and Taichung County respectively. After all positive cases showed negative results in two consecutive tests, the surveillance for the outbreak in Nanjhuang Township of Miaoli County was ended on Dec. 29, 2005, and surveillance for the outbreak in Heping Township of Taichung County was concluded on Feb. 14, 2006.

Case definition

“Confirmed cases” had more than two diarrhea episodes in one day and were positive for *Shigella* five days before or after the onset date of the index case. “Suspected cases” had the symptom of diarrhea, but were negative for *Shigella* in two consecutive examinations. “Asymptomatic carriers” had no diarrhea, but were positive for *Shigella*.

Tracing infection sources

- (1) Water sources at the residences and schools of the index cases were examined for residual chloride, and 82 water specimens taken from the kitchens, bathrooms, water towers and fountains were examined in the CDC laboratory.
- (2) Local medical doctors around the houses of index cases were surveyed in order to find suspected bacillary dysentery cases before the onset of symptoms in the index cases. Neighbors of the index cases were also surveyed to identify possible infection sources, including foreign brides, foreign labors, or diarrhea patients with relevant travel history.

Bacteria isolation and identification

Rectal swabs were delivered in Cary-Blair transport media at low temperature to the Central Taiwan Laboratory of the CDC Laboratory Center. Specimens were then cultured in Hekton Enteric (HE) and *Shigella-Salmonella*

(SS) media at 35 °C overnight. Suspected non-fermenting colonies were cultured in Triple Sugar Iron (TSI), Lysine Iron (LI) and Sulfite- Indol-Motility (SIM) media for biochemical identification. Colonies that showed red/yellow on TSI or were negative for H₂S, for lysine fermentation on LI and for motility on SIM were further identified and typed serologically by Shigella antiserum (Denka Seiken, Japan) [13] and biochemically by API20E (bioMérieux, France).

Drug sensitivity test

Drug sensitivity test was done by the disk diffusion method [10]. Eighteen antibiotics including amikacin (AN, 30 μg), ampicillin (AM, 10 μg), cefazolin (CZ, 30 μg), cefixime (CFM, 5 μg), cefotaxime (CTX, 30 μg), cephalothin (CF, 30 μg), chloramphenicol (C, 30 μg), ciprofloxacin (CIP, 5 μg), enrofloxacin (ENO, 5 μg), gentamicin (GM, 10 μg), kanamycin (K, 30 μg), nalidixic acid (NA, 30 μg), norfloxacin (NOR, 10 μg), ofloxacin (OFX, 5 μg), penicillin (P, 10 U), streptomycin (S, 10 μg), tetracycline (TE, 30 μg) and tobramycin (NN, 10 μg) were used.

PFGE map analysis and comparison

Standardized PulseNet method from the US CDC was used for bacteria embedding, lysis and gel washing. Chromosomal DNA was digested by NotI and XbaI and analyzed by CHEF MAPPER (Bio-Rad Laboratories, Hercules, CA, USA). After ethidium bromide staining, gel images were taken by Kodak Electrophoresis Documentation and Analysis System 290 (Kodak; Rochester, NY) and saved as TIFF files. PFGE maps were then analyzed by BioNumerics (Applied Maths, Kortrijk, Belgium), saved into the Shigella DNA fingerprint database of the CDC, and compared with other saved maps to identify the possible origin of the strains.

Results

Epidemiological investigation of the bacillary dysentery outbreak in Nanjhuang Township of Miaoli County:

Index case 1 (N1, table 1) lived in the East Village of Nanjhuang Township.

The symptoms occurred on Nov. 18, 2005. There were four people in the family. The younger (case N2) and older brothers of the index case developed diarrhea on Nov. 23 and 26 respectively, and were sent to Wei-Gong Hospital. This suggested transmission between family members. Index case 1 and his/her older brother were attending elementary school, and their younger brother was not. There were 299 teachers and students in the elementary school. Health surveillance revealed no symptoms among teachers, but three classmates of index case 1 (dates of onset were between Nov. 26 and 28; lived in East Village, West Village and Nanjiang Village respectively), eight students in other classes (dates of onset between Nov. 24 and Dec. 1; lived in East Village and Nanjiang Village; one of them is case N8), and one toddler in the kindergarten of the elementary school (date of onset was Nov. 28; lived in Nanjiang Village) developed diarrhea after Nov. 24, 2005. We suspected that the disease had spread in the school, and hence we further investigated the health status of family members of the symptomatic students. However, no other diarrhea patients were found. Since index case 1 was admitted, his/her two brothers lived with their grandfather temporarily, and the family members in their grandfather's house (in Nanjiang Village, 19 people) had also been investigated. Six were found to have diarrhea, and three neighbors (belonging to two families, living with other four and five people respectively) were also found to have the same symptom, suggesting that the disease might have spread from East Village to Nanjiang Village (See Fig. 1 for geological locations of positive cases) and from within a single family to the community. Between Nov. 28 and Dec. 5, rectal swabs were taken from contacts, including the family members of index case 1, all students having diarrhea, and family members as well as neighbors of the grandfather of index case 1, by the Health Bureau. On Dec. 1, 2005, one *S. sonnei* positive case, the younger brother of index case 1 (case N2), was identified. Seven cases were identified on Dec. 4. Among them, four (case N3 - N6, Table 1) were family members of the grandfather of index case 1, and the other 3 (case N8 - N10) were neighbors of the grandfather. Another *Shigella* positive case (case N7) was found on Dec. 6,

who was a family member of the grandfather of index case 1 and was an asymptomatic carrier. Students in the elementary school were not infected except index case 1 and one neighbor of the grandfather of index case 1 (case N8), suggesting that the disease was not spread to the school.

On Dec. 8, 2005, Wei-Gong Hospital in Miaoli County reported another 77-year-old case (case N11), who lived in Dongho Village of the Nanjhuang Township. The date of onset was Dec. 1. Disease surveillance revealed that the case was also a stroke patient and the main care giver was his/her daughter-in-law, who lived in Nanjiang Village and was a neighbor of the grandfather of index case 1. In order to take care of her mother-in-law (case N11), she together with her grandson lived alternatively in Nanjiang Village and Dongho Village, and her grandson often played with children in the family of the grandfather of index case 1. Although no positive case in this family was identified by the Health Bureau, they were still considered a possible infection source for case N11. Besides, on Dec. 1 and 2 when case N11 was ill, his/her granddaughter-in-law (case N12) from Kongkung Village acted as the care-giver. Case N12 began to have diarrhea on Dec. 4. According to the health surveillance conducted by the Health Bureau of Miaoli County, cases N11 and 12 were infected by *S. sonnei*, suggesting that the disease had spread from Nanjiang Village to Dongho Village and Gonggung Village.

The outbreak in Nanjiang Village of Miaoli County had 11 confirmed cases, 11 suspected cases, and 1 asymptomatic carrier, with an infection rate of 14.5% (Table 3). The geographical distribution (Fig. 1) and the epidemiological curve (Fig. 2A) showed that the outbreak might have three waves of infection. The first one (Nov. 18 to 23, 2005) occurred in the family of index case 1, the second (Nov. 23 to 29, 2005) spread to neighbors and family members of the grandfather of index case 1, and the third (Dec. 1 to 4, 2005) occurred in the family members, who lived in Dongho Village and Gonggung Village, of the grandfather of index

case 1. The mode of transmission was human-to-human transmission by contact of family members and neighbors. The area of infection covered three villages of two townships (Table 3).

Epidemiological investigation of the bacillary dysentery outbreak in Heping Township of Taichung County

The five family members of index cases 1 and 2 had no diarrhea. Between Jan. 7 and 8, 2006, index case 2 was living with his/her grandfather (who stayed with four family members). The grandfather had diarrhea on Jan 11, 2006. Index case 2 was attending an elementary school which, together with its kindergarten, had 60 students. The class director and one classmate of index case 1, and eight students in the other classes and one of their family members had diarrhea. Between Jan. 12 and 24, 2006, the Taichung County Health Bureau subsequently examined rectal swabs taken from index case 2, and his/her grandfather, class director, contacts of his/her symptomatic classmates, and teachers and students from his/her elementary school. On Jan. 20, 2006, two cases, a classmate and the class director of index case 2 (cases H2 and H3, Table 2), were identified as positive for *S. sonnei*. On Jan. 23, two other cases, family members of index case 2 (cases H4 and H5), were identified as positive for *S. sonnei*. In summary, five confirmed cases and nine suspected cases were identified in this outbreak, with an infection rate of 14.7% (Table 4). The epidemiological curve (Fig. 2B) suggested that this outbreak might include two to three waves of infection. The first was contact transmission in school (Jan. 7 and 8, 2006) and the second was transmission within family (Jan. 12 to 17, 2006) (Table 4).

Laboratory diagnosis and strain analysis

The pathogens in these two outbreaks were *S. sonnei* as confirmed by bacterial culture. Twelve positive cases were identified in Nanjhuang Township of Miaoli County (Table 1), and five were identified in Heping Township of Taichung County (Table 2). Four cases in Nanjhuang Township were later

confirmed to be positive for *S. sonnei*. In summary, 21 strains were isolated in these two outbreaks. All strains were subjected to PFGE map analysis and drug sensitivity test for 18 drugs. We filed the PFGE maps obtained into the Shigella DNA fingerprint map database at the CDC and compared them with other 1,700 maps within the database to look for closely-related strains. The dendrogram (Fig. 3) revealed that the 21 strains isolated had 4 different PFGE maps (I, II, III and IV, C05.2121 and C05.2163 had one different DNA fragment, but the software failed to discriminate between them). There was a high homology between these four maps, with differences ranging from one to three fragments. Strains isolated from Nanjhuang Township and Heping Township shared the same PFGE genotype (I), but later cases (N6, N9 and N10) in Nanjhuang Township had variant strains with new PFGE maps (Table 1, Fig. 3). Case N9 had two different strains with different PFGE maps (III and IV) isolated four days apart. According to the drug sensitivity test (Fig. 3), these two strains should have simultaneous presence in the strains isolated in the first time.

While comparing with maps in the Shigella DNA fingerprint database, the strains isolated were found to have close relationship with a strain from Indonesia (N05.0012, Fig. 3). The strain from Indonesia was taken into Taiwan by an airline worker in March 2005. The strain from Indonesia and genotype I had difference in one DNA fragment (indicated by arrow in Fig. 3) and different drug sensitivity.

The results of drug sensitivity test revealed that, among the 21 strains, 20 of them had the same drug sensitivity map (when differences in intermediate, sensitive and resistant phenotypes are not considered, Fig. 3), but strain C05.2121 did not have amikacin (AN) and gentamicin (GM) resistance, similar to the Indonesia strain (N05.0012). C05.2121 and Indonesia strain did not have one common DNA fragment (indicated by arrow in Fig. 2), suggesting that the DNA fragment might have resistance gene for AN and GM. Another character of the Indonesia strain was its sensitivity to nalidixic acid.

Infection source and transmission route of the outbreak in Nanjhuang Township of Miaoli County

In this outbreak, all of the five families of the cases had tap water. The average amount of residual chloride was 1.0 ppm. The elementary school also had tap water with residual chloride 0.5 ppm. In addition, water samples from the five families and the school were all negative for *S. sonnei*, and hence it was unlikely that contaminated water was the cause of this outbreak.

To trace of possible infection source, the Third Branch of CDC conducted interviews with neighbors of the index case in East Village of Nanjhuang Township in Miaoli County. The interviews showed that in the area surrounding the index case's residence, the population density was low, ranging from 7 to 8 families within 500m. Except for the family of the index case, who was of an aboriginal background, other neighbors all belonged to the Hakka ethnicity, and hence they seldom interact. After interviewing the five neighboring families and local doctors, we knew that no case of diarrhea occurred in Nov. 2005. Two weeks before the onset of symptoms in the index case, only three to four people went to the doctor due to respiratory tract diseases and chronic diseases. Classmates of the index case also had no diarrhea.

To investigate whether the infection came from foreign countries, we also asked about the immigration status of people around the area. No foreign brides or workers lived in the area. The family of the index case had hired a nurse from Indonesia in 2003, but the nurse had returned to Indonesia in Sep. 2003.

All twelve cases were infected by a single strain of *Shigella* (*S. sonnei*). According to their dates of onset (Table 1), we suspected that the mode of transmission was human-to-human contact. There were many people in the families of the index case. For example, 19 people lived in the 66 m² two-floor townhouse of the grandfather. In addition, there were 12 people in the family of a case (N11) in Dongho Village, and 8 in the family of a case (N 12) in Gonggung Township. There were 4 to 5 people in the families of other confirmed cases. In such crowded living conditions and due to frequent personal interaction, contact transmission should be the mode of transmission. The epidemiological curve also

supports this hypothesis.

Infection source and transmission route of the outbreak in Heping Township of Taichung County

The two families and one elementary school having cases of bacillary dysentery were all using boiled mountain spring water. Water specimens taken from the families and school were negative for *S. sonnei* in tests conducted by the Health Bureau of Taichung County. According to the dates of onset and distribution of positive cases, we suspected that the mode of transmission was contact transmission among teachers and students and among family members.

The results of drug sensitivity tests and PFGE map analyses (Fig. 3) showed that the *Shigella* in Nanjhuang Township of Miaoli County and Heping Township of Taichung County should have come from the same origin, suggesting a relationship between these two outbreaks. Investigations revealed that case N7 and N9 in the outbreak in Nanjhuang Township used to live in Heping Township of Taichung County. Case N7 was a family member of the grandfather of the index case in Nanjhuang Township, and case N9 was a neighbor of the grandfather of the index case in Nanjhuang Township (Table 1). They were infected while living in Nanjhuang Township temporarily in November and December 2005. After Dec. 13, 2005, they returned to Heping Township of Taichung County. They two had three children attending the elementary school and its kindergarten of the index case, and one of them was a classmate of the index case. Although no positive case was identified in the 11 family members of cases N7 and N9 in Heping Township, based on the homology of strains, the temporal and spatial relationships of cases and the interaction between related personnel, we suspected that the strain in the outbreak in Heping Township was from Nanjhuang Township.

Discussion and Suggestion

According to the results of drug sensitivity tests, positive adult cases and their close contacts were treated with ciprofloxacin, and children younger than 8

years of age were treated with azithromycin or cefixime by health authorities. Positive cases and students with diarrhea were asked to leave the school temporarily until negative results were obtained in two consecutive tests. If they were unable to cooperate, schools were asked to isolate them. To reduce the opportunity for contact transmission, schools and houses of positive cases were treated with bleach by health authorities. Water towers in the communities having positive cases were also treated with chloride to increase residual chloride to above 0.5 ppm. Meanwhile, health status of family members and classmates of cases were continuously monitored by health authorities. Examination would be undertaken once a diarrhea case was identified. Health education targeting family of the cases, dwellers in their community and students was also intensified.

In this study, we used field epidemiological surveillance together with PFGE to investigate the *S. sonnei* strains isolated and revealed that the strains from Heping Township had the same PFGE maps as strains isolated from Nanjhuang Township earlier, and that the two outbreaks were related. Hence, disease prevention professionals conducted disease surveillance again to find the epidemiological relationship between these two outbreaks. The two outbreaks demonstrated the typical mode of transmission of bacillary dysentery in Taiwan: cluster outbreaks caused by human-to-human contact between family members, neighbors and classmates, and spread by cases or asymptomatic carriers to a new place to repeat the transmission cycle in the family and the community.

Taiwan is not a *S. sonnei* prevalent area. Although *S. flexneri* is prevalent in mountainous areas, cases of bacillary dysentery are still rare. Hence once there is an outbreak of *S. sonnei* infection, we are interested in identifying the origin of the causative strains. In 2002, Taiwan CDC adapted a standardized PFGE protocol from US CDC [11] to analyze strains of *Salmonella*, *Shigella*, *Neisseria meningitidis* and *Streptococcus pyogenes* in Taiwan, and used BioNumerics to analyze and establish DNA fingerprint maps of bacterial strains. At the end of 2005, more than 10,000 maps have been stored in the database. Among them, there are more than 1,700 *Shigella* maps. This database can be used to compare

and trace strains, monitor *Shigella* in our country, investigate the mode and route of transmission [8, 12, and 13], and exchange disease status and information with international disease prevention organizations. In this outbreak, after comparing the PFGE maps, we found that one strain in the database has a similar map with the strains in this outbreak. The strain was brought into Taiwan in Mar. 2005 by an airline worker who was infected in Indonesia. The Indonesia strain has a DNA fragment different from the strains isolated from Nanjhuang Township and Heping Township (Fig. 3), and hence the fragment may be related to amikacin (AN) and gentamicin (GM) resistance. According to the *Shigella* epidemiology and laboratory data from the Third Branch of CDC, almost all imported strains are sensitive to nalidixic acid. However, strains become resistant to nalidixic acid after having been imported into Taiwan. For example, the sh18922 strain in Fig. 3 was from India and caused an epidemic in eastern and central Taiwan in 2000 to 2002. However, strains isolated later are resistant to nalidixic acid. This observation requires further investigation.

According to the PFGE and drug resistance maps, the strains in the two outbreaks in Nanjhuang and Heping Township might be from Indonesia. The family of the index case in Nanjhuang Township had hired a foreign worker from Indonesia until Sep. 2003. Although *Shigella* can survive in carriers for a long period [14], the relationship between this outbreak and the Indonesia foreign worker is difficult to establish after two years. Alternatively, the strain might have been imported from Indonesia by other people and cause infection in many areas in Taiwan.

This study demonstrated the value of a *Shigella* DNA fingerprint database in epidemiology. Although the *Shigella* DNA database has more than 1,7000 maps collected since 1996, most strains came from central and eastern Taiwan. There are few *Shigella* cases in southern Taiwan, and northern Taiwan has the most *Shigella* cases. However, most strains isolated from northern Taiwan do not have PFGE maps in the CDC database. Between 2004 and 2005, the Central Taiwan Laboratory of CDC had collected *Shigella* maps from all areas in Taiwan.

However, the program was discontinued in 2006 due to lack of manpower. The program was very successful and impressed health workers in other countries. Unfortunately, we are unable to continue to program.

S. sonnei is an important pathogen of travel-associated transmittable disease. The DNA fingerprint database of the Central Taiwan Laboratory of CDC showed that most *S. sonnei* strains in Taiwan come from southeastern Asia, China and South Asia. At the end of 2003, several traveler groups were infected by *S. sonnei* in Bali, Indonesia [15]. Currently, several hundred thousand Taiwanese are doing business or traveling in China or Southeast Asia. More than four hundred thousand foreign workers also come to Taiwan each year, in addition to illegal immigrants and foreign brides. Hence, Taiwan is facing the challenge of pathogen importation from many sources that would threaten our national health unless we pay enough attention to it.

Acknowledgement

The authors thank the Health Bureaus of Miaoli County and Taichung County and students at the Epidemiology Training Center for their help in disease surveillance, epidemiological investigation and disease prevention. The authors also thank We-Gong Hospital and Fong-Yen Hospital for their prompt report of cases and provision of results from drug sensitivity tests.

References

1. Kotloff KL, Winickoff JP, Ivanoff B, et al: Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bull World Health Organ* 1999; 77:651-666.
2. Edwards BH: *Salmonella* and *Shigella* species. *Clin Lab Med* 1999; 19:469-487.
3. DuPont HL, Levine MM, Hornick RB, et al: Inoculum size in shigellosis and implications for expected mode of transmission. *J Infect Dis* 1989; 159:1126-1128.
4. Hale TL: Genetic basis of virulence in *Shigella* species. *Microbiol Rev* 1991;

55:206-224.

5. Mohle-Boetani JC, Stapleton M, Finger R, et al: Communitywide shigellosis: control of an outbreak and risk factors in child day-care centers. *Am J Public Health* 1995; 85:812-816.
6. Chiou CS, Shen YM, Yang LJ, et al: Epidemiology of bacillary dysentery in Nanto County – from 1995 to 1999. *Disease Report* 2001; 17: 341-358
7. Yu TH, Lai MH, Wu BH et al: Prevention of bacillary dysentery in mountainous areas in Taiwan between 2001 and 2004, *Disease Report* 2005; 12: 665-689
8. Chen JH, Chiou CS, Chen PC, et al: Molecular epidemiology of *Shigella* in a Taiwan township during 1996 to 2000. *J Clin Microbiol* 2003; 41:3078-3088.
9. Pan TM: Epidemiology and prevention of dysentery. *Disease Report* 1996; 12: 212-219
10. NCCLS: Performance standards for antimicrobial disk susceptibility tests. Approved standard, 7th ed. NCCLS document M2-A7. Wayne, Pa: NCCLS; 2000.
11. Centers for Disease Control and Prevention: One-day (24-28 h) standardized laboratory protocol for molecular subtyping of *Escherichia coli* O157:H7, non-typhoidal *Salmonella* serotypes, and *Shigella sonnei* by pulsed field gel electrophoresis. In: <http://www.cdc.gov/pulsenet/protocolshtm>. 2004.
12. Lee YS, Liu MC, Ko CF, et al: Molecular epidemiology of *Shigella flexneri* in a long-stay psychiatric nursing center during 2001 to 2003. *J Clin Microbiol* 2005; 43:1353-1360.
13. Chiou CS, Hsu WB, Wei HL, et al: Molecular epidemiology of a *Shigella flexneri* outbreak in a mountainous township in Taiwan, Republic of China. *J Clin Microbiol* 2001; 39:1048-1056.
14. Levine MM, DuPont HL, Khodabandelou M, et al: Long-term *Shigella*-carrier state. *N Engl J Med* 1973; 288:1169-1171.
15. Li SJ, Chen KL, Tsai JL et al: Molecular Epidemiology study of imported *Shigella sonnei* infection from Bali Island. *Disease Report* 2004; 20: 56-73.

Table 1 Profiles including symptoms, age, date of onset, residential area, PFGE genotype, family type and epidemiological relationship between cases in Nanjhuang Township of Miaoli County in 2005

| Case | Symptoms | Strain | PFGE genotype | Family | Age | Date of onset | Area | Epidemiological relationship |
|-------|--|----------------------|---------------|------------|------|-----------------|---------------------------|--|
| N1 | Diarrhea, abdominal pain, fever, cough, headache | C05.2105 C05.2161 | I, I | A | 9 | 2005/11/18 | East Village | Index case 1 |
| N2 | Diarrhea, abdominal pain | C05.2108 | I | A | 5 | 2005/11/23 | East Village | Younger brother |
| N3 | Diarrhea, abdominal pain, fever, cough | C05.2134 | I | B | 3 | 2005/11/23 | Nanjhuang Township | Family member of the grandfather |
| N4 | Diarrhea | C05.2120 | I | B | 15 | 2005/11/26 | Nanjhuang Township | Family member of the grandfather |
| N5 | Diarrhea, cough, headache | C05.2135 | I | B | 29 | 2005/11/27 | Nanjhuang Township | Family member of the grandfather |
| N6 | Diarrhea, abdominal pain, fever, cough | C05.2132 | IV | B | 14 | 2005/11/29 | Nanjhuang Township | Family member of the grandfather |
| N7* | Asymptomatic | C05.2168 | I | B | 24 | Asymptomatic | Nanjhuang Township | Family member of the grandfather |
| N8 | Diarrhea, abdominal pain, fever, cough, headache | C05.2119 C05.2185 | I, I | C | 10 | 2005/11/27 | Nanjhuang Township | Family member of the grandfather |
| N9* | Diarrhea, fever, cough | C05.2121 C05.2163 | III, IV | D | 3 | 2005/11/26 | Nanjhuang Township | Neighbor of the grandfather |
| N10 | Diarrhea | C05.2128 C05.2166 | II, II | D | 18 | 2005/11/28 | Nanjhuang Township | Neighbor of the grandfather |
| N11 | Diarrhea, vomiting | C05.2224 | I | E | 77 | 2005/12/1 | Donghe Township | Mother-in-law of the neighbor of the grandfather |
| N12 | Diarrhea | C05.2226 | I | E | 24 | 2005/12/4 | Gonggung Village | granddaughter-in-law of N11 |
| Total | | 16 strains | 4 genotypes | 5 families | 3-77 | 2005/11/18-12/4 | 2 Townships 3 Villages | |

*positive cases who returned to Heping Township of Taichung County after Dec. 13, 2005

Table 2 Profiles including symptoms, age, date of onset, residential area, PFGE genotype, and epidemiological relationship between cases in Heping Township of Taichung County in 2006

| Case | Symptoms | Strains | PFGE genotype | Age | Date of onset | Epidemiological relationship |
|-------|---|-----------|---------------|------|---------------|------------------------------|
| H1 | Diarrhea, abdominal pain, mucoid stool, fever, cough | C06.0082 | I | 7 | 2006/1/7 | Index case 2 |
| H2 | Diarrhea | C06.0168 | I | 7 | 2006/1/7 | Classmate |
| H3 | Diarrhea, abdominal pain, bloody stool, fever, headache, vomiting | C06.0167 | I | 25 | 2006/1/8 | Class director |
| H4 | Diarrhea, abdominal pain | C06.0180 | I | 59 | 2006/1/12 | Grandmother of H2 |
| H5 | Diarrhea | C06.0179 | I | 9 | 2006/1/17 | Sister of H2 |
| Total | | 5 strains | 1 genotype | 7-59 | 2006/1/7-17 | |

Table 3 Statistics of bacillary dysentery infections in Nanjhuang Township of Miaoli County in 2005

| Infection waves | Date of onset | A(R%) | Residual Chloride in water (ppm) | PFGE genotype | positive cases |
|-----------------|-----------------|--|----------------------------------|----------------|-----------------------------|
| 1 | 2005/11/18-23 | AR_0 (family of index case 1) =40 | 1.0 | I | N1, N2 |
| | 2005/11/18 | AR_1 (classmates and class director) =4.3 | 0.5 | I | N1 |
| 2 | 2005/11/23-29 | AR_2 (family of grandfather of index case 1) =26.3 | 1.0 | I, IV | N3-7 |
| | 2005/11/26-28 | AR_3 (neighbors of grandfather of index case 1) =15.8 | 1.0 | I, II, III, IV | N8-10 |
| 3 | 2005/12/1-4 | AR_4 (families of neighbors of grandfather of index case 1 in Donghe Village and Gonggung Village) =11.8 | 1.0 | I | N11-12 |
| total | 2005/11/18-12/4 | AR_{total} =14.5 | 0.5-1.0 | 4 genotypes | 12 confirmed positive cases |

Table 4 Statistics of bacillary dysentery infection in Heping Township of Taichung County in 2006

| Wave | Date of Onset | AR(%) | Residual chloride in water (ppm) | PFGE genotype | Confirmed positive cases |
|---------|---------------|--|---|------------------|-------------------------------|
| 1 | 2006/1/7 | AR ₀ (family of index case 2) =16.7 | 0 | I | H1 |
| | 2006/1/7-8 | AR ₁ (classmates and class director) =50 | 0 | I | H1-3 |
| | 2006/1/8 | AR ₂ (family of class director of index case 2) =20 | 0 | I | — |
| | 2006/1/11 | AR ₃ (family of the grandfather of index case 2) =0 | 0 | I | H3 |
| | 2006/1/7-17 | AR ₄ (family of H2) =60 | 0 | I | H2, 4 and 5 |
| 2 and 3 | 2006/1/17 | AR ₅ (classmates and class director of H5) =8.3 | 0 | I | H5 |
| Total | 2006/1/7-17 | AR _{total} =14.7 | 0 | 1 genotype | 5 confirmed positive cases |



Fig. 1 Geographic distribution of confirmed cases in Nanjhuang Township of Miaoli County and Heping Township of Taichung County. One black dot represents one positive case.

Fig. A (Nanjuang Township of Miaoli County)

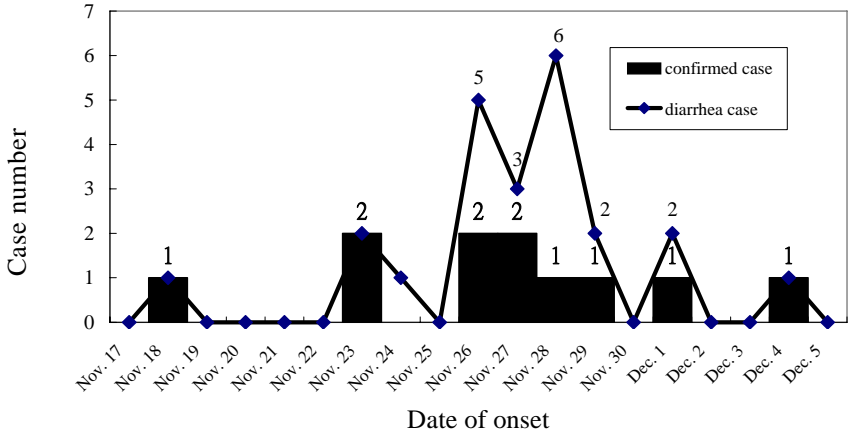


Fig. B (Hoping Township of Taichung County)

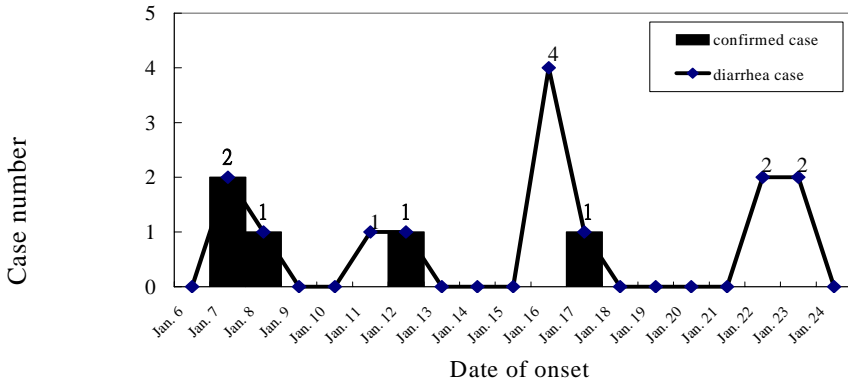


Fig. 2 Epidemiology curves of bacillary dysentery outbreaks in Nanjuang Township of Miaoli County (A) and Heping Township of Taichung County (B)

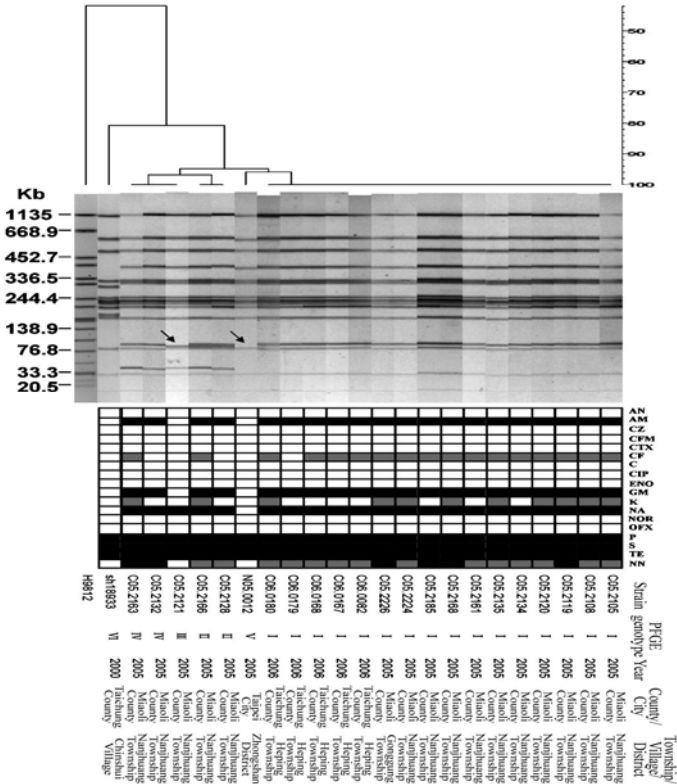


Fig. 3 PFGE dendrogram and drug sensitivity maps of *S. sonnei* strains isolated from outbreaks in Heping Township of Taichung County and Nanjuhuang Township of Miaoli County . Strain sh18933 (from India) was used as the control. H9812 is *Salmonella enterica* subsp. *enterica* serotype Braenderup and used as a DNA fragment size marker. The arrows indicate amikacin (AN) and gentamicin (GM) resistance-related DNA fragments. In the drug sensitivity test, blank squares represent sensitivity, gray squares represent intermedia phynotype, and black squares represent resistance. The drugs tested were Amikacin (AN), ampicillin (AM), cefazolin (CZ), cefixime (CFM), cefotaxime (CTX), cephalothin (CF), chloramphenicol (C), ciprofloxacin (CIP), enrofloxacin (ENO), gentamicin (GM), kanamycin (K), nalidixic acid (NA), norfloxacin (NOR), ofloxacin (OFX), penicillin (P), streptomycin (S), tetracycline (TE) and tobramycin (NN).