Molecular Epidemiological Study of Outbreaks of *Shigella sonnei* infection between 1995 – 2003 in Taiwan

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Abstract

Shigella sonnei, the most common intestinal and diarrhea-inducing pathogen in Taiwan, often results in major infection clusters or outbreaks. In this study, we use three genotyping methods including M13 arbitrary primer polymerase chain reaction (AP-PCR), plasmid profile assay (PPA), and pulsed-field gel electrophoresis (PFGE) to analyze the molecular relationship between strains of *Shigella sonnei* outbreaks during the years 1995 – 2003 in Taiwan. Of the six strains isolated from *Shigella sonnei* outbreaks, the results of Xba I and Sfi I PFGE showed that they could be separated into six subtypes (X-I ~ VI, S₁ - S₆). The dendrogram indices (DI) among strains were between 0.25 and 0.65. Their

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plasmid profiles could also be separated into seven subtypes ($P_1 - P_7$). Comparing each outbreak with the seven molecular types (X1 - X6) isolated in northern Taiwan in 2001, the strains from a kindergarten in I-lan (X-I) and an elementary school in Ping-Chen, Tao-Yuan were closely related to X2 and X3 strains in Northern Taiwan. The outbreak in a Hualien Senior High School in eastern Taiwan was closely related to local sporadic cases, and had greater differences from the strains in northern Taiwan. Outbreaks in Mountain A-Li and Ba-li Island (X-IV and X-VI) were significantly different from that in northern Taiwan. The outbreak in Dan-Shuei had 93% homology with the strain in metropolitan Taipei, suggesting its cause to be by regional water contamination.

Our results showed that, between 1995 and 2003, six strains isolated from outbreaks of bacillary dysentery at distinct times and locations in Taiwan belonged to local endemic outbreaks, since they had no molecular relationship. Nevertheless, they had close relationships with local strains. The difference in molecular typing suggested that strains of *Shigella sonnei* have local variations, including variations in drug resistance. Therefore, to prevent bacillary dysentery, in addition to strengthening public health education and diarrhea syndrome monitoring, local, rapid and small scale isolation measures are suggested.

Introduction

According to epidemiological studies related to bacillary dysentery in Taiwan, *Shigella sonnei* and *S. flexneri* are the two predominant strains (1, 2). Between 1992 and 1999, *S. flexneri* was the most common cause of bacillary dysentery, and the others causes were *Shigella sonnei*(3). However, after the outbreak of *Shigella sonnei* infection in a senior high school in Hualien in November 2000, which resulted in 65 cases, sporadic cases began to emerge in

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eastern Taiwan. Cases of bacillary dysentery began to rise in northern Taiwan since the beginning of 2001. In March 2001, 20 confirmed cases of bacillary dysentery occurred in Mountain A-Li, Chia-Yi, and this was the first *Shigella sonnei* outbreak in southern Taiwan (4). The water contamination which occurred in Dan-Shuei, Taipei County in June 2002 was confirmed to have caused an outbreak of *Shigella sonnei*. In 2001, there were almost one thousand cluster and sporadic cases reported in northern Taiwan (Table 1). I-Lan had the most numerous cases. There were also multiple cases in Taipei, Tau-Yuan and Shing-Ju. The endemic areas were not restricted to mountainous regions.

Why did cases of *Shigella sonnei* infection increase dramatically in northern Taiwan? According to Chen et al., strains resulting in sporadic cases in mountainous areas of three districts in northern Taiwan had close molecular relationships, which may indicate a spreading of local clusters(5). Because bacillary dysentery is extremely infectious with person to person or person to water to person modes of transmission, it usually results in large scale outbreaks (6-8), which impose great challenges to disease prevention. Moreover, imported infections were usually caused by travel to countries in southeastern Asia where many kinds of diseases are endemic. In November 2003, 38 travelers were infected during their trip to Ba-li Island(9). Whether there is any molecular relationship between strains in domestic and imported infections requires further analysis.

We then utilized molecular epidemiological methods to analyze six outbreaks which occurred between 1995 and 2000 in Taiwan, and which included cases from an elementary school in Tau-Yuan and a kindergarten in I-Lan in 1995 (10), a senior high school in Hualien in 2000, Mountain A-Li in Chia-Yi in 2001, Dan-Shuei in Taipei in 2002, and imported cases from Ba-li in 2003. Relationship

326 Epidemiology Bulletin Octtober 25,2005 between each outbreak was compared with their molecular relationship with local sporadic cases discussed. Moreover, each strain collected was compared with six main strains (X1 - X6) resulting in sporadic cases in 2001 in northern Taiwan to analyze their relationship and infection sources with outbreaks in recent years.

Material and Methods

1. Case analysis

Six outbreaks of *Shigella sonnei* infection from different time periods between 1995 and 2003 and different locations were chosen. They included cases from an elementary school in Tau-Yuan and a kindergarten in I-Lan in 1995 (10), a senior high school in Hualien in 2000, Mountain A-Li in Chia-Yi in 2001, Dan-Shuei in Taipei in 2002, and imported cases from Ba-li in 2003. Relationship between each outbreak was compared, with their molecular relationship with local sporadic cases discussed.

2. Strain isolation and biochemical and serotype analysis

Strains were cultured on SS-medium (Salmonella-Shigella agar) at 36° C for 16-18 hr, then were subjected to triple sugar iron (TSI) test. After that, Shigella Antisera (II) kits (Denka Seiken Co., ltd. Tokyo, Japan) were used for serotyping agglutination analysis. Automatic microbial biochemical confirmation system (Vitek system, GNI) was used for final confirmation.

3. Agar disc diffusion test

Ampicillin (AM) $10\mu g$, chloramphenicol (C) $30\mu g$, trimethoprim/ sulfamethoxazole (SXT) $1.25\mu g$ / $23.75\mu g$, third generation cephalosporin ceftriaxone (CRO) $30\mu g$ and ceftazidine (CAZ) $30\mu g$, first generation quinolone nalidixic acid (NA) $30\mu g$, and second generation quinolone ciprofloxacin (CIP) $5\mu g$ and norfloxacin (NOR) $10\mu g$ were used for agar disc

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diffusion test. Fresh bacteria suspension was blended into Mc Fraland standard $0.5(about 1.5 \times 10^8 \text{ CFU/ml})$ standard $BaSO_4$ suspension turbidity, and was spread by sterile method onto Mueller-Hinton agar (M-H medium, Difco). Drugs were fixed to plates by dispersors, and the plates were cultured at 35°C for 16 to 18 hrs. The size of inhibition rings was recorded, and analyzed according to criteria set by NCCLS antibiotics sensitivity standards.

4. plasmid profile analysis

High pure plasmid isolation kit (Roche, Mannheim, Germany) was used to extract plasmid DNA. Electrophoresis was done in 0.5X Tris-borate-EDTA (TBE) buffer and 1.2 % SeaKem LE agarose (BioWhittaker Molecular Applications, Rockland, ME, USA). Voltage was set at 100 V for 3.5 hr. The size of plasmids (1 - 20 kb) was used for typing.

5. AP-PCR

Single plus strand M13 primer (5'- TTA TGT AAA ACG ACG GCC AGT-3') was used for polymerase chain reactions. Reactions were done at 94° C for 60 sec, 36° C for 60 sec, and 72° C for 120, with total 45 cycles. 7 l of PCR products were used for analysis. Electrophoresis was done with 1.5 % Agarose SFRTM Bioteriolgical grade (AMRESCO, Solon, OHIO, USA) in 1× Tris-acetate-EDTA (TAE) solution with 100 V for 35 min.

6. Pulsed-field gel electrophoresis

Protocol for pulsed-field gel electrophoresis was adapted for the CDC, USA with sight modification (Gautom, 1997). Chromosomal DNA was digested by Xba I and then subjected to electrophoresis for 24 hr with transition time ranging from 5 to 35 sec. 1.2% SK Gold agarose and $0.5 \times$ TBE buffer were used for electrophoresis. Gels were subjected to photography after EtBr staining.

7. Dendrogram

Restriction maps generated form Xba I digestion were analyzed by UPGMA (unweighted pair group method using mathematical averages) with Phoretix 1D Advanced Version 5.01 to generate a dendrogram.

Results and discussion

In this study, we used molecular epidemiological methods to analyze outbreaks of *Shigella sonnei* infection occurring between 1995 and 2004 in Taiwan, which included cases from an elementary school in Tau-Yuan, a kindergarten in I-Lan, a senior high school in Hualien, Mountain A-Li in Chia-Yi in 2001, Dan-Shuei in Taipei in 2002, and imported cases from Ba-li Island (Table 2). Relationship between each outbreak and molecular relationship with local sporadic cases were compared. Moreover, molecular typing and relationship between sporadic cases occurring in northern Taiwan in 2001 were also discussed.

I. Cases from an elementary school in Ping-Chen, Tau-Yuan and a kindergarten in I-Lan, northern Taiwan

In 1995, two outbreaks of *Shigella sonnei* infection occurred in northern Taiwan (10), including cases from an elementary school in Ping-Chen, Tau-Yuan and a kindergarten in I-Lan. The outbreaks were due to improper handling of fecal material by children or those who had mental illness. The modes of transmission were person to person and person to water to person. In July 1995, a child from a kindergarten in Lo-Dong, I-Lan was brought to St. Mary's Hospital because of diarrhea. Infection caused by *Shigella sonnei* was confirmed by the Institute of Preventive Medicine, Department of Health (predecessor of the Center for Disease Control). Examination of more than 400 children was immediately performed, and 25 confirmed cases were found. In November 2000, a child from

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a class for mentally challenged at an elementary school in Ping-Chen, Tau-Yuan was brought to an emergency room because of high fever and severe diarrhea. After investigation, it was found that symptomatic cases had begun to emerge in the middle of November, and the peak occurred in late November. *Shigella sonnei* was found in a well at the back of the school. The outbreak was caused by contaminated underground water. 404 children were infected in this outbreak.

Strains of *Shigella sonnei* in these two outbreaks were analyzed by Xba-I pulse electrophoresis map analysis. The strain in the kindergarten in I-Lan was type X-I, and that of the elementary school was type X-II. Their molecular characters (Fig. 1) and plasmids types were different. There were also significant differences in their antibiogram. The strain of the kindergarten outbreak was resistant to nalidixic acid (NA) and sensitive to ampicillin (AM), chloramphenicol (C) and trimethoprim-sulfamethoxazole (SXT). The strain sensitivities from the elementary school outbreak were completely reversed: resistant to AM, C, and SXT, and sensitive to NA. Because of their different molecular types, times of isolation and geographic areas, we can deduce that there was no relationship between these two outbreaks.

II. Outbreak of bacillary dysentery at a senior high school in Hualien

An outbreak of *Shigella sonnei* occurred in Hualien in October 2000. Contamination of underground water was the suspected cause. 65 positive cases were confirmed. During November and December of 2000, sporadic cases were confirmed in Hualien City, Shiu-Lin, Ji-An, Cho-Shi and Shin-Chen. After pulse electrophoresis analysis, the strain was confirmed to be type X-III, and the outbreak was a cluster case. While comparing the molecular types of pulse electrophoresis of contemporary sporadic strains (Fig. 4), we confirmed that the sporadic strain was spread from the outbreak strain. Moreover, the antibiogram of the outbreak strain was tetracycline (TE), SXT, and NA-resistant, and AM-sensitive.

III. Outbreak of bacillary dysentery on Mountain A-Li

An outbreak of bacillary dysentery occurred in April 2001 in Chia-I, southern Taiwan. 20 positive cases were reported from Da-Bun and Shian-May in the Mountain A-Li area. This outbreak was the first episode of cluster cases of Shigella sonnei in southern Taiwan. The antibiogram was AM, TE, SXT, and NA-sensitive, different from that of northern Taiwan, which was resistant to AM, TE, SXT, and NA. The strains found in cases of the outbreak were all type X-IV, as shown by pulse electrophoresis. Because no sporadic strains could be used as comparison in southern Taiwan, 10 strains isolated in July and August of 2001 and January to May of 2002 from central Taiwan (Tai-Chun and Nan-Tou) were used for comparison (6). As shown by PPA and M13 AP-PCR, the strain isolated on Mountain A-Li outbreak was very similar to the five strains isolated in July and August of 2001 in central Taiwan, and different from those isolated in 2002. Similar results were obtained with PFGE (Fig. 5). Accordingly, from geographic location and isolation time frame, we deduce that a certain molecular relationship existed between the strain found in the Mountain outbreak and the strains found in July and August of 2001 in central Taiwan.

IV. Outbreak of bacillary dysentery in Dan-Shuei

In June 2002, 44 people in Dan-Shuei, Taipei were infected with *Shigella sonnei*. This outbreak had two peaks: 6 were infected before May 28 by suspected contaminated water in an apartment, and students of an elementary school were found to be infected after May 28. After analysis, the strain showed resistance to TE and SXT and was sensitive to AM and NA. Plasmid profile analysis showed mainly five plasmid fragments: 1.7, 2.4, 10, 21, and > 40 kb. Xba-I PFGE showed

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type X-V. These results indicated that a single infection source was responsible for the outbreak (Fig. 6). Nevertheless, the index case was suspected to be infected in central Taiwan. The sensitivity to AM and NA of the strain in this outbreak was different to strains isolated in northern Taiwan in recent years. To discriminate between the infection source of this outbreak, molecular relationship between strains in northern, central, and southern Taiwan needs further comparison.

1. Comparison between the strain in theDan-Shuei outbreak and those in northern Taiwan (I-Lan, Tao-Yuan, Shin-Ju, and MetropolitanTaipei)

Comparison has been made between 19 strains sensitive to AM and NA and isolated in northern Taiwan between September and October of 2001, and7 strains sensitive to those two drugs isolated from sporadic cases. Results of Xba-I PFGE showed that significant differences existed between strains from northern Taiwan and those from Dan-Shuei. Similarity index was between 0.40 and 0.62, which showed minimal relationship between them (Fig. 7). There results indicated that the strains in northern Taiwan were not responsible for the outbreak which occurred in Dan-Shuei.

2. Comparison between the strain in the Dan-Shuei outbreak and those in central Taiwan (Tai-Chun and Nan-Tao)

Among the ten sporadic strains isolated in central Taiwan, 5 isolated between July and August of 2001 were sensitive to AM and NA, and the remaining 5 strains isolated between January and May of 2002 were sensitive to AM and resistant to NA. When they were compared with the strain from the outbreak in Dan-Shuei, the results of M13-PCR showed the strain from the outbreak was similar to the 5 strains isolated in 2002, with only one difference in a 1.5 kb DNA band. On the other hand, difference in 2 bands, 1.5k and 2.5k, was revealed when

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compared to the strains isolated in 2001. The results plasmid profiles showed that the strain was different from the strains isolated in 2002 in 5k and 11k plasmids, and from the strains in 2001 in 1.5k, 2k, and 10k plasmids. Further, PFGE also showed differences in 2 to 4 bands. They were closely related in types, and their relationship indices were between 0.42 and 0.80 (average 0.58). The results of comparison showed that the strain in the outbreak in Dan-Shuei had no molecular relationship with the strain of *Shigella sonnei* isolated from central Taiwan.

3. Comparison between the strain in the Dan-Shuei outbreak and those in southern Taiwan (Mountain A-Li)

While comparing the strains from Mountain A-Li and that from the Dan-Shuei outbreak, M13-PCR maps showed a difference in 1.5k and 2.5k products, plasmid profiles showed differences in 1.7k, 2.3k, 2.7k and 4k, and PFGE showed differences in more than 4 bands. Therefore, their types were possibly unrelated. Their relationship index was only 0.72. Because of their obvious differences, they were unrelated (Fig. 8).

4. Comparison between the strain in the Dan-Shuei outbreak and those in metropolitan Taipei (Taipei City and Taipei County)

After we compared the Dan-Shuei strain with *Shigella sonnei* strains from northern, central, and southern Taiwan, their relationship indices were between 0.40 and 0.80. Although there were some similarities, we were not sure about the infection source of the Dan-Sheui outbreak. Further, we chose AM and NA sensitive strains isolated from metropolitanTaipei in August of 2001, and found the results of M13-PCR, plasmids profile, and PFGE showed very similar molecular types. Their relationship indices were also as high as 0.93 (Fig. 8). The results of comparison indicated that the infection source of the Dan-Shuei outbreak probably resulted from spreading of local water contamination.

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V. Imported cases from Ba-Li Island

Between November 7 and 17 in 2003, among 4,107 travelers to Bali Island who belonged to 5 travel agents including Shih-Yu, Shih-Bao, Guei-Guan, Kang-Fu, and Shiung-Shih, 176 had symptoms including vomiting, fever, and diarrhea. After being examined by disease prevention personnel at the airport, 38 travelers were confirmed to have *Shigella sonnei* infection (9). Antibiogram showed SXT-resistance, and AM and NA-sensitivity. This was significantly different from strains, which showed resistance to these three drugs, isolated in northern Taiwan. Moreover, their plasmids profile (P-VI) and PFGE (X-VI) (Fig. 1) showed differences from major types (P1-P7, X1-X7, S1-S7) of strains isolated from northern Taiwan. Since all of the cases had a history of travel to Bali Island, we proposed that they were all imported cases from cluster infection on Bali Island.

VI. Comparison between *Shigella sonnei* strains in each outbreak and major strains in northern Taiwan (I-Lan, Ta-Yuan, and Shing-Ju)

According I-Lin and colleagues (7, 10), outbreaks in Tao-Yuan between 95 and 96 had a molecular relationship with local sporadic cases. Trends in *Shigella sonnei* infection showed there was a significant increase of *Shigella sonnei* infection cases in 2001-2002 (3). Among those cases, most occurred in I-Lan County. Several cases were also found in metropolitan Taipei, Tau-Yuan, and Shin-Ju. What were the causes that led to an increase of infection? Was there a common infection source or an environmental factor? Chen and colleagues reported that sporadic strains in northern Taiwan, including I-Lan, Tao-Yuan, and Shin-Ju, had certain molecular similarities with sporadic cases which occurred in mountainous areas of Shin-Ju. Similar infection sources may account for the spreading of local cluster cases (5).

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PFGE of six strains of *Shigella sonnei* outbreaks (Fig. 1) showed relationship indices between 0.25 and 0.65, which signified no relationship (Fig. 3). When compared with six pulse types (X1-X6) (Fig. 9) and three subtypes (X1a, X1b, and X1c) isolated in northern Taiwan, Xba-I PFGE mainly showed 48kb to 450 kb DNA fragments. Two strains in 1995 showed similarity with X2 and X3 from northern Taiwan. Strains from a senior high school in Hualien and Mountain A-Li showed less relationship with strains from northern Taiwan. Thus, they were local strains. Strains in the Dan-Shuei outbreak showed significant similarity with strains isolated from Taipei. Relationship indices also showed similar results: two strains isolated in 1995 and in Dan-Shuei showed significant similarity with strains isolated in Taipei. Strains from Hualien, Mountain A-Li, and Bali Island showed no molecular relationship with those from northern Taiwan. We suspected that the increased sporadic cases in 2001 - 2002 may have been caused by spreading of two cluster strains in 1995. Together with other relative environmental factors and foreign strains, strains in northern Taiwan showed larger strain types. Strains from Hualien and Mountain A-Li showed significant differences from those in northern Taiwan in molecular types and antibiogram. They may arise from local strains or hybrids with foreign strains.

Conclusion and Suggestions

The results of PFGE showed that 6 strains from *Shigella sonnei* outbreaks occurring at different times and in different areas could be separated into 6 molecular types (X-I ~ X-VI, S1 ~ S6). Their dendrogram Indices were between 0.25 and 0.65. Therefore, they belonged to single endemic cases, no molecular relationship among them. Nevertheless, strains from outbreaks had molecular and dendrographic relationship with local sporadic strains. For example, the strain in

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the outbreak of a high school in Hualien had the same molecular type with sporadic strains, and the strain from Dan-Shuei outbreak had high similarity with strains from metropolitan Taipei. Spreading of outbreak strains may lead to spreading of sporadic cases.

Strains from different geographic had different molecular types. X-III strains from eastern Taiwan were different from major strains (X1 – X6) in northern Taiwan. Strains from Mountain A-Li in southern Taiwan also had significant differences from those from northern and eastern Taiwan. This implied that *Shigella sonnei* had local differences, which may suggest more virulence and spreading ability. Moreover, antibiogram of different strains also differed significantly. Northern strains were resistant to AM, TE, SXT, and NA; eastern strains were resistant to TE, SXT, and NA, and sensitive to AM; southern strains were sensitive to AM, TE, SXT, and NA. These results suggested improper drug usage or plasmid exchange with foreign strains as the causes of difference. It was worthwhile to know that in Jian-Shih, Shin-Ju, 40% of strains showed multiple drug resistance, and they were even resistant to third generation cephalosporin CRO. Where the multiple drug-resistant strains originated and whether this means increased virulence requires further investigation.

The results of strain maps of *Shigella sonnei* isolated in northern Taiwan in 2001 showed that there wase more variation among strains from I-Lan area (X1 – X4). The main strains, X1 and P1, existed among three aboriginal tribes in mountainous area. This suggested that close molecular relationship between sporadic strains or mutual spreading of strains occurred in northern Taiwan. The results of the antibiogram showed that in Jian-Shih, Shin-Ju, up to 40% (8/20) of strains had multiple drug resistance (AM, SXT, TE, C, CRO, etc.). This may have been caused by improper drug usage or cross infection by foreign strains.

336 Epidemiology Bulletin Octtober 25,2005 Whether these strains arose locally or were imported from foreign regions, and whether they had more virulence requires further investigation.

Our results showed that strains from northern, eastern, and southern Taiwan had certain molecular types. This suggests local differences exist between strains of *Shigella sonnei* in Taiwan. Cluster outbreaks had a certain relationship with local sporadic strains, indicating spreading of local latent strains. Bacillary dysentery transmission was by the person-to-person or person-to-water-to-person route, and outbreaks were often caused by the person-to-water-to-person route. We suggest that to prevent dysentery, besides intensifying public education and health facility construction, local, rapid, and small-scale isolation strategies should be used to actively monitor diarrhea syndromes.

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Table 1Statistics of strains resulting in bacillary dysentery between 1992 and 2003 in
Taiwan.

| Types \Ye | ar 19 | 92 19 | 93 1994 | 1995 | 2000 | 2001 | 2002 | 2003 |
|----------------------------|-------------|----------------|-------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|
| Group A (S. dysente | riae) (0. | 1 4%) | 0 0 | 0 | 0 | 0 | 0 | 1 (0.4%) |
| Group B | 4 | 9 7. | 3 52 | 104 | 53 | 136 | 35 | 38 |
| (S. flexneri | i) (66 | %) (31 | %) (76% | (18% |) (16%) |) (10%) |) (8%) | (11%) |
| Group C | 4 | 4 |) 3 | 3 0 | 0 | 0 | 0 | 0(<i>S</i> . |
| boydii) | (5%) | | (0.5%) | | | | | |
| Group D <i>sonnei</i>) | 22 (29%) | 161 (68.6%) | 16 (24%) | 473 ₍₁₎ (81.5%) | 264 ₍₂₎ (83%) (| 1221 ₍₃ (89%) (|) 401 ₍₄₎ 91%) (| 304 ₍₅₎ (S. 88%) |

 ⁽¹⁾Including two outbreaks in a kindergarten in I-Lan and an elementary school in Tao-Yuan •

- ⁽²⁾Including the outbreak in a senior high school in Hualien
- ⁽³⁾Including the outbreak in Mountain A-Li
- ⁽⁴⁾Including the outbreak in Dan-Shuei
- ⁽⁵⁾Including the imported outbreak from Bali Island

| Table 2 | Analysis of outbreaks of Shigella sonnei infection occurring between 1995 and | |
|---------|---|--|
| | 2003 in Taiwan | |

| Outbrooks | Number | Isolation | Pulsed | DDA | M13-PCR | Dendrogram |
|-------------------------|------------|-----------|--------|-------|---------|------------|
| Outbreaks | of strains | Date | types | rrA | | Indices |
| Clusters | | | | | | |
| Kindergarten in I-Lan | 25 | 1995/7 | X-I | А | - | 0.65 |
| Elementary school in | 404 | 1995/11 | X-II | В | - | 0.50 |
| Tao-Yuan | | | | | | |
| High school in Hualien | 65 | 2000/10 | X-III | С | M1 | 0.58 |
| Mountain A-Li in Chia-I | 20 | 2001/3 | X-IV | D | M2 | 0.33 |
| Dan-Shuei, Taipei | 44 | 2002/6 | X-V | Е | M3 | 0.65 |
| Bali Island | 38 | 2003/11 | X-VI | F | - | 0.40 |
| Sporadic cases | | | | | | |
| Eastern Taiwan | 10 | 89/11-12 | X-III | С | M1 | |
| Northern Taiwan | 19 | 90/9-10 | X1-X7 | P1-P6 | _ | |
| Metropolitan Taipei | 6 | 90/3 | X-V | Е | M3 | 0.93 |
| Central Taiwan | | | | | | |
| Sing-I, Nan-Tao | 5 | 90/7-8 | E1 | P4 | _ | 0.36 |
| Tai-Chun and Nan-Tao | 5 | 91/1-5 | D1 | P2-P3 | _ | 0.80 |

* Dendrogram Index (DI) was calculated from the dendrogram formed by UPGMA (unweighted pair group method using mathematical)analysis of PFGE of two strains, and analysis by Phoretix 1D Advanced Version 5.01.



Fig. 1: Xba-I PFGE maps of six strains from Shigella sonnei outbreaks

Lane 1 and 15: λ Ladder 48 kb Marker; Lane 2 and 3: kindergarten from I-Lan; Lane 4 and 5: elementary school in Tao-Yuan; Lane 6 and 7: senior high school in Hualien; Lane 8 and 9: Mountain A-Li in Chia-I; Lane 10 and 11: outbreak in Dan-Shuei, Taipei; Lane 12 and 13: outbreak on Bali Island; Lane 14: ATCC 15394 standard strain.





Lane 1, 9: 100 bp and 1 Kb Ladder Markers; Lane 2: kindergarten from I-Lan; Lane 3: elementary school in Tao-Yuan; Lane 4: senior high school in Hua-lien; Lane 5: Mountain A-Li in CHia-I; Lane 6: outbreak in Dan-Shuei, Taipei; Lane 7: outbreak on Bali Island; Lane 8 : ATCC 15394 standard strain.



Fig. 3: PFGE maps of six strains of *Shigella sonnei* outbreaks. Dendrogram indices were between 0.25 and 0.65



Fig. 4: Xba-I restriction enzyme PFGE analysis of the outbreak in a senior high school in Hualien and local sporadic strains in Hualien

Lane 14: λ Ladder 48 kb Marker; Lane 1~13: strains form a senior high school in Hualien; Lane 15-29: sporadic strains





PPA



Fig. 5: PFGE, PPA and AP-PCR analyses of *Shigella sonnei* strains from the outbreak on Mountain A-Li and central Taiwan

Lane $1 \sim 3$: strains from Mountain A-Li; Lane 15 - 29: strains from central Taiwan



Fig 6 Xba-I restriction enzyme PFGE of *Shigella sonnei* strains from outbreak in Dan-Shuei, Taipei

Lane 1, 26 : λ Ladder 48 kb Marker; Lane 2 ~ 23, 25 : strains from outbreak in Dan-Shuei, Taipei ; Lane 24 : sporadic strains from Tao-Yuan



Fig. 7 PFGE and dendrogram analyses of *Shigella sonnei* strains from outbreak in Dan-Shuei, Taipei and sporadic cases in three counties in northern Taiwan.
The dendrogram indices between the strain from the outbreak in Dan-Shuei and those from sporadic cases in northern Taiwan were between 0.40 and 0.62.



Fig. 8 PFGE and dendrogram analyses of *Shigella sonnei* strains from sporadic cases in Taipei area. The dendrogram indices between the strain from the outbreak in Dan-Shuei and those from Sporadic cases in Taipei area aer were 0.93.



Fig. 9 Xba-I restriction enzyme PFGE of *Shigella sonnei* strains from sporadic cases in northern Taiwan

Lane $1 \cdot 9 \cdot 12$: λ Ladder 48 kb Marker; Lane 2, $4 \sim 6$: strains from sporadic cases in I-Lan; Lane 3: strains from sporadic cases in Tao-Yuan