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Imported Infection of *Shigella sonnei*
Molecular Epidemiological Investigation of Cases of the Bali Tours

Abstract

The 38 strains isolated from five members of the tour groups to the Bali Island collectively infected with bacillary dysentery on November 7 through 17, 2003, were confirmed *Shigella sonnei*. To understand the sources of infection, the present study selected two strains each from the Hsinchu and Yilan areas of the northern part of Taiwan known to have had outbreaks of *Shigella sonnei* infection confirmed by molecular typing, and eight strains of sporadic *Shigella sonnei* infection in the northern part of Taiwan in 2003 collected by the Kungyang Laboratory of the Center for Disease Control (the Laboratory) for analysis, to understand if the strains of the present incident were associated with the indigenous strains isolated in the northern part of Taiwan. The present study used the phenotype disk antimicrobial susceptibility test, and the genotype AP-PCR, plasmid profile analysis, and pulsed-field gel electrophoresis (PFGE) methods for the analysis of molecular epidemiological association.

Testing by the disk antimicrobial susceptibility test showed that the 38 *Shigella sonnei* strains isolated from the Bali tourists demonstrated drug

resistance only to Sulfamethoxazone (SXT); they were significantly different from the *Shigella sonnei* strains isolated in the northern part of Taiwan, which demonstrated drug resistance to Ampicillin (AM), Sulfamethoxazone (SXT) and Nalidixic acid (NA). Analysis by plasmid typing of 20 kb and under showed that the Bali isolates showed a consistent P8 type, quite different from the isolates of the northern part of Taiwan, which showed P1 to P7 types. P8 type has mainly five plasmids of 2.8 kb, 4 kb, 9 kb, 12 kb and 20 kb. The PFGE profiles of *Xba*-I showed, when analyzing the DNA sections of 48 kb to 485 kb, that the main difference was in four DNA sections. The Bali isolates showed a consistent X7 type; they were different from the Taiwan isolates, which were primarily of X1, X1a, X1b, X1c, X1d, X2, X3, X4, X5 and X6. The primary difference was that the Bali isolates had three more sections between 242.5 kb and 291.5 kb. The PFGE profiles of *Sfi*-I showed that the Bali isolates showed a consistent S7 type; they were different from the Taiwan isolates with S1 to S5 types. By the disk antimicrobial susceptibility test, it was noted that the Bali isolates though were similar to the Taiwan isolates in their phenotypes; they were different in their drug resistance. The genotype analysis of AP-PCR, plasmid profile analysis, and PFGE showed that the primary molecular types of the Bali isolates were constantly P8, X7 and S7 profiles. The PFGE profiles of *Xba*-I showed that the phylogenecity was 95% similar, suggesting a close molecular association between the strains. In short, testing of both the phenotype and genotype of the strains of the collective infections of bacillary dysentery in the Bali Island suggested that the infections were of the same source, and they were most probably imported from the Bali Island of Indonesia.

Introduction

Shigella is a gram-negative bacillus of the Enterobacteriaceae family. Shigellosis is a highly infectious bacterial disease involving intestinal and gastric

tracts characterized by vomiting, fever, diarrhea or bloody stools of different degrees[1]. The genus *Shigella*, by biochemical and serological characteristics, is composed of four species, Group A, *S. dysenteriae*; Group B, *S. flexneri*; Group C, *S. boydii*; and Group D, *S. sonnei*. Diarrhea of infections by *S. boydii* and *S. sonnei* is milder and of shorter duration though often with watery or bloody stools[2]. Diarrhea of *S. flexneri* is more serious and of longer duration, with bloody stools. Infection of *S. dysenteriae*, particularly serotype 1, has a high fatality[3,4]. By O-antigen, *S. dysenteriae, S. flexneri* and *S. boydii* can be further divided into 45 serotypes; while *S. sonnei* has only one serotype[5]. Worldwide, *S. flexneri* and *S. sonnei* have higher incidence. *S. flexneri* is more common in developing countries; whereas *S. sonnei* is most common in developed countries[6].

In the period between November 7 and 17, 2003, of the 4,107 members of five tour groups to the Bali Island of Indonesia, 176 had developed symptoms of vomiting, fever, and diarrhea. Specimens collected at airport by quarantine officers and follow-up specimens collected were tested, and confirmed by the Laboratory that 38 of them were *S. sonnei* infection. Molecular biological techniques commonly used in the typing of pathogenic agents, such as ribotyping, polymerase chain reaction (PCR), plasmid profile analysis (PPA), and pulsed-field gel electrophoresis (PFGE)[10-16] can be used to clear the molecular associations between strains. The present study also collected *S. sonnei* strains of outbreaks and sporadic infections in the northern part of Taiwan for analysis by the most effective method currently available of PFGE, together with PPA and AP-PCR, to investigate the molecular associations of the sources of infection of the outbreaks, herd infections and sporadic infections in the northern and north-eastern parts of Taiwan. The Bali isolates were confirmed *S. sonnei*. For the early understanding of the sources of infection in outbreaks, the Laboratory

investigated, by using strains of some major types from the already established data banks of *Shigella* fingerprints, the molecular epidemiological associations between these strains and the strains isolated from the imported Bali infections.

Materials and Methods

1. Isolation, biochemical and serological assessment of *Shigella sonnei*

Fecal or anal swabs of suspected patients or close contacts of patients were collected, transported under frozen condition by Cary-Blair medium to the Laboratory for testing. Patients already showing symptoms of bacillary dysentery infection upon entry were collected of specimens at the airport. Specimens were also collected from suspected patients and close contacts of patients. Specimens collected were placed in *Salmonella-Shigella* agar and Hektoen Enteric agar (Difco Laboratories, Detroit, MI, USA) under 35℃ for 18-24 hours. Suspected non-fermented colonies were picked up and inoculated on Triple Sugar Iron agar (TSI, Difco), Lysine Iron agar (LIA, Difco), and Sulfide-Indole-Motility medium (SIM, Eiken Chemical Co., Tokyo, Japan) for biochemical testing. When the colonies showed biochemical reactions such as, in TSI, red/yellow and H_2S negative, no mobility of SIM, and fermentation reaction in Lysine of LIA negative, they were conducted agglutination testing for serological typing with *Shigella* Antisera (II) reagents (Denka Seiken Co., Ltd., Tokyo, Japan). Colonies were finally confirmed by SYSTEK No. 1 biochemical reagent kit (Eiken Chemical Co., Ltd., Tokyo, Japan).

2. Agar disc diffusion test

In the disk antimicrobial susceptibility test, eight antibiotics, 10 μg of Ampicillin (AM), 30 μg of Chloramphenical (C), 1.25 μg/23.75 μg of Trimethoprim/sulfamethoxazole (SXT), 30 μg of the third generation Ceftiriaxone (CRO), 30 μg of Ceftazidine (CAZ), 30 μg of Nalidixic acid of the first

generation Quinolone, 5 μg of Cirprofloxacin (CIP) of the second general Quinolone, and 10 μg of Norfloxacin (NOR) were used. In the Tryptic Soy Broth (TSB), the fluid concentration was adjusted to McFarland No. 0.5 barium sulfate, the fluid was then evenly placed on the Mueller-Hinton medium (M-H medium, Difco), placed in the ordinary culture box under 35℃ for 16-18 hours to observe the size of the inhibition ring. The result was read by the NCCLS standards as S (susceptible), I (intermediate), and R (resistant).

3. Plasmid profile analysis

The High Pure Plasmid Isolation Kit (Roche, Mannheim, Germany) was used to collect the DNA of the strain plasmid. Testing was conducted according to the standard operational procedures of the kit. 0.5x Tris-borate-EDTA (TBE) electrophoresis buffer was used to prepare 1.2% SeaKem LE agarose (BioWhittaker Molecular Applications, Rockland, ME, USA). Conditions for electrophoresis were 100 volts for 3.5 hours. Electrophoresis profiles were used to analyze the size of the strain plasmid. The size of the plasmid carried by strains was used for typing (at a range of 1-20 kb).

4. AP-PCR

M13 single positive ply 21 bp (5'-TTATGTAAAACGGCCAGT-3') was used as primer for AP-PCR under 94℃ for 60 seconds, 36℃ for 60 seconds, and 72℃ for 120 seconds, for 45 rounds. 7 μl of the product was collected for analysis. 1x Tris acetate-EDTA (TAE) electrophoresis buffer was used to prepare 1.5% Agarose SFR™ Bioteriological grade (AMRESCO, Solon, Ohio, USA), under 100 volts for 35 minutes for the analysis of the size of the DNA sections.

5. Pulsed-field gel electrophoresis (PFGE)

The US CDC's PFGE standard operational procedures were used with slight modification. Strains were inoculated on Tryptic Soy Agar (TSA, Difco

Laboratories, Detroit, MI, USA) for overnight culturing under 35℃. Colonies were placed in the cell suspension buffer (100 mM Tris: 100 mM EDTA, pH 8.0) to test wave length of 610 nm. Concentration of the fluid was adjusted to 1.15-1.25, added 1% of SeaKem Gold agarose (BMA, Rockland, ME, USA) in 20 μl Proteinase K (20 mg/ml) (Sigma, St Louis, MO, USA), mixed evenly with the fluid, and placed in plug molds (plug mold, Biometra, Goettingan, Germany), placed under room temperature for 10 minutes for coagulation, placed it in Lysis buffer (50 mM Tris: 50 mM EDTA, pH 8.0, + 1% Sarcosine, +20 μl 20 mg/ml Proteinase K/reaction), shaken under 54℃ for 2 hours, washed with dH_2O twice for 15 minutes each, and washed again with TE buffer (10 mM Tris: 1 mM EDTA, pH 8.0) four times for 15 minutes each, all in water at 50℃. Slices of 2.0-2.5 mm were taken for digestion with *Xba-1* (37℃) (MBI Fermentas, Hanover, MD, USA) and *Sfi-1* (50℃) (New England Biolabs Inc., Beverly, MA, USA) 30 units, shaken in water for four hours. Rotaphor Type V (Biometra, Goettingan, Germany) was used with 1% SeaKem Gold agarose (BMA, Rockland, ME, USA), 0.5x Tris-Borate-EDTA (TBE) buffer, under 13℃ for 5-30 seconds (logarithmical change), for 110-120 seconds (linear change), for 23 hours. Lambda Ladder PFG Marker (New England Biolabs Inc., MA, USA) was used as index for the size of the sections. The product was then dyed with 0.5 μg/ml ethidium bromide for 30 minutes, washed for 2 hours, for reading by ultra-violet radiation.

6. Dendrogram of associations

Profiles of *Xba-1* was used and stored in computers. The soft, Phoretix 1D gel analysis advanced version 5.01 (Nonlinear Dynamics, UK), was used for the analysis of associations between strains. Similarity in the profiles of electrophoresis was used to draw dendrograms by using the UPGMA (unweighted pair group method using arithmetic averages). From dendrograms, associations between strains of the same incident and strains of different incidents can be

noted.

Results

Agar disc diffusion test

Drug resistance patterns of the 38 *Shigella sonnei* strains isolated from the Bali tour members were all the same, resistant only to Sulfamethoxazone (SXT); and susceptible to Ampicillin (AM), third generation Ceftitriaxone (CRO), Nalidixic acid (NA) of the first generation Quinolone, and Ciprofloxacin (CIP) of the second generation Quinolone. The *Shigella sonnei* strains collected from outbreaks in 2001 in the northern part of Taiwan were drug resistant to Sulfamethoxazone (SXT) and Nalidixic acid (NA); of them, No. 6 strain collected from the Hsinchu outbreak was multi-resistant to AM, SXT, CRO and NA. The eight *Shigella sonnei* strains collected from sporadic cases in the northern part of Taiwan in 2003 were resistant to AM, SXT and NA. What was worth noting was that the third generation Ceftitriaxone (CRO) became intermediate from susceptible, suggesting that the abuse of antibiotics in Taiwan required more monitoring for improvement. Although NA of the first generation Quinolone commonly used for the treatment of bacillary dysentery is no longer effective, no strains have been found to be drug-resistant to the CIP of the second generation Quinolone. The disk antimicrobial susceptibility test showed that the phenotype of the drug resistance of strains isolated from the Bali tourists was different from that of the strains of the northern part of Taiwan.

AP-PCR

16 strains of the same batch conducted of M13 AP-PCR were analyzed by observing their DNA of sizes 1-10 kb. They could be, from their profiles, differentiated into four M1-M4 groups of M13 AP-PCR subtypes (Figure 2). Of them, No. 1 of the Hsinchu outbreak, No. 2 of the Yilan outbreak, and No. 4 of

the 2003 sporadic infections in the northern part of Taiwan were of M1 type; No. 6 of the Hsinchu outbreak, standard strain ATCC 25931, and Nos. 1, 2, 3, 5, 6, 7, and 8 of the 2003 sporadic infections in the northern part of Taiwan were all of M2 type; No. 16 of the Yilan outbreak was of M3 type; and Nos. 1, 2, and 12 of the Bali incident were of M4 type.

Plasmid profile analysis

Sections larger than 20 kb could be chromosomes of bacteria and could be lost in the culturing process of larger plasmids, they were not stable for extraction under high pH, they could have some impact on typing, and therefore were not used as a basis for typing [10,15,16]. The present study focused on the analysis of plasmids smaller than 20 kb. The plasmid patterns of the Bali isolates showed a consistent P8 type, and P8 was composed primarily of five plasmids, 2.8 kb, 4 kb, 9 kb, 12 kb, and 20 kb (Figure 3). The isolates of outbreaks and sporadic infections in the northern part of Taiwan were primarily of P1 type; and P1 type was composed of four plasmids, 1.7 kb, 2.8 kb, 9 kb, and 20 kb. The No. 6 strain of the Hsinchu outbreak and the No. 2 strain of the Yilan outbreak were of P1 type. The No. 1 strain of the Hsinchu outbreak was of P7 type (1.7 kb, 2.3 kb, and 9 kb); and the No. 16 strain of the Yilan outbreak was of P7 type (7 kb, 9 kb, and 20 kb). Nos. 1 and 3 of the 2003 sporadic infections in the northern part of Taiwan were of P7 type; the rest were of P1 type.

Pulsed-field gel electrophoresis (PFGE)

The PFGE profiles sectioned by *Xba*-I to analyze DNA sections of sizes 48 kb to 480 kb, by the relative positions of the DNA sections, found that a difference of four DNA sections was a cutoff point. The Bali isolates showed a consistent X7 type (Figure 1). *Sfi*-I section also gave the same PFGE profiles. The main difference between the Taiwan isolates, which were of X1, X1a, X1b, X1c, X1d, X2, X3, X4, X5, and X6 types, the Bali isolates had additional three

sections of 242.5 kb – 291.5 kb. *Sfi-I* profiles also indicated that the Bali strains were of a consistent S7 type; whereas the Taiwan strains were of S1 – S6 types.

Dendrograms of associations

The dendrograms of associations showed that the plasmid profiles of the Bali isolates and the reference strains were 16-70% associated (Figure 4); and the PFGE profiles sectioned by *Xba-I* of the Bali isolates and the reference strains were 35-80% associated; suggesting that the Bali strains were different from the strains of the northern part of Taiwan (Figure 5).

Discussion

Symptoms of Shigellosis are diarrhea, accompanied with fever, nausea, and sometimes toxemia, vomiting, cramps and tenesmus. The incubation period is for 1-3 days, and symptoms may last for 3-5 days. *Shigella* spp. is classified as a Group B notifiable disease of Category 2, is a highly infectious communicable disease of the intestinal and gastric tracts requiring reporting and isolation care. Statistics of the Center for Disease Control for the period 1995 to 1999 shows that *S. flexneri* is more common in the northern and central parts of Taiwan, with a few *S. sonnei* infections. However, since a major outbreak of *S. sonnei* infection in 2000 in a high school in Hualien, there have been sporadic cases of *S. sonnei* in the eastern part of Taiwan. Major outbreaks occurred in 2001 involving Taipei City and County, Taoyuan, and Hsinchu. *S. sonnei* infection was no longer restricted to the aboriginal townships in the mountain areas; and sporadic cases were also reported from urban areas. With the increase in international tours, particularly to the southeastern countries, importation of foreign laborers in large numbers, and illegal migrants, more diseases have been imported to pose challenges to the disease control systems that the CDC has established. The Bali incident was, next to the SARS outbreaks, a major imported case of communicable disease in 2003. To understand if the infection was actually

imported, the present study used the phenotype of drug susceptibility, AP-PCR, plasmid profile analysis, and PFGE methods to conduct a molecular epidemiological analysis of the gene fingerprints to investigate the associations.

The reference strains used in the study included Nos. 1 and 6 strains collected from the 2001 sporadic infections of Hsinchu, and Nos. 2 and 16 strains collected from the 2001 sporadic infections of Yilan, all kept in the *S. sonnei* strain fingerprint databank of the Laboratory. These four reference strains were selected after having compared with the 89 strains of 75 sporadic *S. sonnei* infections in 2001 and 2002 in the northern part of Taiwan (involving Hsinchu City and County, Taoyuan City and County, Taipei City and County, and Yilan City and County), and 14 outbreaks in the same period, they covered 80% of the major PFGE profile types, and were used as a reference of comparison for *S. sonnei* strains in the northern part of Taiwan. At the same time, eight strains of sporadic infections recently collected from the northern part of Taiwan were also used for comparison. For details of the strains, please refer to Table 1. In the drug susceptibility test, the 38 isolates of the Bali tours showed a consistent pattern of drug susceptibility, all susceptible only to Sulfamethoxazone (SXT). They were different from the Taiwan strains, which were susceptible to Ampicillin (AM), Sulfamethoxazone (SXT), and Nalidixic acid (NA). Studies show that there are on the Bali Island of Indonesia *S. dysenteriae, S. flexneri* and *S. sonnei* infections, and that *S. sonnei* is 32% and 79% susceptible respectively to Ampicillin (AM) and Sulfamethoxazone (SXT), but not resistant to Nalidixic acid (NA)[17]. Findings of the present study corresponded. No specific drug resistance plasmid types were identified by comparing the different drug resistance patterns of the phenotype of drug susceptibility test, plasmid profile analysis, and PFGE. M13 AP-PCR typing of the Bali isolates and reference strains of the northern Taiwan by the same batch (Figure 2) showed that Nos. 1, 2

and 12 of the Bali isolates were of M4 type. By the total number of types, M13 AP-PCR was poorer in typing than the plasmid profiles analysis or the PFGE methods. The plasmid profiles analysis analyzes primarily plasmids of 2.8-20 kb size, because larger plasmids become unstable along with changes of the pH values. They are likely to lose the ability to re-emerge, and are less used for typing. Plasmid types of the Bali isolates were of a consistent P8 type, and P8 is composed of primarily five plasmids, 2.8 kb, 4 kb, 9 kb, 12 kb, and 20 kb (Figure 4). Dendrograms of associations of plasmid types showed that the plasmid profiles of the Bali bacillary dysentery infections were identical to the plasmid profiles of the present incident, and were 16%-70% similar (Figure 4) to the reference strains of the northern Taiwan, suggesting that the plasmid profiles of the Bali isolates were different from that of the reference strains of the northern Taiwan.

PFGE profiles sectioned by *Xba*-I showed that, when analyzing DNA sections of 48 kb to 480 kb, by the relative positions of DNA sections, the cutoff point was four DNA sections. The Bali strains showed a consistent X7 type (Figure 1). Sections by *Sfi*-I also showed similar PFGE profiles in the Bali isolates, different from the isolates of the northern Taiwan, which were primarily of X1, X1a, X1b, X1c, X1d, X2, X3, X4, X5 and X6. The major difference was the Bali isolates had three additional sections of 242.5 kb-291.5 kb. The *Sfi*-I PFGE profiles showed that the Bali isolates had a consistent S7 type, significantly different from the Taiwan isolates, which were of S1-S6 types. Dendrograms developed by UPGMA showed that the PFGE profiles sectioned by *Xba*-I of the Bali isolates were 35%-80% similar in associations with the reference strains, suggesting that the Bali isolates were different from the Taiwan isolates (Figure 5). Phenotype of drug resistance and genotype preliminarily showed that the source of infection of the present collective *S. sonnei* incident in the Bali Island was

imported *S. sonnei* strains.

The source of infection was in another country, the epidemiological information of *S. sonnei* in the Bali Island of Indonesia was unavailable, the Laboratory conducted comparison of the available strains of the incident and the Taiwan strains to initially clear up the molecular association of the source of infection. By the information collected from tour agents, and the fact that the incubation period of *S. sonnei* infection is 1-3 days, the duck meal supplied by the Dirty Duck Restaurant was the likely source of infection. Quarantine officers immediately gave health education to passengers to Bali, recommended then to change either itineraries or restaurants, or having food in hotels, and drinking only bottled water to be safe. Since November 25, 2003, no more positive cases have been reported. The timely assessment of the source of infection by the Center for effective control measures has successfully prevented the spread of the infection in the county.

Conclusion

By the isolation and statistical analysis of the Center, the collective infection *of S. sonnei* occurred on November 7 to 24, 2003, on the Bali Island of Indonesia. Of the returned tourists, 111 were positive cases, and three were contacts. Since November 25, no more positive specimens have been detected. For the timely assessment of the source of infection, the present study decided to focus on the five tour groups to the Bali Island during November 7 and 17, 2003 for study. 38 strains of *S. sonnei* were isolated from them for molecular epidemiological investigation. For the assessment of the source of infection, two strains were selected each from the *S. sonnei* outbreaks of Hsinchu and Yilan of the northern part of Taiwan, and eight strains of the sporadic infections in the northern part of Taiwan in 2003 for comparative study, to understand if the Bali isolates were associated with the Taiwan isolates. Methods used in the study for molecular

epidemiological investigation of associations were phenotype disk antimicrobial susceptibility test, and genotype of AP-PCR, plasmid profile analysis, and pulsed-field gel electrophoresis.

The disk antimicrobial susceptibility test showed that the phenotype of the 38 *S. sonnei* strains isolated in the Bali incident was identically resistant to Sulfamethoxazone (SXT), significantly different from the Taiwan isolates, which were resistant to Ampicillin (AM), Sulfamethoxazone (SXT) and Nalidixic acid (NA). The AP-PCR, plasmid profile and PFGE typing showed that the 38 strains of the Bali incident were identically of M4, P8 and X7 types, significantly different from those of the Taiwan reference strains. Their *Xba-I* PFGE profiles of dendrograms of association were 95% similar, suggesting that the Bali isolates were closely associated to each other. By the phenotype and genotype of the imported collective infection of bacillary dysentery, it was speculated that the incident was of one source of infection, and was likely to have been imported from the Bali Island of Indonesia.

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Table 1. Basic Information and Molecular Typing of *S. sonnei* of the Bali Incident and the Taiwan Infections (in Yilan, Taipei, Taoyuan and Hisnchu)

No.	County/City	Date of Isolation	Drug Resistance Test					M13	PPA	PFGE
			AM	SXT	CRO	NA	CIP			
1	Hsinchu Co.	Oct 30, 2001	S	R	S	R	S	M1	P7	X1
6	Hsinchu Co.	Oct 30, 2001	R	R	R	R	S	M2	P1	X1c
2	Yilan Co.	Sep 10, 2001	S	R	S	R	S	M1	P1	X1
16	Yilan Co.	Sep 13, 2001	S	R	S	R	S	M3	P3	X1
1	Taoyuan Co.	Jan 16, 2003	R	R	I	R	S	M2	P7	X1d
2	Taipei Co.	Jan 27, 2003	S	R	S	R	S	M2	P1	X1b
3	Taoyuan Co.	Jan 28, 2003	R	R	R	R	S	M2	P1	X1d
4	Taoyuan Co.	Jan 30, 2003	R	R	I	R	S	M2	P7	X1d
5	Taoyuan Co.	Jan 30, 2003	R	R	I	R	S	M1	P1	X1d
6	Hsinchu Co.	Apr 11, 2003	R	R	I	R	S	M2	P1	X1a
7	Hsinchu Co.	Apr 17, 2003	R	R	I	R	S	M2	P1	X1d
8	Taipei Co.	Jul 12, 2003	S	R	S	R	S	M2	P1	X1d
1	Bali (Hsinchu C.)	Nov 11, 2003	S	R	S	S	S	M4	P8	X7
2	Bali (Taipei Co.)	Nov 12, 2003	S	R	S	S	S	M4	P8	X7
3	Bali (Taoyuan Co.)	Nov 11, 2003	S	R	S	S	S	M4	P8	X7
4	Bali (Taipei Co.)	Nov 12, 2003	S	R	S	S	S	M4	P8	X7
5	Bali (Taoyuan Co.)	Nov 12, 2003	S	R	S	S	S	M4	P8	X7
6	Bali (Taoyuan Co.)	Nov 11, 2003	S	R	S	S	S	M4	P8	X7
7	Bali (Taoyuan Co.)	Nov 7, 2003	S	R	S	S	S	M4	P8	X7
8	Bali (Taipei Co.)	Nov 7, 2003	S	R	S	S	S	M4	P8	X7
9	Bali (Taipei Co.)	Nov 7, 2003	S	R	S	S	S	M4	P8	X7
10	Bali (Taoyuan Co.)	Nov 7, 2003	S	R	S	S	S	M4	P8	X7
11	Bali (Hsinchu C.)	Nov 20, 2003	S	R	S	S	S	M4	P8	X7
12	Bali (Taipei C.)	Nov 20, 2003	S	R	S	S	S	M4	P8	X7

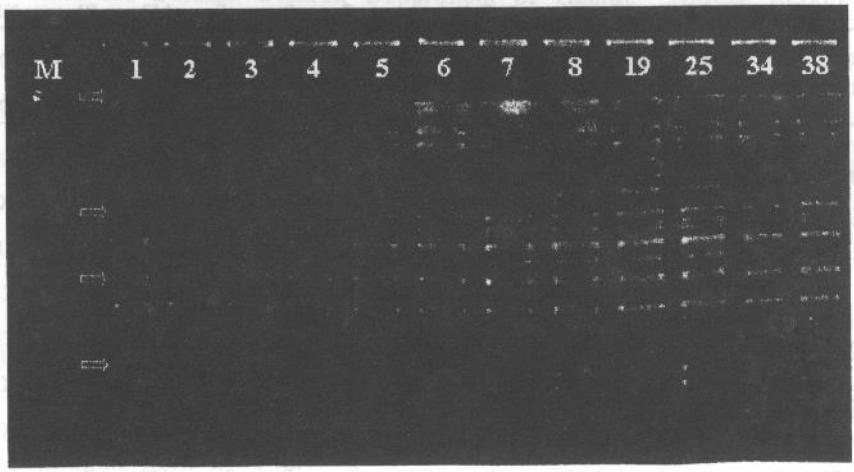

Figure 1. PFGE *Xba*-1 Profiles of *Shigella sonnei* Infections in Bali

(1) Lane 1: λ Ladder PFG Marker, Lanes 2-13, Bali isolates, number of strains as shown above.

(2) 1% SeaKem Gold agarose, 0.5x Tris-Borate-EDTA (TBE) electrophoresis, at 13℃, for 5-30 seconds (changed logarithmically), 110-120 volts (changed linearly), electrophoresis time, 23 hours.

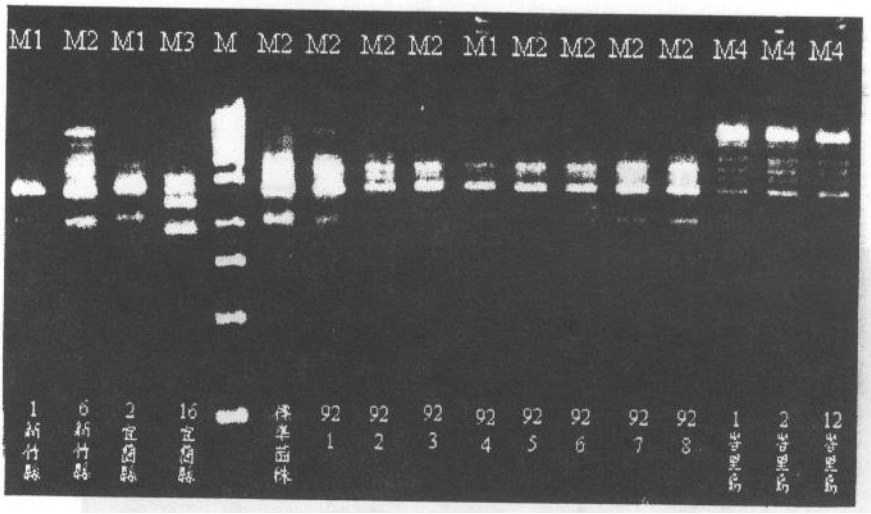

Figure 2. M13 AP-PCR Typing of the *Shigella sonnei* Infections in Bali

Lane 5 M: 1 kb DNA Ladder; Lanes 1 and 2, strains of Hsinchu sporadic cases in 2001; Lanes 3 and 4, strains of Yilan sporadic cases in 2001; Lane 6, *Shigella sonnei* standard strains ATCC 25931; Lanes 7-14, strains of the sporadic cases of the northern part of Taiwan in 2003; Lanes 15-18, specimens of Bali infections. Run 1.5% Agarose SFR™ Biological grade gel; electrophoresis at 100 volts for 35 minutes.

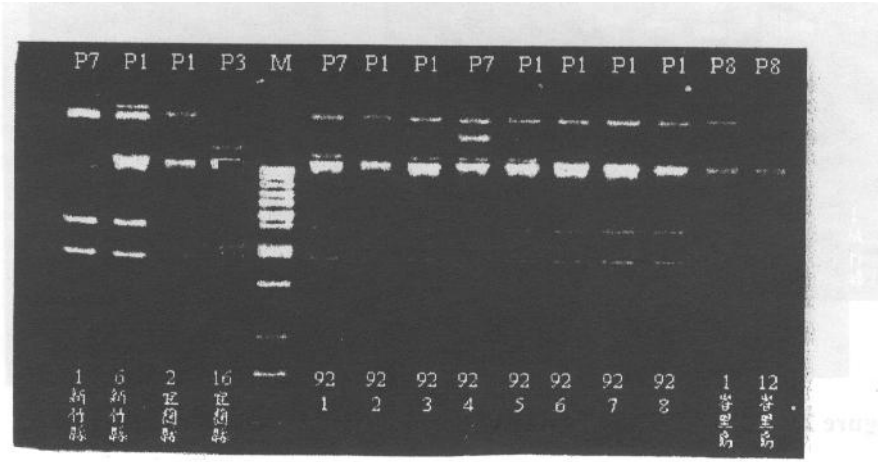

Figure 3. Plasmid Profiles of the *Shigella sonnei* Infections in Bali

Lane 5 Marker: 1 kb DNA Ladder; Lanes 1 and 2, strains of Hsinchu sporadic cases in 2001; Lanes 3 and 4, strains of Yilan sporadic cases in 2001; Lanes 6-13, strains of the sporadic cases of the northern part of Taiwan in 2003; Lanes 14 and 15, specimens of Bali infections.

Run 1.2% SeaKem LE agarose gel, electrophoresis at 100 volts for 3.5 hours; sizes of plasmids carried by strains are used for basis of typing (at a range of 1-20 kb).

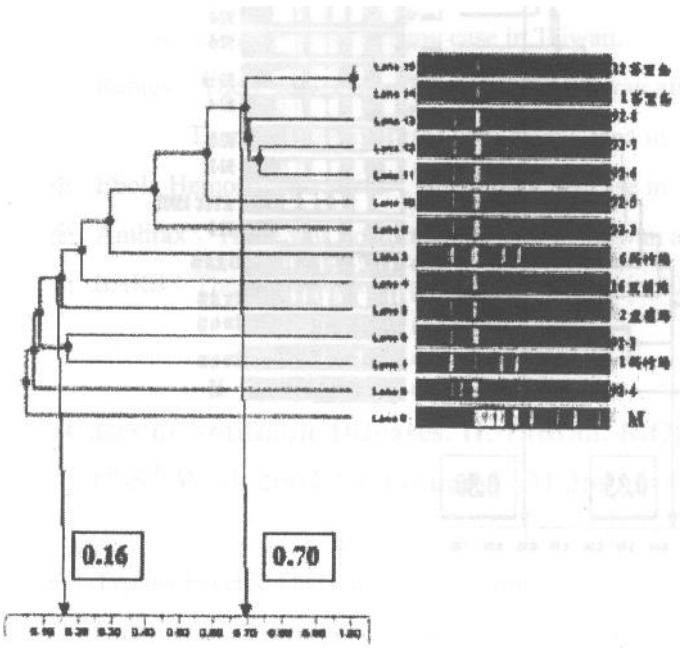

Figure 4. Dendrogram of Associations of Plasmid Profiles of the *Shigella sonnei* Infections in Bali.

Using plasmid profiles of the *Shigella sonnei* strains imported from Bali for analysis by UPGMA (unweighted pair group method using arithmetic averages) with Phoretix 1D Advance Version 5.01 soft to draw dendrogram; similarity index of strains is used to express associations.

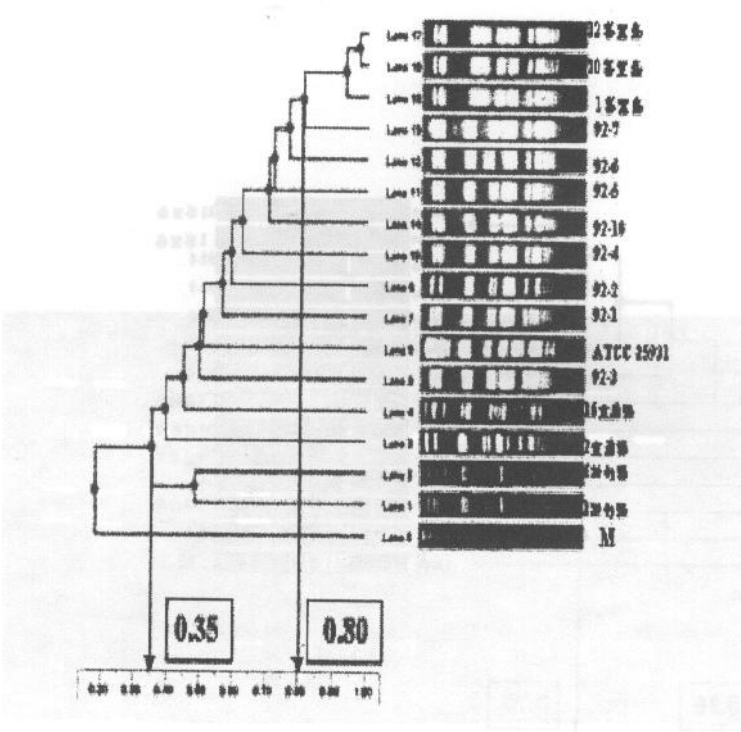

Figure 5. Dendrogram of PFGE Profiles of *Shigella sonnei* Infections in Bali

Using PFGE profiles of the *Shiegella sonnei* infections of Bali for analysis by UPGMA (unweighted pair group method using arithmetic averages) with Phoretix 1D Advanced Version 5.01 soft, to draw dendrogram; similarity index is used to express associations.