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A Molecular Fingerprint Analysis of *Shigella flexneri* 1b in Bacillary Dysentery Outbreaks in a Nursing Home in Lungchi Township, Tainan County Introduction

Abstract

Bacillary dysentery is both a common and important public health issue. Bacillary dysentery outbreaks caused by certain specific serotypes of *Shigella* spp., primarily *Shigella flexneri*, are frequently reported in nursing homes, homes for the mentally retarded, and rehabilitation institutions. Two bacillary dysentery outbreaks were reported in a nursing home in Lungchi Township of Tainan County from August through December 2003. The present study conducted a molecular fingerprint analysis of the 13 *Shigella flexneri* 1b strains isolated in the outbreaks using drug resistance testing, pulsed-field gel electrophoresis (PFGE), and enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) methods. Results show that, in drug resistance tests, two types of antibiogram were noted. Eleven strains were of type I. They were resistant to ampicillin and chloramphenical, and susceptible to cefotaxime, ciprofloxacin, and sulfamethoxazole/trimethoprim. Two strains were of type II. They were resistant to ampicillin, chloramphenicol, and sulfamethoxazole/trimethoprim, susceptible to ciprofloxacin, and showed intermediate sensitivity to cefotaxime. In PFGE testing, after cleavage with restriction enzyme *Xba*I, a main X-type pattern appeared. From the slight

differences in PFGE- patterns between strains, this X- pattern could further be divided into three subtypes. Subtype X1 had eight strains; subtype X2 had three strains; and subtype X3 had two strains. If the strains were cleavaged with restriction enzyme *Not*I, an N-type pattern with three subtypes appeared. Subtype N1 had 11 strains; subtype N2 had one strain; and subtype N3 had one strain. In ERIC-PCR test, five main fingerprint types appeared. Type I had two strains; type II had one strain; type III had three strains; type IV had five strains; and type V had one strain. From the findings of PFGE and ERIC-PCR, it could be concluded that the first bacillary dysentery case surnamed Wong, of the nursing home in Lungchi Township in whom *Shigella flexneri* 1b was isolated on August 29, should be considered an independent case; whereas the case surnamed Kuo, diagnosed at the same time could be considered the index case of the first outbreak. The suspected index case surnamed Yang of the second outbreak on December 3 showed the same PFGE or ERIC-PCR fingerprints as those of case Kuo; therefore case Yang should not be considered the index case of the second outbreak. By date of onset, case Chen with strain No. 9203471 should be considered to be the index case of the second outbreak. ERIC-PCR technique has been found to be a useful tool in rapidly screening index cases at the early stage of disease outbreaks.

Introduction

Bacillary dysentery is an acute intestinal tract infection. Incubation period is from one to three days, and can be as long as seven days. Infection may be asymptomatic or present with symptoms such as fever, vomiting, diarrhea, and bloody stool. Infection may last from four to seven days or even up to several weeks. Usually, 10 to 100 pathogenic bacilli are enough to cause the spread of this highly infectious disease [4,11]. From an epidemiological standpoint, the infection is transmitted through person-to-person contact in overcrowded

places or institutions with inadequate sanitation such as primary schools, nurseries, and nursing homes, leading to major epidemics; small-scale outbreaks among family members may also occur[3,12]. Infection may be transmitted via contaminated drinking water or food[5,13]as well.

Shigella spp. is a gram-negative bacillus. Classified according to it's biochemical and serological characteristics, the bacillus is divided into four species, *Shigella dysenteriae* (Group A), *Shigella flexneri* (Group B), *Shigella boydii* (Group C), and *Shigella sonnei* (Group D). By its O antigen, groups A, B and C can be classified further into 15, 13 and 18 serotypes respectively; whereas Group D has only one serotype[14]. According to statistics of the number of patients with notifiable diseases in the Taiwan Area in the last three years, the confirmed bacillary dysentery cases rank third[1]. More than 95% of all confirmed bacillary dysentery cases are caused by Groups B and D species[2].

In epidemiology, outbreaks of infectious diseases are normally the result of exposure to a common etiologic agent. It is therefore necessary, to clarify by typing, the clonal relationship of the common etiologic agents in order to understand causes or patterns of the epidemic, it's routes of transmission, as well as to identify the real source of infection, to discriminate between the virulent strains. By doing this, it is possible to adjust and monitor vaccination programs, and thus to effectively control an epidemic[15]. Molecular typing focuses on the nucleic acid of bacteria to determine the clonal relationship between organisms according to differences or varieties of nucleic acid arrangements in bacteria[7]. In recent years, the conventional bacterial assessment typing methods such as drug resistance pattern[16], bacteriophage typing[17], or serotyping have been replaced by emerging molecular techniques such as plasmid profile analysis (PPA)[18], ribotyping, pulsed-field gel electrophoresis (PFGE)[8,19,40], and PCR-based methods[20]. In the last 20

years, molecular typing techniques have been widely used in epidemiological studies, and the five techniques currently used for the molecular typing of *Shigella* are PFGE, PPA, ribotyping[21], ERIC-PCR(enterobacterial repetitive intergenic consensus-PCR)[22], RAPD (random amplification of polymorphic DNA)[23],insertion sequence polymorphism[24],and *ipaH*(*ipaH* polymorphism)[25].

Taking into account the discriminatory power, reproducibility, typeability, readability, and operational procedures, the present study decided to use PFGE and ERIC-PCR for the molecular epidemiological study of the *Shigella flexneri* 1b outbreaks in a nursing home in Lungchi Township of Tainan County.

The Background

On August 28, 2003, the Fourth Branch Bureau of the Center for Disease Control of the Department of Health, received a report from the Tainan Municipal Hospital of a suspected case of bacillary dysentery. The case, an inmate of a nursing home in Lungchi Township, developed diarrhea on August 23, and was admitted to the Tainan Municipal Hospital for treatment on August 25. The Hospital, suspecting bacillary infection, collected rectal swabs for bacterial culture. The strains were sent to the Fourth Branch Bureau for confirmation on August 29, and this first reported case was confirmed *Shigella flexneri* 1b positive on September 1 (case surnamed Wong, index case of the first outbreak). Later, one case of *Shigella flexneri* 1b was detected from specimens of a contact (surnamed Kuo). The remaining 52 specimens were all negative. On December 2, the Tainan Municipal Hospital reported again a new suspected case of bacillary dysentery, and sent specimens to the Branch Bureau for confirmation on December 3. The new case was confirmed *Shigella flexneri* 1b positive on December 5 (case surnamed Yang, index case of the second outbreak). From 48 specimens of contacts, *Shigella flexneri* 1b was isolated in four. In the subsequent investigation by the Branch Bureau, from the 165 specimens collected from contacts and environmental specimens

on December 4, 7, 8 and 15, four more *Shigella flexneri* 1b cases were detected. Inmates of the nursing homes are patients with moderate, severe and extremely severe mental retardation; they have difficulty expressing clearly their physical problems. To screen out all carriers, the Branch Bureau decided for the second time to collect specimens from all inmates and staff of the nursing home on December 17. Findings of laboratory testing on December 19 detected two new *Shigella flexneri* 1b cases from the 164 specimens of inmates and environment. In the period between August 29 and December 17, a total of 13 *Shigella flexneri* 1b positive cases were detected. All environmental specimens were negative.

Materials and Methods

Targets for Epidemiological Investigation

The nursing home in Lungchi Township, Tainan County, had at the time 130 inmates and 34 staff members. The nursing home is registered, and is located on the outskirts of Lungchi Township, Tainan County. The buildings and the environment are satisfactory. The nursing home accepts patients with moderate, severe and extremely severe mental retardation with ages ranging from 16 to 94 years. Eight patients share a room; and two rooms share a bathroom. All inmates live in the home. Families visit them occasionally. The last inmate was admitted on July 15. Staff members commute to work.

Definition of Case

Confirmed case: an inmate or staff member of the nursing home who developed one of these symptoms: diarrhea, abdominal pain, fever, vomiting, and tenesmus, and was confirmed positive by laboratory testing.

Suspected case: an inmate or staff member of the nursing home who developed one of these symptoms: diarrhea, abdominal pain, fever, vomiting, and tenesmus, but was found negative by laboratory testing.

Collection of Specimens

Human specimens: rectal swabs of all inmates and staff members of the nursing home.

Environmental specimens: specimens of food, drinking water in the dining room, water in kitchen, water from outlets of the water tower, and specimens from the handles of the wash basins in the toilets were collected in sterilized bags.

Laboratory Methods

Human specimens: specimens were sent in Carry-Blair transport medium to the laboratory; they were tested using the standard operational procedures for the laboratory testing of bacillary dysentery[9].

Water specimens: a 250 mL water sample was taken, filtered through 0.45 μm filters (Whatman®, England). Filters were stuck separately with Salmonella-Shigella agar (SS), Deoxycholate Hydrogen Sulfide Lactose agar (DHL), Xylose Lysine Desoxycholate agar (XLD), and Hektoen Enteric agar (HE). They were placed in 10 mL of nutrient broth, and tested using the standard operational procedures for laboratory testing of bacillary dysentery. Another sample of 250 mL of water was taken for the testing of coliform[10] bacteria.

Food specimens: same as human specimens.

Environmental specimens: same as human specimens.

Drug resistance analysis: Drug susceptibility testing of the isolated *S. flexneri* 1b was conducted by the dilution and disk diffusion method[26]. Antibiotics used and their concentrations were, Ampicillin (AM) 10 μg, Cefotaxime (CTX) 30 μg, Ciprofloxacin (CIP) 5 μg, Chloramphenicol (C) 30 μg, and Sulfamethoxazole/Trimethoprim (SXT) 1.25 μg/23.75 μg.

Pulsed-field gel electrophoresis: The US CDC procedures[27] were partially modified for use. A single colony was picked up from the overnight culture

plate, inoculated on 2 mL of nutrient broth, shaken for culturing at 37℃, and centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded. 0.5 ml of SE buffer (75 mM NaCl, 25 mM EDTA, pH 8.0) was added, the broth washed again, centrifuged at 10,000 rpm for 5 minutes, and the supernatant was again discarded. SE buffer was added, and tested for light absorbance under 610 nm wave lengths. Fluid concentration was adjusted to OD values between 1.15 and 1.25. 1.6% Chromosomal Grade agarose (Bio-Rad Laboratories, Richmond, CA, USA) of equal volume was taken and mixed evenly with the fluid. It was then put in plug mold (Bio-Rad Laboratories), placed at 4℃ for 10 minutes for coagulation. To the plug was added lysis buffer (50 mM Tris, 50 mM EDTA, 1% Sodium lauryl sarcosine, 1 mg/mL proteinase K, pH 8.0), and shaken for overnight reaction at 50℃. The lysis buffer was discarded, TE buffer (1 M Tris base, 0.37 g EDTA, pH 8.0), containing 1mM phenyl methylsulfonyl fluoride (Sigma Chemical Co., St Louis, MI, USA), was added, then washed twice, each time placed at room temperature for 30 minutes. The PMSE/TE buffer was thrown away, and washed four times with TE buffer, 30 minutes each time. A half of the plug was taken, placed in reaction solution not containing either *Xba*I or *Not*I restriction enzymes, placed at room temperature for 30 minutes. The reaction solution was discarded, 20 U of reaction solution containing *Xba*I or *Not*I restraint enzymes was added, placed at 37℃ for overnight reaction. The reaction solution was discarded, 0.5 X TBE buffer was added, placed at room temperature for use. BioMetra (Rotaphor Type V, Germany) was used with 1.2 % agarose (Pulsed Field Certified DNA Grade Agarose, Bio-Rad Laboratories) at 14℃, electric field angle 120^0, changing at 5 to 33 seconds, electrophoresis time for 22 hours, and voltage at 200 V. Lambda Ladder PFG marker (BioLabs, New England) was used as marker of molecular weight. Finally, at a concentration of 1.0 μg/mL ethidium bromide, it was stained for 30

minutes, de-colorized for 30 minutes, and irradiated with ultraviolet rays for imaging.

ERIC-PCR analysis: Analysis followed the method published by Liu et al. (1995)[28]. Three to five fresh colonies were picked up from the nutrient plate, 50 μL of sterile distilled water was added, mixed evenly and heated to boiling for 15 minutes, centrifuged at 4℃ and 12,000 rpm for 5 minutes. 1*uL* of the supernatant was taken as template and mixed evenly with the reaction solution to a volume of 50 μL. The reaction solution contained 1 U of *Taq* polymerase (Applied Biosystems, USA), 10 mM Tris (pH 8.3), 50 mM KCl, 5 mM $MgCl_2$, 0.01% gelatin, 250 uM deoxynucleoside triphophates (each), and a single primer of 5'-GTGAATCCCCAGGAGCTTACAT-3' of 1 uM concentration. The amplification reaction was conducted in HYBAID (USA), under (1) template denature at 95℃ for 5 minutes, (2) low products amplification at 94℃ for 1 minute; 26℃ for 1 minute; 72℃ for 2 minutes, in four cycles, (3) amplification reaction at 94℃ for 30 seconds; 40℃ for 30 seconds; 72℃ for 1 minute, in 40 cycles, (4) the final extension at 72℃ for 10 minutes. Sterile distilled secondary water not containing DNA was used as control. After the amplification reaction, 10 uL of PCR reaction product was taken, mixed with 1.6% agarose, and 100 bp DNA Ladder (Violet BioScience, Inc., USA) was used as markers of molecular weight. Electrophoresis was conducted at 50 V for 95 minutes, stained with 1.0 μg/mL ethidium bromide for 5 minutes, de-colorized for 10 minutes, and irradiated with ultraviolet rays for imaging.

Results of Laboratory Testing

Water, food and environmental specimens were all negative for *coliform*, *E. coli*, *Salmonella* and *Shigella bacteria*.

In the drug sensitivity testing, the 13 *Shigella flexneri* 1b strains isolated from the nursing home showed two antibiogram patterns. The first pattern

with 11 strains was resistant to ampicillin and chloramphenicol, and susceptible to cefotaxime, ciprofloxacin and sulfamethoxazole/trimethoprim. The second pattern with two strains was resistant to ampicillin, chloramphenical and sulfamethoxazole/trimethoprim, susceptible to ciprofloxacin, and showed intermediate sensitivity to cefotaxime (Table 1).

In the PFGE testing, the 2nd through the 14th strains were positive cases of the 13 *Shigella flexneri* 1b cases; and the first strain was the *Shigella flexneri* 1b isolated and retained by the Branch Bureau in 2001. It was isolated from a positive case of A-lien Township, Kaohsiung County, who was working in Tainan City. It was used as a reference strain in the present study. Results of the testing showed that when the strains were cleavaged with restriction enzyme *Xba*I, they showed a major X-pattern. From the slight differences of strains in PFGE patterns, the X- pattern could be further divided into three subtypes (Figure 1). Subtype X1 had 8 strains, X2 had 3 strains, and X3 had 2 strains. The X2 subtype had one more section than the X1 subtype in the area between 48.5 and 97.0 kb; and the X3 subtype had one more section each than the X1 subtype in areas 48.5 and 97.0 kb and 145.5 and 197.0 kb. Though the second and the 13th strains were of the same X3 subtype, for deletion mutation, the 13th strain had a small section in the area between 97.0 and 145.5 kb that was different from that of the 12th strain, and was closer to 97.0 kb. The reference strain (the first) was of X2 subtype. It had one more section than the X1 subtype in the area between 48.5 and 97.0 kb. When strains were bisected with restraint enzyme *Not*I, a major PFGE gram of N type appeared. The type could also be divided into three subtypes (Figure 2). Subtype N1 had 11 strains, N2 had 1 strain, and N3 had 1 strain. Subtype N2 had one more section than subtype N1 in the area between 97.0 and 145.5 kb. Though two subtypes had the same number of sections in the area between 48.5 and 97.0 kb, they were located differently, and their sizes were different.

Subtype N3 had one more section each than subtype N1 in areas between 48.5 and 97.0 kb and 97.0 and 197.0 kb. The reference strain (the first) was of N2 subtype. It had one more section than subtype N1 in the area between 197.0 and 232.5 kb. Compared with the 12th strain of the same N2 subtype, the 12th had one fewer section in the area between 197.0 and 242.5 kb, and one more small section in the area between 97.0 and 145.5 kb. In the area between 48.5 and 97.0 kb, the number of sections was the same, though located differently. It was hypothesized that the 13th strain could have mutated from the reference strain.

The ERIC-PCR testing showed that there would be 6 to 10 PCR sections in the area between 300 and 2,000-bp, making the 13 *Shigella flexneri* 1b strains demonstrate five major ERIC-PCR fingerprints, two strains in type I, one strain in type II, four strains in type III, five strains in type IV, and one strain in type V (Figure 3). Of them, the 3rd strain (of case surnamed Wong, the index case of the first outbreak), the 2nd strain (case Kuo of the first outbreak), the 4th strain (case Yang, index case of the second outbreak), and the 7th strain (case of the second outbreak) were of type III. However, the molecular fingerprint of case Wong, index case of the first outbreak, had no 600-bp PCR section but had 500-bp PCR section; it was different from the other three. The other three had 600-bp PCR section but no 500-bp PCE section. From the outcome of ERIC-PCR testing, case Wong, diagnosed with *Shigella flexneri* 1b on August 29, should be considered an independent case. Case Kuo of the same time was probably the index case of the first outbreak. The suspected index case of the second outbreak, case Yang, had similar PFGE and ERIC-PCR findings as case Kuo, it was postulated that case Yang was not the index case of the second outbreak. The index case must have been someone else. By the date of onset, the case with strain No. 9203471 should be considered the index case of the second outbreak.

Discussion

By drug susceptibility testing, the 13 *Shigella flexneri* 1b isolated from the nursing home showed two antibiogram patterns. Type I, the main pattern, had 11 strains, and was resistant to ampicillin and chloramphenicol, and susceptible to cefotaxime, ciprofloxacin and sulfamethoxazole/trimethoprim. Type II, with two strains, was resistant to ampicillin, chloramphenical and sulfamethoxazole/trimethoprim, susceptible to ciprofloxacin, and showed intermediate sensitivity to cefotaxime. Ciprofloxacin (Superocin) was the antibiotic used by the DOH Tainan Hospital Hsinhua Branch. The use of the findings of drug susceptibility test in the present epidemiological investigation was limited, as the drug susceptibility patterns of type II (RISRR) and type I (RSSRS) were different, and their reasons for the differences in phenotypes could not be differentiated in the PFGE and ERIC-PCR. However, Villalobo et al. (1998) pointed out that *Shigella* bacillary dysentery with large size virulence plasmid should be considered a very virulent strain[29,30]. Anderson et al. (1999) further pointed out that virulent strains were more drug resistant and of higher transmissibility[32]. When considering the use of drug susceptibility in epidemiology, it would be more meaningful to use plasmid virulence genes for the study of the virulence, epidemicity and antibiotic resistance of pathogenic bacillary dysentery[33,34,35,36]. Moreover, the pathogenicity island on the chromosomes often contained transposons and Integron (gene cassettes that integrate drug resistance genes that are acquired horizontally), study of the gene structure, virulence and horizontal transmission of the pathogenicity island could be an important marker of epidemiology[31]. Study of plasmid virulence genes and pathogenicity island will be the next step of the present project.

In the PFGE, the 2nd through 14th strains were the positive cases of 13 *Shigella flexneri* 1b isolated from the nursing home. The 1st strain was the only *Shigella flexneri* 1b strain isolated and retained by the Branch Bureau in 2001. The strain was isolated from a positive case from A-lien Township, Kaohsiung County, working in Tainan City. The strain was used in the present study as a reference strain. Cleavage with *Xba*I restriction enzyme showed that there was only one type of strain in the bacillary dysentery outbreaks of the nursing home, that is, there was only one major X-pattern. Subtype X1 had 8 strains, subtype X2 had 3 strains, and subtype X3 had 2 strains. The reference strain (the first) was of X2 subtype. If strains were cleavaged with *Not*I, there was also one type of strain in the bacillary dysentery outbreaks of the nursing home, that is, there was only one major N- pattern. Subtype N1 had 11 strains, subtype N2 and subtype N3 had one strain each. The reference strain was of subtype N2. The Branch Bureau had not, prior to 2000, detected any *Shigella flexneri* 1b in its jurisdiction. According to the restraint enzyme *Xba*I and *Not*I- patterns, it could be postulated that the bacillary dysentery outbreaks in the nursing home of Lungchi Township, Tainan County, were one single incident. The reference strain was similar to a certain subtype; it could have originated from the same source of the main strains of this incident, though there were slight differences due to mutation[(39)]. If information about *Shigella flexneri* 1b in other areas and at different times had been available, the relationship between the two could probably be explained, and the epidemic situation of *Shigella flexneri* 1b in Taiwan could be better understood.

PFGE typing[(6)] is based primarily on the existence and number of sections at the relative position. If the difference is larger than three sections, the strains are classified under different biograms. If the difference is from one to three sections, they are subtyped. By analyzing the similarities of PFGE- patterns,

the relatedness of strains, and whether they come from the same source of infection can be understood. This is calculated by the D coefficients formula[38]. That is, the number of sections at the same relative position in two isolated strains is multiplied by 2 and divided by the total number of sections. When D is $\geqq$ 0.8, the strains are considered to come from related strains. In the relatedness of the 13 *Shigella flexneri* 1b strains of the present study, the D coefficient was between 0.92 and 0.96, larger than 0.8, and therefore, these strains were considered to be significantly related.

The ERIC sequences are in the non-coding regions of genes, or above and below the open reading frames, or between operons. It is a repetitive sequence of 126 bp. In the middle of the sequence, there is a section of conserve sequence of 40 bp[37]. ERIC sequences are located differently in different species or strains; the frequency of repetition is also different. By PCR amplification, strain-specific fingerprints will appear. This method is, therefore, widely used in the typing of isolated gram-negative and positive strains. The present study also used this method to analyze the genomic variability of ERIC sequences. Findings of laboratory testing suggested that case Kuo was most likely the index case of the first outbreak; and case Chen of strain No. 9203471 was the index case of the second outbreak.

Since all food, water and environmental specimens were negative, the possibility of transmission by food or water was therefore excluded. The drug susceptibility test was found to be of limited use in typing. If the test was matched with analysis of the genomic types of drug resistance to comprehend drug resistance mechanisms, the functions of the drug susceptibility test would be more complete. Furthermore, though drug susceptibility testing could predict the clinical results of drug use, their clinical indexes could not explain the new significantly reduced susceptibility. That is, though clinical indexes

may indicate susceptibility, yet the strains are still likely to be resistant. The antibiotic used by the Municipal Tainan Hospital was Ciprofloxacin, tested for susceptibility in the present study. This is a second-generation Quinolone. A recent study of the National Health Research Institute shows that of a total of 1,203 strains of *Escherichia coli*, 22% showed reduced susceptibility to Ciprofloxacin. In the case of *Shigella*, further studies are needed, because *Shigella* is a virulent strain of a high virulence plasmid. Generally speaking, virulent strains are more antibiotic resistant and of higher epidemicity. Studies of the interactions of virulence, epidemicity and antibiotic resistance of the pathogenic agents of bacillary dysentery, and the gene structure, virulence and horizontal transmission of the pathogenicity island can function as important epidemiological markers. Corresponding to the D coefficient of PFGE, these isolated strains were found to be significantly related and of the same source of infection. The reference strain was similar to certain strains, suggesting the reference strain was of the same source as strains of the main types of this incident, the slight differences being due to mutation. If information about *Shigella flexneri* 1b occurring in other areas and at different time periods becomes available, the relationship between the two might be explained, and the epidemic situation of *Shigella flexneri* 1b in Taiwan could be understood better. Case Wong, diagnosed with *Shigella flexneri* 1b on August 29, should be considered an independent case. Case Kuo of the same time period was probably the index case of the first outbreak. Since case Yang of the second outbreak on December 3 had the same PFGE and ERIC-PCR fingerprints as case Kuo, it was postulated that he was not the index case of the second outbreak. By date of onset, case Chen with strain No. 9203471 should be considered the index case of the second outbreak. The ERIC-PCR method, due to its easy performance and reproducibility, is considered a good method to screen index cases at the early stage of outbreaks.

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Table 1. Information of *Shigella flexneri* 1b Cases and Findings of Laboratory Testing

No.	Strain No.	Age Sex	Date of Onset	Specimen Received	Epidemiological Relatedness	PFGE *Xba*I / *Not*I	ERIC-PCR	AM/CTX/CIP/C/SXT Susceptibility*
1**	9003009	47 F	900801	900807	Control	X2/N2	None***	None
2	9202041 (Case Kuo)	18 M	N0ne	920829	Inmate 1st outbreak	X1/N1	III	RSSRS****
3	9202177 (Case Wong)	62 M	920823	920829	Inmate、index case、1st outbreak	X1/N1	III	RSSRS
4	9203440 (Case Yang)	19 M	921127	921203	Inmate、index case、2nd outbreak	X1/N1	III	RSSRS
5	9203470	50 M	921125	921203	Inmate 2nd outbreak	X1/N1	IV	RSSRS
6	9203471	52 M	921121	921203	Inmate 2nd outbreak	X2/N1	IV	RSSRS
7	9203481	49	921113	921203	Inmate 2nd outbreak	X1/N1	III	RSSRS
8	9203483	70 M	921201	921203	Inmate 2nd outbreak	X1/N1	I	RSSRS
9	9203580	52 M	921204	921204	Inmate 2nd outbreak	X1/N1	V	RSSRS
10	9203725	74 F	None	921207	Inmate 2nd outbreak	X1/N1	IV	RSSRS
11	9203761	44 M	921204	921208	Inmate 2nd outbreak	X2/N1	IV	RSSRS
12	9203980	83 M	921211	921215	Inmate 2nd outbreak	X3/N3	II	RISRR
13	9204042	64 M	921215	921217	Inmate 2nd all-home screening	X3/N2	IV	RISRR
14	9204053	28 F	None	921217	Inmate 2nd all-home screening	X2/N1	I	RSSRS

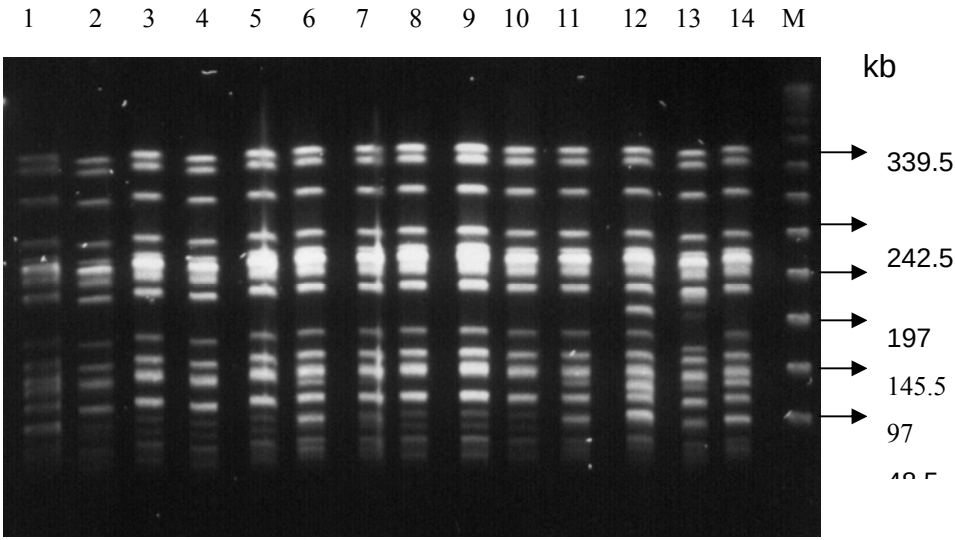
* AM: Ampicillin; CTX: Cefotaxime; CIP: Ciprofloxacin; C: Chloramphenical; SXT: Sulfamethoxazole/Trimethoprim, None.

** This strain was isolated by the Branch Bureau in 2001 from a positive case of A-lien Township, Kaohsiung County then working in Tainan City.

*** None: No experimental value.

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

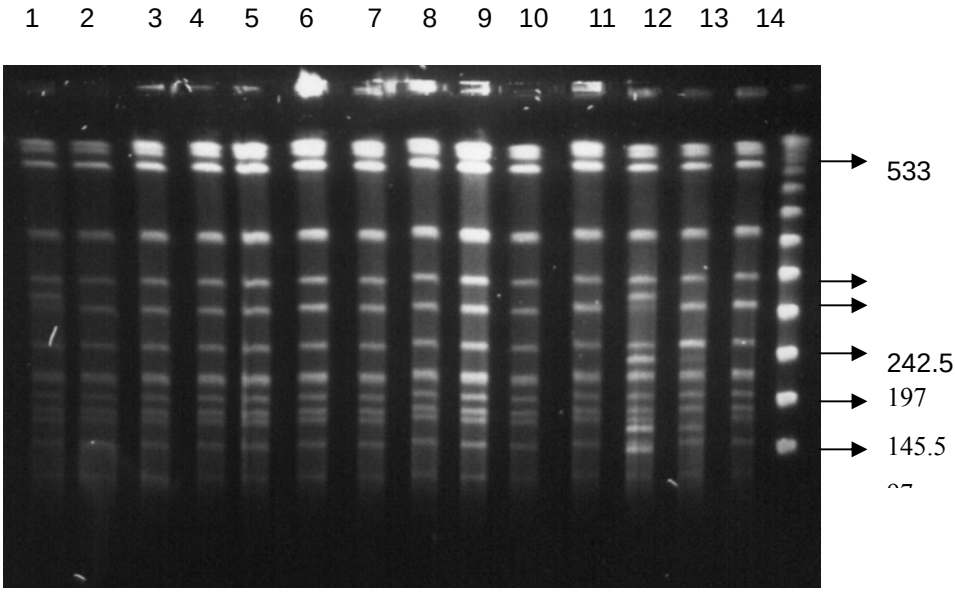
Figure 1. *Xba*I-PFGE-gram



M：Lambda Ladder PFG marker (BioLabs, New England)。

No 1: Controls (strain No 9003009, isolated by the Branch Bureau in 2001 from a positive case of A-lien Township, Kaohsiung County, then working in Tainan City); Nos 2-14: 13 confirmed *Shigella flexneri* 1b strains of bacillary dysentery outbreaks in a nursing home in Lungchi Township, Tainan County; No 2: case Kuo of strain No 9202041; No 3: case Wong of strain No 9202177; No 4: case Yang of strain No 9203440; No 5: strain No 9203471; No 6: strain No 9203471; No 7: strain No 9203481; No 8: strain No 9203483; No 9: strain No 9203580; No 10: strain No 9203725; No 11: strain No 9203761; No 12: strain No 9203980; No 13: strain No 9204042; and No 14: strain No 9204053.

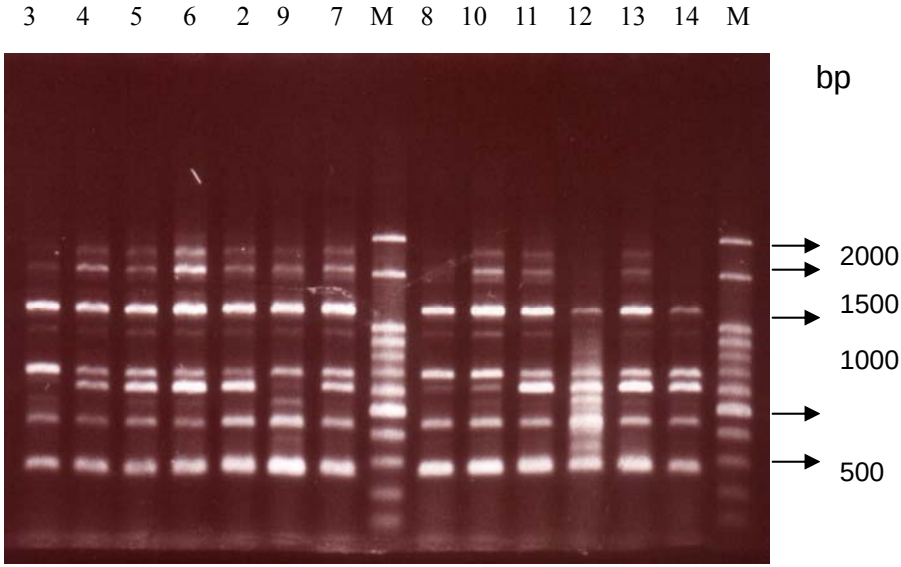
Figure 2. *Not*I-PFGE-gram.



M：Lambda Ladder PFG marker (BioLabs, New England)。

No 1: Controls (strain No 9003009, isolated by the Branch Bureau in 2001 from a positive case of A-lien Township, Kaohsiung County, then working in Tainan City); Nos 2-14: 13 confirmed *Shigella flexneri* 1b strains of bacillary dysentery outbreaks in a nursing home in Lungchi Township, Tainan County; No 2: case Kuo of strain No 9202041; No 3: case Wong of strain No 9202177; No 4: case Yang of strain No 9203440; No 5: strain No 9203471; No 6: strain No 9203471; No 7: strain No 9203481; No 8: strain No 9203483; No 9: strain No 9203580; No 10: strain No 9203725; No 11: strain No 9203761; No 12: strain No 9203980; No 13: strain No 9204042; and No 14: strain No 9204053.

Figure 3. ERIC-PCR-gram



M：100 bp DNA Ladder (Violet BioScience, Inc, USA)。

No 2: case Kuo of strain No 9202041; No 3: case Wong of strain No 9202177; No 4: case Yang of strain No 9203440; No 5: strain No 9203471; No 6: strain No 9203471; No 7: strain No 9203481; No 8: strain No 9203483; No 9: strain No 9203580; No 10: strain No 9203725; No 11: strain No 9203761; No 12: strain No 9203980; No 13: strain No 9204042; and No 14: strain No 9204053.