

A Shigellosis Outbreak Caused by *S. flexneri* X Variant at a Psychiatric Hospital in Miaoli County

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Abstract

In March 2008, there was an outbreak of acute diarrhea among patients of a psychiatric hospital located in Miaoli County, Taiwan. The outbreak was caused by *Shigella flexneri*. A total of 15 cases were identified in the outbreak, all of them were inpatients of the hospital, with an attack rate of approximately 5% (15/307). Among those cases, 9 were symptomatic (6 of the specimens collected were cultured positive) and the other 6 were apparently asymptomatic (contacts tested positive). Of 10 *S. flexneri* isolates obtained, one was subserotype 2b and 9 were the extremely rare X variant type. This was the first shigellosis outbreak caused by X variant type ever reported in Taiwan. The isolates were subjected to antimicrobial susceptibility testing and pulsed-field gel electrophoresis (PFGE). The isolates were resistant to first-line antimicrobials, including ampicillin, streptomycin, tetracycline,

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chloramphenicol, and trimethoprim/sulfamethoxazole. DNA fingerprint comparison with those in the *Shigella* DNA fingerprinting database, established by Taiwan Centers for Disease Control, revealed that the outbreak isolates shared more closely pattern similarity than those in the database and suggested that the isolates of subserotypes 2b and serotype X should be derived from a common recent ancestor. In order to search the infection source, the Second Branch Office of Taiwan CDC carried out an epidemiological investigation. Unfortunately, the investigation failed to identify the source of infection. However, reviewing the fact that the X variant was rare in Taiwan and its DNA fingerprints shares high level of pattern similarity to some imported 2b isolates in the database, inferring that the outbreak strain most likely came from abroad.

Keywords: outbreak, shigellosis, pulsed-field gel electrophoresis (PFGE), DNA fingerprinting

Introduction

Shigellosis is an infectious disease caused by *Shigella* spp.; it is one of the most common diarrheal diseases in the developing world [1]. The typical symptoms of shigellosis include fever, diarrhea, nausea, vomiting, stomach cramps, and mucous-like blood stool. Infections are often asymptomatic. Based on the antigenic characteristics, *Shigella* is classified into four species: *S. dysenteriae* (group A), *S. flexneri* (group B), *S. boydii* (group C), and *S. sonnei* (group D). Only one subserotype has been found so far in group D while groups A, B, and C contain 15, 13, and 20 subserotypes, respectively. The 13 subserotypes that have been identified within *S. flexneri* are 1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b, 5a, 5b, 6, X variant, and Y variant, but there are still certain strains nontypable. However, those so-called nontypable isolates

can eventually be designated as new serotypes. Examples are *S. flexneri* 1c, recently prevalent in Egypt [2], Bangladesh [3], and Vietnam [4] and *S. flexneri* 4c [5], which caused a shigellosis outbreak in Mainland China [6]. In 2008, at the 5th annual conference of PulseNet Asia Pacific held in Calcutta, India, there were reports about recent Chinese epidemics of shigellosis caused by *S. flexneri* 4c.

According to the data from the Infectious Disease Data Warehouse of Centers for Disease Control, Taiwan (Taiwan CDC), the total number of confirmed shigellosis cases across Taiwan in 2007 was 246, of which 45 cases were imported and the other 201 cases were indigenous. The majority of the *Shigella* isolates were *S. flexneri* and *S. sonnei*. *S. flexneri* mainly caused endemic infections among aboriginal tribes in the mountainous area [7], while *S. sonnei* accounted more often for outbreaks among people living in townships, and occurred at populous institutions like schools, psychiatric centers, nursing homes, prisons or military camps [8]. Main transmissions occur from human to human contact and transmission through contaminated food and water often lead to large-scale epidemic [9]. In recent years, due to the progress in food preservation technology, international food trade has been flourishing; incidents of international shigellosis outbreaks occurred through contaminated foodstuffs from time to time. An example was, in 2007, outbreaks by *S. sonnei* took place in Denmark and Australia, which were later linked to contaminated baby corn exported from Thailand [10]. Contaminated meals offered on board of airplanes can also cause international shigellosis outbreaks, as in 2004, a *S. sonnei* outbreak happened in the United States and Japan [11]. In Taiwan, most imported shigellosis cases were from Southeast Asian countries. According to the statistics



between 2002 and 2006, the top six countries for imported shigellosis cases, in decreasing order, are Indonesia, China, Vietnam, Thailand, Cambodia, and India.

Pulsed-field gel electrophoresis (PFGE) is a powerful subtyping technique that can be used to effectively track down the transmission route of a specific bacterial strain and thus is helpful in investigating outbreaks. PFGE is the current standard typing method of PulseNet International, an international molecular typing network for surveillance of foodborne diseases. Since 2002, the Taiwan CDC had established the standardized PulseNet PFGE protocols for routine analysis and surveillance of various bacterial pathogens. The resulting DNA fingerprints are analyzed by computer software named BioNumerics and save with the epidemiological information for the isolates in a DNA fingerprint database for several bacterial pathogens including *Shigella*. This database is used as technical platform for disease surveillance and epidemiological investigation of infections.

Materials and Methods

Outbreak report:

On March 26, 2008, several inpatients of a psychiatric hospital located in Miaoli County suddenly displayed symptoms of fever, diarrhea, vomiting, and blood stool one after another. On that day, the hospital's infection control team suspected it was a cluster of acute gastrointestinal infection. They notified the local health bureau immediately, and collected stool specimens to the Central Regional Laboratory of Taiwan CDC for further testing. Soon the laboratory verified that the incident was a shigellosis outbreak caused by *S. flexneri*. During this outbreak, there were 9 persons showing

symptoms and from 6 of them, *S. flexneri* was isolated. The pathogen was also isolated from 6 asymptomatic contacts. After implementation of infection control measures, no new cases appeared after April 3 and two weeks quarantine for all cases was finally lifted on April 17.

Background of the institution:

The institution was a newly established psychiatric hospital for treating and caring patients suffering from acute and chronic psychiatric disorders, drug abuse, and alcoholism. The hospital is a 5-floor building. The first floor of the building was the ward for those patients only staying at the hospital during the daytime, whereas the 2nd to 5th floors were beds for acute cases. At the time when the incident took place, there were 210 inpatients and 97 at the place.

Environment inspection and specimen collection:

1. Environment inspection: Water samples were taken from tap water and well water, along with those from drinking water machines and bathroom faucets on each floor of the building. Those water samples were tested for total bacterial counts and cultured for pathogenic microorganism. Aside from that, the inspector released some red No. 5 edible dyestuff into 6 toilets on different floors on April 2 and watched carefully at all faucets from then on till April 5 to see if any of the red dye reappeared in the tap water. The purpose of the test was to find out whether the water supply, disposal tubing networks, and storage water tank had any leaks.
2. Personal specimen collection: The hospital took rectal swab specimens from all symptomatic cases and their contacts. All of those specimens were preliminarily screened at the hospital's own microorganism



laboratory, some were also sent directly to the Central Regional Laboratory of Taiwan CDC for bacterial examination. Isolated strains were also shipped over to the latter PFGE molecular typing analysis. Miaoli County Health Bureau followed 14 employees of the contracted box meal supplier of the hospital with their swab specimens taken and examined by the hospital.

Bacterial isolation, cultivation, and identification:

After the Central Regional Laboratory of Taiwan CDC had received the specimens (either in the form of a rectal swab or an isolate), it was first cultivated in Hekton Enteric (HE) selective medium (Creative Media Products, Ltd., Taiwan) at 37°C for 18-20 hours, and then selected for suspicious fermentative colonies (*Shigella* grown on HE medium would appear in the same dark green background color as that of the medium). Next, it was cultivated separately in three presumptive identification media, i.e. TSIA (Triple Sugar Iron Agar), LIA (Lysine Iron Agar), and SIM (Sulfite-Indol-Motility), all of them from Creative Media Products, Ltd., Taiwan. The typical biochemical reactions of *Shigella* to these medium reagents are as follows: red/yellow response in TSIA, H₂S negative, and generating no gas; the lysine in LIA does not ferment (negative); and the SIM mobility is also negative. Once the strain had met all these three essential biochemical requirements, it was then mixed with *Shigella* antiserum (DENKA SEIKEN Co., Ltd., Japan) in a slide agglutination test for the purpose of identification and serotyping. They are also processed for biochemical catabolism verification using API20E (a commercial kit from bioMerieux, France).

Antimicrobial susceptibility testing:

Disk diffusion method was performed for antimicrobial susceptibility testing, with a total of 16 antimicrobials that included 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), gentamicin (GM, 10 µg), kanamycin (K, 30 µg), nalidixic acid (NA, 30 µg), norfloxacin (NOR, 10 µg), ofloxacin (OFX, 5 µg), streptomycin (S, 10 µg), tetracycline (TE, 30 µg), tobramycin (NN, 10 µg), and trimethoprim/sulfamethoxazole (SXT). *Escherichia coli* ATCC 25922 was used as the standard reference strain. After cultivated for 18-24 hours, resistance levels were measured by the size (mm) of the inhibition zone, following the guidelines published by Clinical and Laboratory Standard Institute (CLSI, used to be NCCLS). The verdicts are expressed as R (resistant), S (susceptible), or I (intermediate).

PFGE and pattern comparison:

The isolates were embedded and plugs were washed, followed the standardized PulseNet PFGE protocol employed by PulseNet laboratories of the US CDC for *E. coli* O157:H7, *Salmonella*, and *Shigella* [12]. Chromosomal DNA of the analyzed isolates was digested separately with restriction enzymes *NotI* and *XbaI* before applying to the PFGE analysis. The resulting gel was stained with ethidium bromide before being photographed and the image stored as a TIFF file. Then the image was analyzed with software BioNumerics (Applied Maths, Kortrijk, Belgium), and the result along with epidemiological information of the strain, was fed into the Taiwan CDC's *Shigella* DNA fingerprint database. Phylogenetic tree for isolates was established using PFGE patterns and UPGMA algorithm.

Results and Discussion

The 10 *S. flexneri* isolates recovered from the incident were confirmed to be subserotype 2b (1 isolate) and X variant (9 isolates).

The cases appeared on different floors and wards. Looking into the epidemic curve of this outbreak (Figure 1), we noticed that the daily number of patients seemed to be slowing down after the initial burst, and the trend appeared to be a single left-lopsided peak, which leads us to suspect that some residents were exposed simultaneously to a common infection source and then the disease spread through person-to-person contacts.

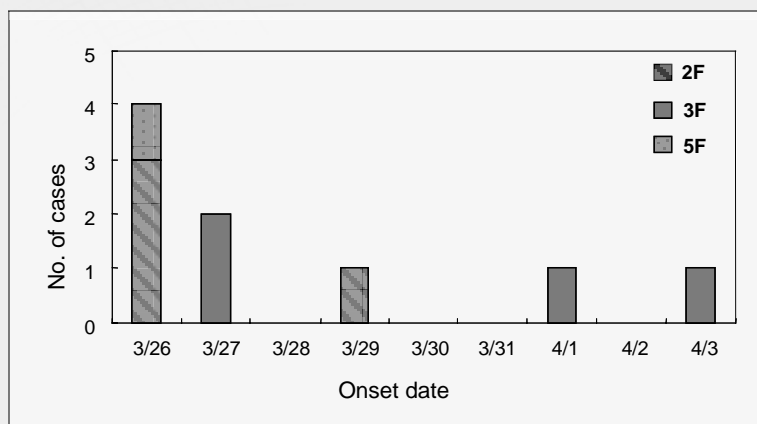


Figure 1. Number of shigellosis patients in a psychiatric hospital, by onset date and floor, in Miaoli County, Taiwan, 2008. Among the 15 cases found in this incident, 9 people displayed symptoms.

To search for the source of infection, several water samples were taken from the hospital and the microbiological examinations showed that only one specimen containing abnormal numbers of *E. coli* but no *Shigella* was isolated. The results of tossing red dyestuff into the septic tanks indicated that the water supply tubing system was not contaminated.

Therefore, the possibility of infection is low by coming from contaminated water source. Food Hygiene Section of Miaoli County Health Bureau tracked down the health of each employee of the contracted meal box company but no abnormality was found. The laboratory test results of the employees' rectal swabs were all negative. That removed the meal box makers out as a source of infection. There were regular gatherings of inpatients on the first floor for group occupational therapeutic activities, and patients admitted to and discharged from the hospital from time to time. But there is a limitation to investigate infection source from the psychiatric patients. Since the serotypes of isolates recovered in this incident were 2b and X variant that were very rare in Taiwan before, it was suspected that those pathogens were most likely from abroad. Investigators checked this hypothesis, finding that no employees or imported foreign care workers showed any relevant symptoms throughout the outbreak and no one had traveled abroad in the previous 2 months. All foreign employees of the hospital were hired by the founding and took regular physical examinations as requested by the relevant regulations, and no new employees were hired recently. As for the possibility of transmission from foreign visitors, investigation of the family members of all the infected and newly joined inpatients showed no definite information associated with the infection. All in all, the source of infection was still unknown in spite of multiple investigations conducted.

At present, there are a total of 2,130 *Shigella* isolates with DNA fingerprints in the database. There are 5, 856, 3, and 1,266 isolates for *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*, respectively. Within the *S. flexneri*, there are 807 isolates with serotyping information. Of these, 585



isolates are 2a (mainly circulating among aboriginal tribes in mountainous area), 136 isolates are 4a (prevalent in eastern Taiwan), 29 isolates are 1a and 17 isolates are 1b (found in southern Taiwan). Other *S. flexneri* isolates in the database include 3b, 2b, 3a, Y variant, and 6. Since the isolates obtained in this incident were subserotype 2b and X variant, in order to probe the phylogenetic connection between these two serotypes and the previously recovered *Shigella* isolates in Taiwan, the outbreak isolates were subjected to PFGE fingerprint analysis in Central Regional Laboratory of Taiwan CDC and the PFGE patterns were compared with those in the *Shigella* DNA fingerprint database. Figure 2 is a phylogenetic dendrogram for the outbreak isolates and those closely related isolates in the database. As shown in Figure 2, the 10 outbreak isolates fell into 4 PFGE patterns and they were more closely related than others in the database. The 2b outbreak isolate shared an indistinguishable PFGE pattern with two X isolate. These results indicated that the 2b and the X isolates recovered from the incident should be derived from a common recent ancestor. The outbreak isolates were most closely related to a cluster of 2b isolates recovered in Taiwan or imported from Vietnam and Cambodia, strengthening a speculation that the X variants should be evolved from a subserotype 2b strain.

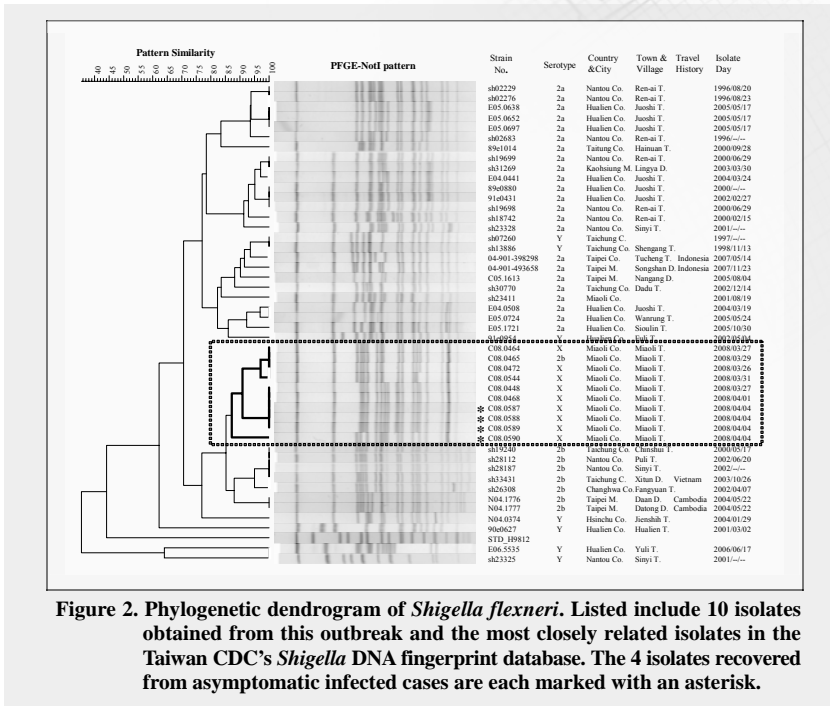


Figure 2. Phylogenetic dendrogram of *Shigella flexneri*. Listed include 10 isolates obtained from this outbreak and the most closely related isolates in the Taiwan CDC's *Shigella* DNA fingerprint database. The 4 isolates recovered from asymptomatic infected cases are each marked with an asterisk.

The discrimination between the 2b and X variant is decided by whether they make a type factor antigen II or not. Subserotype 2b is both type factor antigen II positive and group factor antigen 7,8 positive, while serotype X variant is type factor antigen II negative but group factor antigen 7,8 positive. Then, by the mechanism of converting of serotype from 2a to Y variant [13], we can reasonably assume that it is possible that a 2b strain should have evolved into the X variant through gene mutation. However, this hypothesis still needs further studies to verify it. Since the 10 outbreak isolates fell into four genotypes, the outbreak strains could be genetically extreme unstable or they had been circulating in the hospital for a while. Multiple similar PFGE patterns displayed among *S. sonnei*



isolates recovered from a common outbreak have been previously reported in a shigellosis outbreak in Nanjuang, Miaoli County and Heping, Taichung County [14]. These two cases demonstrate how a high degree of variation of *Shigella* could be and the high discriminatory power of PFGE for subtyping *Shigella*. Since 3 isolates from asymptomatic cases and 2 isolates from symptomatic patients displayed an identical PFGE pattern, they were evident of the same common origin.

Figure 2 also reveals that the outbreak isolates were most closely related to a cluster of subserotype 2b isolates in the database, recovered during 2000-2004, some imported from Vietnam and Cambodia. Shigellosis was not an endemic disease in Taiwan and most *S. flexneri* infections which occurred mostly in the aboriginal villages were caused by subserotype 2a. Subserotype 2b in Taiwan is rare and most 2b isolates are associated with imported cases, so the outbreak strains should most likely originated from outside of Taiwan.

As to the results of antimicrobial susceptibility testing, the outbreak isolates showed resistance to the first-line antimicrobials, including ampicillin, streptomycin, tetracycline, chloramphenicol, and trimethoprim/sulfamethoxazole. The outbreak isolates with distinct PFGE patterns showed no obvious differences in their resistance patterns. Trimethoprim/sulfamethoxazole had been used broadly in intestinal infections, but almost all *Shigella* strains are resistant to this agent now. According to antimicrobial resistance data for the isolates in the *Shigella* DNA Fingerprint database, most isolates from indigenous cases have displayed resistance to nalidixic acid and almost all those from imported case are sensitive to this agent. The rarity of 2b/X variant strains Taiwan and the sensitivity to

nalidixic acid suggest that the outbreak strain should come from abroad.

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