Epidemiol. Infect. (2005), **133**, 611–615. © 2005 Cambridge University Press
doi:10.1017/S0950268805003985 Printed in the United Kingdom

Sentinel surveillance for international *Shigella* by a quarantine station in Japan

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(*Accepted 18 January 2005*)

SUMMARY

The Japanese quarantine system monitors incoming passengers to detect imported pathogens at international airports. At one airport, we found that 74% of 13315 travellers returning with diarrhoea had visited only one country before entering Japan. On the basis of our results, we hypothesized that the international distribution and potential source of bacterial strains could be inferred by analysing strains isolated from travellers returning to Japan. In order to demonstrate the potential for this system, we randomly selected five *Shigella sonnei* strains and examined their restriction fragment length polymorphism patterns. One set of strains appeared to be closely related, while three sets, isolated from travellers who visited different countries were possibly related. These results suggest that international distributions and potential sources of *S. sonnei* may be inferred by monitoring isolates from passengers arriving at a Japanese quarantine station.

INTRODUCTION

Shigellosis is a diarrhoeal disease that is highly endemic in developing Southeast Asian countries. In addition, sporadic cases and outbreaks of shigellosis have been reported from developed countries, such as Japan [1–3], Taiwan [4, 5] and North America [6, 7]. Outbreaks associated with imported food products have been reported [8]. With the development and international spread of drug-resistant strains of *Shigella* spp. [9, 10], the potential to identify the international distribution and source of *Shigella* is important in developing effective public health surveillance and control programmes.

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Molecular subtype-specific surveillance based on the comparison of restriction fragment length patterns among *Shigella* strains from patients is an important epidemiological approach to understanding the distribution of *Shigella* in endemic areas [11–15]. Traditionally, surveillance of bacterial diseases is performed by analysis of isolated bacteria in each designated centre of each country. Attempting to conduct surveillance over a large endemic area such as Southeast Asia requires collection and subtyping of bacterial isolates from each country. For the surveillance of selected foodborne disease agents in the United States, PulseNet has been developed as a national molecular subtyping network [16]. PulseNet's methods are also being applied to international surveillance, such as PulseNet Asia Pacific [17]. However, since technical harmonization of multiple laboratories and the cost of equipment

and personnel may be obstacles to its implementation, alternative surveillance methods are still needed.

As millions of travellers enter Japan from around the world, the Japanese quarantine system has the potential to serve as a surveillance system for isolates of pathogenic bacteria from the travellers [18, 19].

In this study, we analysed the number of countries visited by travellers arriving with diarrhoea and isolated *Shigella* spp. from diarrhoeal patients who entered Japan. Based on the results, we evaluated the potential for using the Japanese quarantine system to determine the international distribution and potential source of *Shigella* based on restriction fragment length pattern polymorphism (RFLP) assay using pulsed field gel electrophoresis (PFGE).

METHODS

Number of symptomatic travellers

The number of symptomatic travellers was obtained from data for the year 2002. The travellers were divided into two categories: the first category consisted of travellers who visited only one country 21 days before their arrival and the second category consisted of travellers who visited two or more countries. From among the symptomatic travellers, the number of diarrhoeal travellers was also obtained from the 2002 data, and the number of countries visited was analysed.

Isolation and identification of *Shigella* spp.

Stool samples were collected from overseas travellers who reported a history of diarrhoea upon arrival at Kansai International Airport Quarantine Station between November 2001 and January 2002. In this study, 10 samples were examined by direct plating on *Salmonella–Shigella* (SS) agar plates (Eiken Chemical Co. Ltd, Tokyo, Japan) and desoxycholate hydrogen sulphide lactose (DHL) agar plates (Eiken Chemical Co. Ltd) at 37 °C for 18 h. *Shigella*-like colonies were screened using triple sugar iron agar (TSI; Eiken Chemical Co. Ltd) and lysine-indole-motility medium (LIM; Eiken Chemical Co. Ltd). Suspected isolates were examined by serotype testing using specific *Shigella* O-antisera (Denka Seiken Co. Ltd, Tokyo, Japan). The serotyped strains were identified using biochemical tests such as amino acid and sugar assimilation tests.

Preparation of DNA for PFGE

Agarose plugs containing DNA for PFGE analysis were prepared using CHEF Bacterial Genomic DNA Plug kits® (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. In brief, bacterial cells were grown with agitation at 37 °C in heart infusion broth (Difco, Detroit, MI, USA) at McFarland 2 concentration. These bacterial cells were then plugged into agarose plugs, and bacterial genomic DNA was extracted with lysozyme and proteinase K solution provided in the kits. The bacterial DNA in the agarose plugs was then digested with *Xba*I and *Sfi*I restriction enzymes (TaKaRa Shuzo Co. Ltd, Otsu, Japan) at 37 and 50 °C overnight respectively.

PFGE

Electrophoresis was carried out by the contour-clamped homogeneous electric field method on a CHEF-DR II system (Bio-Rad Laboratories) at 200 V with a pulse time of 5–8 s at 14 °C for 20 h for *Xba*I-restricted fragments in 1% agarose gel (pulsed field certified agarose; Bio-Rad Laboratories) using 0·5% TBE buffer (pH 8·3) containing 44·5 mM Tris, 44·5 mM boric acid and 1 mM EDTA, and at 200 V with a pulse time of 5–35 s at the same temperature for 30 h for *Sfi*I-restricted fragments in 1·5% agarose gel. The gels were stained with SYBR Green nucleic acid gel stain (BMA, Rockland, ME, USA) for 30 min and photographed with a UV transilluminator (302 nm). DNA fragment patterns were visually analysed and the relationship was determined using the criteria for bacterial strain typing of Tenover et al. [20].

RESULTS

In 2002, 22 282 symptomatic travellers arrived at Kansai International Airport. Among these travellers, 13 315 (59·8%) had diarrhoea; 9793 (73·5%) of the diarrhoeal travellers visited a single country 21 days before their arrival. Among the countries visited by the diarrhoeal travellers, 94·7% of the countries were located in Southeast and East Asia. Among the 13 315 travellers, 5080 (38·2%) had a stool specimen examined for enteric pathogens. A total of 65 strains of *Shigella* spp. (1·3%) were isolated from 5080 travellers. Of the 65 of *Shigella* strains, 48 (73·9%) were isolated from travellers who visited a single country.

https://doi.org/10.1017/S0950268805003985 Published online by Cambridge University Press

Table. *Difference in* (*a*) *Xba*I *and* (*b*) *Sfi*I *RFLP*

(*a*) *Xba*I	Isolate no.				
	1 (A)	2 (B)	3 (C)	4 (D)	5 (B)
Isolate no.					
1 (A)	0				
2 (B)	>7	0			
3 (C)	**6**	>7	0		
4 (D)	>7	**4**	>7	0	
5 (B)	**2**	>7	**5**	>7	0

(*b*) *Sfi*I	Isolate no.				
	1 (A)	2 (B)	3 (C)	4 (D)	5 (B)
Isolate no.					
1 (A)	0				
2 (B)	**6**	0			
3 (C)	**5**	>7	0		
4 (D)	**4**	**6**	>7	0	
5 (B)	**1**	**4**	**4**	**5**	0

Number of different bands among isolates using *Xba*I and *Sfi*I is shown.
A, B, C, D denotes country of origin. All countries (A–D) are in Southeast Asia.
The strains shown in bold face meet the modified criteria of Tenover et al. [20] as possibly related or closely related strains.

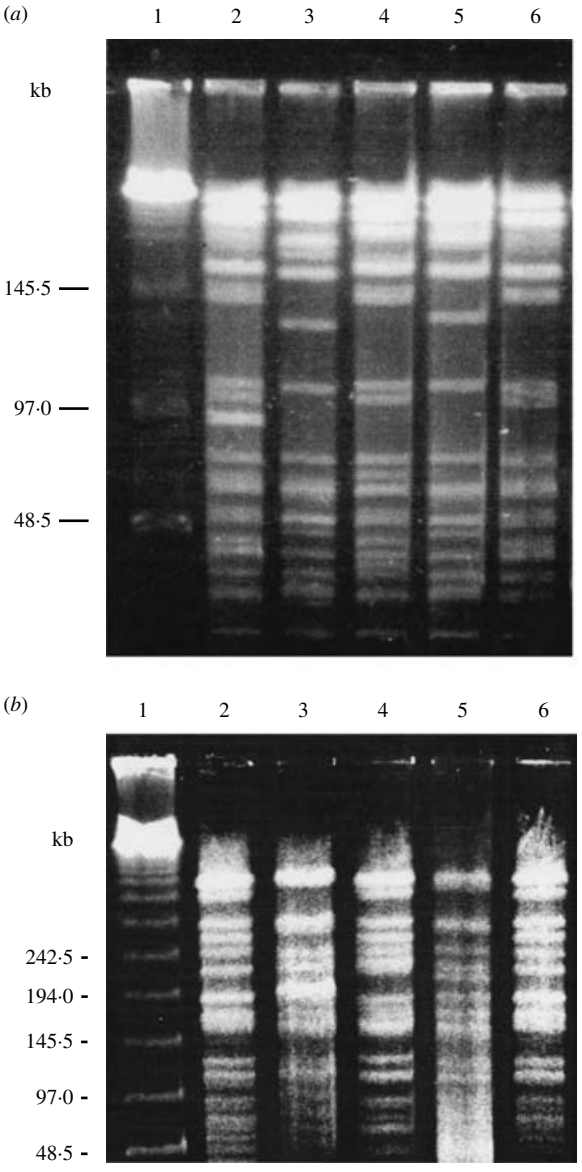

Fig. Representative RFLP pattern of *Shigella sonnei* isolates. Lane 1, markers for fragments; lanes 2–6, DNA fragments digested with (*a*) *Xba*I and (*b*) *Sfi*I. Lane 2, isolate no. 1; lane 3, isolate no. 2; lane 4, isolate no. 3; lane 5, isolate no. 4; lane 6, isolate no. 5.

Forty-three strains of *Shigella sonnei*, two strains of *S. flexneri*, two strains of *S. boydii*, and one strain of *S. dysenteriae* were isolated from travellers who visited a single endemic country. Since *S. dysenteriae*, *S. flexneri*, and *S. boydii* can be divided into serotypes and the number of isolates was small in this study, we focused on molecular subtyping of *S. sonnei*.

In order to demonstrate the potential to link specific strains to exposure in different countries, five *S. sonnei* isolates were randomly chosen and subjected to RFLP assay. Pairwise comparisons of these isolates are presented in the Table. Only two different bands were detected between *Xba*I-digested DNA fragments from isolate nos. 1 and 5, four between those from isolate nos. 2 and 4, six between those from isolate nos. 1 and 3, and five between those from isolate nos. 3 and 5 (Fig. *a*, Table). According to the criteria of Tenover et al. [20], only isolate nos. 1 and 5 were closely related. Similarities suggesting possible relationships were observed between isolate nos. 2 and 4, 1 and 3, and 3 and 5. In comparison using other combinations of isolates, more than seven different bands were detected, the isolates were determined to be unrelated. To confirm the relationships in the sets of isolates, these bacterial isolates were analysed by *Sfi*I digestion by RFLP assay. *Sfi*I-digested DNA fragments revealed only one different band between isolate nos. 1 and 5, four between isolate nos. 1 and 4, 2 and 5, and 3 and 5 (Fig. *b*, Table). Also five different bands were detected between isolate nos. 1 and 3, and 4 and 5, and six different bands between isolate nos. 2 and 4.

Tenover et al. [20] recommended their criteria for studies of potential outbreaks in hospitals or communities spanning relatively short time periods (1–3

https://doi.org/10.1017/S0950268805003985 Published online by Cambridge University Press

months). Criteria for evaluating isolates collected over extended periods may need to be modified to accommodate the use of multiple enzymes and analyses. Therefore, for this study, Tenover's criteria were modified to accommodate the use of two restriction enzymes. Strains were considered to be closely related when 0–2 different bands appeared for both restriction enzymes. Strains were considered to be possibly related when 3–6 different bands appeared for both enzymes. These data indicated that isolate nos. 1 and 5 were closely related, and isolate nos. 1 and 3, 2 and 4, and 3 and 5 were possibly related.

DISCUSSION

The quarantine system at Kansai International Airport identifies incoming travellers with diarrhoea, determines where and for how long these travellers stayed abroad, and requires isolation of pathogens from them. *S. sonnei* is the most frequently isolated pathogen from these travellers. These findings confirmed that *S. sonnei* is endemic to Southeast Asia, and that *Shigella* spp. is an important cause of travel-associated diarrhoea in Japan. On the basis of these findings, it appears that the international distribution and potential source of *S. sonnei* may be monitored at quarantine stations.

Of the various tools for discriminating of *S. sonnei* strains, such as plasmid profile, drug resistance pattern, colicin type, and RFLP pattern analysis, it appears that RFLP analysis using *Xba*I and *Sfi*I restriction enzymes and PFGE is sufficiently reliable in discriminating *S. sonnei* strains [16, 20–23]. Although genes responsible for drug resistance are frequently encoded by plasmids, plasmids tend to be unstable markers for epidemiological surveillance [15].

Using these methods, we found that one set of strains was closely related and at least three sets of strains were possibly related using the modified criteria of Tenover et al. [20]. We isolated closely related isolates from countries A and B, and possibly related isolates from countries A and C, B and C, and B and D. The international distribution of related *S. sonnei* strains among countries separated by sea, but connected by established transportation and trade networks suggests that *S. sonnei* is transported between these countries by some manner, such as contaminated food products and infected humans [8]. A similar study on sequential outbreaks and sporadic cases of *S. sonnei* infection performed by Matsumoto et al. [13], collected and genetically analysed 53 bacterial strains from several laboratories, and suggested that extensive movement of people or food caused those infections.

Surveillance of *S. sonnei* at quarantine stations may be useful to monitor changes in the international distribution of the strains. Although PulseNet has been developed as a multi-laboratorial RFLP pattern analysis system using computer technology, it requires standardization of laboratory techniques in each laboratory that applies this system [24, 25]. Quarantine station-based surveillance has the advantage of using a single laboratory. Thus, inter-laboratory standardization is not required. As daily surveillance in a single laboratory is simpler than that in several laboratories, we recommend that a Japanese Quarantine Station continues to monitor the spread of *S. sonnei* in Southeast Asia.

ACKNOWLEDGEMENTS

We thank Messrs K. Taga, H. Miyagi, S. Shiraishi, W. Iizuka, W. Suzuki, K. Nomoto, and H. Kodama for technical support in isolating bacteria at Kansai International Airport Quarantine Station. We also thank Drs C. Morita, T. Kohno, and W. Hong for scientific advice, and Mr Y. Fujioka and Dr E. Daikoku for technical support in the Department of Preventive and Social Medicine, Osaka Medical College.

REFERENCES

1. **Yamada M, Muto T, Kitazume H, Suzuki M.** The molecular epidemiological analysis of *Shigella sonnei* isolates from outbreak cases in Yokohama. Kansenshogaku Zasshi 1999; **73**: 923–929.
2. **Uchimura M, Kishida K, Koiwai K.** Increasing incidence and the mechanism of resistance of nalidixic acid resistant *Shigella sonnei*. Kansenshogaku Zasshi 2001; **75**: 923–930.
3. **Uchimura M, Koiwai K, Yoda K, Kishida K, Tsuruoka Y, Mizuguchi Y.** Usefulness of genotypic analysis for investigation of an epidemic of *Shigella sonnei* infections in Ooami-shirasato Town, Chiba Prefecture. Kansenshogaku Zasshi 1998; **72**: 615–620.
4. **Lin CS, Wang TK, Tsai JL, Ho SI, Lee CL, Lu CH.** Molecular subtyping of *Shigella flexneri 3a* isolates by plasmid profile analysis and pulsed-field gel electrophoresis. J Microbiol Immunol Infect 2001; **34**: 103–108.

5. **Chiou CS, Hsu WB, Wei HL, Chen JH.** Molecular epidemiology of a *Shigella flexneri* outbreak in a mountainous township in Taiwan, Republic of China. J Clin Microbiol 2001; **39**: 1048–1056.
6. **Sobel J, Cameron DN, Ismail J, et al.** A prolonged outbreak of *Shigella sonnei* infections in traditionally observant Jewish communities in North America caused by a molecularly distinct bacterial subtype. J Infect Dis 1998; **177**: 1405–1409.
7. **Preston MA, Borczyk AA.** Genetic variability and molecular typing of *Shigella sonnei* strains isolated in Canada. J Clin Microbiol 1994; **32**: 1427–1430.
8. **Naimi TS, Wicklund JH, Olsen SJ, et al.** Concurrent outbreaks of *Shigella sonnei* and enterotoxigenic *Escherichia coli* infection associated with parsley: implications for surveillance and control of foodborne illness. J Food Protection 2003; **66**: 535–541.
9. **Lima AA, Sidrim JJ, Lima NL, Titlow W, Evans ME, Greenberg RN.** Molecular epidemiology of multiply antibiotic-resistant *Shigella flexneri* in Fortaleza, Brazil. J Clin Microbiol 1997; **35**: 1061–1065.
10. **Navia MM, Capitano L, Ruiz J, et al.** Typing and characterization of mechanisms of resistance of *Shigella spp*. isolated from feces of children under 5 years of age from Ifakara, Tanzania. J Clin Microbiol 1999; **37**: 3113–3117.
11. **Liu PY, Lau YJ, Hu BS, et al.** Analysis of clonal relationships among isolates of *Shigella sonnei* by different molecular typing methods. J Clin Microbiol 1995; **33**: 1779–1783.
12. **Litwin CM, Leonard RB, Carroll KC, Drummond WK, Pavia AT.** Characterization of endemic strains of *Shigella sonnei* by use of plasmid DNA analysis and pulsed-field gel electrophoresis to detect patterns of transmission. J Infect Dis 1997; **175**: 864–870.
13. **Matsumoto M, Suzuki Y, Saito M, Ishikawa N, Ohta M.** Epidemiologic study of *Shigella sonnei* from sequential outbreaks and sporadic cases using different typing techniques. Microbiol Immunol 1998; **42**: 259–264.
14. **Houang ETS, Chu YW, Ng TK, Cheng AFB.** Study of the relatedness of isolates of *Shigella flexneri* and *Shigella sonnei* obtained in 1986 and 1987 and in 1994 and 1995 from Hong Kong. J Clin Microbiol 1998; **36**: 2404–2407.
15. **Talukder KA, Dutta DK, Albert MJ.** Evaluation of pulsed-field gel electrophoresis for typing of *Shigella dysenteriae* type 1. J Med Microbiol 1999; **48**: 781–784.
16. **Arbeit RD.** Laboratory procedures for the epidemiologic analysis of microorganisms. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of clinical microbiology, 6th edn. American Society for Microbiology, Washington, DC, 1995; 190–208.
17. **Centers for Disease Control and Prevention (CDC).** PulseNet News, Spring 2003 (http://www.cdc.gov/pulsenet/newsletter/spring_2003.htm). Accessed 1 Jan. 2005.
18. **Ueda Y, Suzuki N, Miyagi K, et al.** Studies on bacillary dysentery case of overseas travellers – during 1997 to 1995. Saikingaku zasshi 1997; **52**: 735–746.
19. **Ueda Y, Suzuki N, Furukawa T, et al.** Bacteriological studies of travellers diarrhea. (6) Analysis of enteropathogenic bacteria at Kansai Air Port Quarantine Station from 4 September 1994 through December 1996. Kansenshougaku Zasshi 1999; **73**: 110–120.
20. **Tenover FC, Arbeit RD, Goering RV.** Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; **33**: 2233–2239.
21. **Maslow JN, Slutsky AM, Arbeit RD.** Application of pulsed field gel electrophoresis to molecular epidemiology. In: Persing DH, Smith TF, Tenover FC, White TJ, eds. Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, DC, 1993.
22. **Brian MJ, Van R, Townsend I, Murray BE, Cleary TG, Pickering LK.** Evaluation of the molecular epidemiology of an outbreak of multiply resistant *Shigella sonnei* in day-care center by using pulsed-field gel electrophoresis and plasmid DNA analysis. J Clin Microbiol 1993; **31**: 2152–2156.
23. **Lee TM, Chang LL, Chang CY, et al.** Molecular analysis of *Shigella sonnei* isolated from three well-documented outbreaks in school children. J Med Microbiol 2000; **49**: 355–360.
24. **Swaminathan B, Barrett TJ, Hunter SB, Tauxe RB, CDC PulseNet Task Force.** PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg Infect Dis 2001; **7**: 382–389.
25. **Graves LM, Swaminathan B.** PulseNet standardized protocol for subtyping Listeria monocytogenes by macrorestriction and pulsed-field gel electrophoresis. Int J Food Microbiol 2001; **65**: 55–62.