Epidemiol. Infect. (2010), **138**, 318–321. © Cambridge University Press 2009
doi:10.1017/S0950268809990380 Printed in the United Kingdom

SHORT REPORT
Salmonella enterica serovar Typhi in Japan, 2001–2006: emergence of high-level fluoroquinolone-resistant strains

M. MORITA[1], K. HIROSE[1], N. TAKAI[1], J. TERAJIMA[1], H. WATANABE[1], H. SAGARA[2], T. KURAZONO[3], M. YAMAGUCHI[3], Y. KANAZAWA[4], T. OYAIZU[5] AND H. IZUMIYA[1]*

[1] *Department of Bacteriology, National Institute of Infectious Diseases, Tokyo, Japan*
[2] *Department of Infectious Diseases, Yokohama Municipal Citizen's Hospital, Yokohama, Japan*
[3] *Division of Clinical Microbiology, Saitama Institute of Public Health, Saitama, Japan*
[4] *Shizuoka City Institute of Environmental Sciences and Public Health, Shizuoka, Japan*
[5] *Department of Gastroenterology, Shizuoka City Hospital, Shizuoka, Japan*

(*Accepted 19 June 2009; first published online 27 July 2009*)

SUMMARY

The phage types and antimicrobial susceptibilities of 226 isolates of *Salmonella enterica* serovar Typhi from imported cases in Japan between 2001 and 2006 were investigated. Most (93·8%) had travelled to Asian countries, particularly South East Asia. Twenty-one phage types were identified with E1 (30·5%), UVS (15·9%) and B1 (9·3%) being the most common. The frequency of multidrug-resistant strains reached 37·0% in 2006 with phage types E1 and E9 predominating. Almost half (48·2%) of the isolates were resistant to nalidixic acid and two isolates displayed high-level fluoroquinolone resistance. Three mutations, two in *gyrA* and one in *parC*, were identified in both isolates.

Key words: Antibiotic resistance, bacteriology, *Salmonella* (Typhi), surveillance.

Typhoid fever is a systemic infection that causes bacteraemia and inflammatory destruction of the intestine and other organs. *Salmonella enterica* serovar Typhi (*S.* Typhi) is the causative agent and is transmitted from human to human via food or drinking water; therefore, hygiene and sanitary conditions mainly determine its spread [1, 2]. Until the 1960s in Japan, outbreaks of typhoid fever were associated with the ingestion of contaminated food or well water. Thereafter, as a result of improved public sanitation, most of the cases have been sporadic and have come from abroad. Fluoroquinolones have been used for the treatment of typhoid fever as the first drug of choice following the emergence of multidrug-resistant (MDR; resistant to ampicillin, chloramphenicol, and trimethoprim–sulfamethoxazole) *S.* Typhi strains [1, 3–5]. However, the frequency of isolates resistant to nalidixic acid, which exhibit reduced susceptibility to other fluoroquinolones has increased. There have been several clinical treatment failures following the administration of ciprofloxacin and other fluoroquinolones to patients with typhoid fever due to these resistant strains [6, 7]. More recently, high-level fluoroquinolone-resistant *S.* Typhi isolates have also been reported [8]. In this study, we examined *S.* Typhi isolates collected over 5 years from imported cases by Vi-phage typing and determination of antimicrobial susceptibility. This surveillance revealed increases in the frequencies of drug resistance yielded first isolation of high-level fluoroquinolone-resistant *S.* Typhi in

* Author for correspondence: H. Izumiya, Ph.D., Department of Bacteriology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo, 162-8640, Japan.
(Email: izumiya@nih.go.jp)

Table 1. *Presumed region of infection and phage type of isolates in Japan from 2001 to 2006*

	Imported cases									
	South Asia ($n=138$)	South East Asia ($n=70$)	East Asia ($n=6$)	Latin America ($n=4$)	Africa ($n=3$)	Oceania ($n=1$)	Europe ($n=1$)	Unknown* ($n=3$)	Others † ($n=80$)	Total ($n=306$)
Phage type										
A	4	6	1		1				13	25
B1	4	16						1	10	31
B2	3	1							1	5
C4									1	1
C5	2									2
D1	1	1							4	6
D2		16	1			1			9	27
E1	59	6			2			2	12	81
E2	5	4							4	13
E6									1	1
E9	19	1								20
E10		2								2
E14			1							1
F6	6									6
F9				1						1
H									2	2
J1	1		1						1	3
M1	3								8	11
M4	1									1
35				2						2
36	1									1
40	3									3
43		2							2	4
46	2								2	4
56									1	1
DVS	4	2					1		2	9
UVS	20	13	2	1					7	43

* Country visited is not noted.
† Patients in whom travel history was not available or without recent foreign travel.

Japan. Isolates were also characterized by molecular typing and identification of mutations conferring quinolone resistance.

We assembled 226 clinical isolates of *S.* Typhi between 2001 and 2006 from patients with a history of foreign travel. All isolates were collected from regional public health centres and sent to the Department of Bacteriology, National Institute of Infectious Diseases. Isolates were phage typed by the standard technique with the phage set kindly provided by the Health Protection Agency, London, UK [9]. The minimum inhibitory concentrations (MICs) of 15 antimicrobials for all isolates were determined using E-tests (AB Biodisk, Sweden) according to the manufacturer's instructions. The antimicrobials were ampicillin, cefotaxime, ceftriaxone, imipenem, aztreonam, kanamycin, gentamicin, tetracycline, fosfomycin, chloramphenicol, nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin and trimethoprim–sulfamethoxazole. *Escherichia coli* ATCC25922 was included in each test as quality control. Inhibition zones were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) method [10]. Pulsed-field gel electrophoresis (PFGE) of chromosomal DNA digested with *Xba*I was performed as previously described using the *S. enterica* serovar Braenderup H9812 as the standard strain [11]. DNA sequences of the quinolone resistance-determining regions (QRDRs) of the *gyrA*, *gyrB*, *parC*, and *parE* genes were determined as described previously [12].

With the exception of 2005, there were around 50 cases of *S.* Typhi infection in Japan annually, and

https://doi.org/10.1017/S0950268809990380 Published online by Cambridge University Press

Table 2. *Antibiotic susceptibilities of* S. *Typhi from imported cases* (*n* = *226*)

Drug (breakpoint, mg/l)	MIC (mg/l) for *S.* Typhi			
	Range	MIC_{50}	MIC_{90}	Resistant (%)*
Ampicillin (⩾32)	0·064–>256	1	>256	23·3
Cefotaxime (⩾64)	0·032–1	0·125	0·25	0
Ceftriaxone (⩾64)	0·016–0·5	0·125	0·25	0
Imipenem (⩾16)	0·064–0·5	0·25	0·5	0
Aztreonam (⩾64)	<0·016–0·25	0·032	0·25	0
Kanamycin (⩾64)	0·125–8	2	4	0
Gentamicin(⩾64)	0·032–1	0·25	0·5	0
Tetracycline (⩾64)	0·5–>256	2	>256	17·6
Fosfomycin (⩾64)	1–>1024	8	16	3·4
Chloramphenicol (⩾64)	0·25–>256	4	>256	23·3
Nalidixic acid (⩾64)	2–>256	8	>256	48·5
Norfloxacin (⩾64)	0·064–128	1	2	0·9
Ofloxacin (⩾64)	0·032–>32	0·25	1	0·9
Ciprofloxacin (⩾64)	<0·016–>32	0·125	0·5	0·9
Trimethoprim–sulfamethoxazole (⩾4/76)	0·016–>160	0·125	>160	23·3

MIC, Minimum inhibitory concentration.
* Resistance based on CLSI breakpoint.

226/306 patients reported during the past 6 years had a history of foreign travel before onset of typhoid fever. Of these, 212 cases (93·8%) had a history of travel to Asian countries, with South Asia deemed to be a particularly high-risk travel destination for typhoid fever (Table 1). Of the 226 isolates from the imported cases, 140 (61·9%) were from males, and 86 (38·1%) were from females. The patients ranged in age from 1 to 67 years (median age 25 years) and there was no report of death. The bacterial strains were isolated from blood (181 isolates, 80·1%), stool (39 isolates, 17·3%), bile (one isolate, 0·4%), and urine (one isolate, 0·4%). The information on sources was not available for six isolates. Twenty-one phage-type patterns were identified among all and strains of phage type E1 were the most frequent (30·5%) followed by UVS (15·9%), B1 (9·3%), E9 (8·8%), D2 (8·0%), and A (5·3%); seven strains had unique phage types (Table 1).

Table 2 shows that the MIC_{90} of ampicillin, chloramphenicol, trimethoprim–sulfamethoxazole, and tetracycline for *S.* Typhi exceeded the highest concentration tested. This underlines the finding that agents traditionally used for first-line treatment for typhoid fever are no longer effective for this purpose, at least in Japan. The incidence of MDR strains of *S.* Typhi in 2001 was 21·1%, 15·8% in 2002, 13·2% in 2003, 22·2% in 2004, 28·6% in 2005, and 37·0% in 2006. Of the 52 MDR strains identified, 48 were recovered from travellers to South Asia. The most predominant phage types among MDR strains were E1 (27 strains), followed by E9 (14 strains).

Fluoroquinolones and third-generation cephalosporins were the most effective against *S.* Typhi *in vitro*. However, reduced susceptibility to fluoroquinolones must currently be considered for treatment of *S.* Typhi infection as we have previously observed nalidixic acid-resistant *S.* Typhi strains, which had reduced susceptibility to fluoroquinolones [13]. The incidence of nalidixic-acid resistance of *S.* Typhi was 28·9% in 2001, 28·9% in 2002, 39·5% in 2003, 66·7% in 2004, 47·6% in 2005, and 69·6% in 2006; of the 109 nalidixic acid-resistant strains, 96 were imported from South Asian countries with E1 (47·7%), E9 (15·6%), and UVS (14·7%) being the most common phage types. Forty-eight nalidixic acid-resistant strains also exhibited multidrug resistance.

In 2006, two high-level fluoroquinolone-resistant strains of phage type UVS were identified for the first time in Japan; these were isolated from independent travellers to the Indian subcontinent. Their common visited country was India. Both patients were successfully treated with appropriate antibiotics according to the resistant profiles of isolated *S.* Typhi strains. As resistance to fluoroquinolones in Enterobacteriaceae are mostly attributed to mutations in the genes encoding DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) [14], the nucleotide sequences of the QRDRs were determined [12]. Both

strains, showed three identical mutations, two within *gyrA* at codons 83 and 87, and one mutation in *parC* at codon 80. For *gyrA*, TCC of Ser^{83} codon and GAC of Asp^{87} changed to TTC (Phe) and AAC (Asn), respectively. For *parC*, the mutation was a change of AGC (Ser) to ATC (Ile). No alteration in the QRDRs of *gyrB* and *parE* was found.

PFGE genotyping exhibited unique profiles of two high-level fluoroquinolone-resistant strains of serovar Typhi, but the profiles were quite similar to each other (data not shown). Both the fluoroquinolone-resistant strains gave highly related DNA profiles suggesting clonal identity.

Typhoid fever has become a predominantly travel-associated disease in developed countries, and the emergence of strains resistant to and with reduced susceptibility for fluoroquinolones is a matter of grave concern. From 2001 to 2006 in Japan, 73·9% of cases were related to foreign travel and 48·2% of isolates showed reduced susceptibility to fluoroquinolones. Further, we demonstrated the presence of *S.* Typhi resistant to fluoroquinolones, including norfloxacin, ofloxacin, and ciprofloxacin. All isolates investigated here were susceptible to third-generation cephalosporins (cefotaxime and ceftriaxone), which might indicate that these antibiotics could still provide an appropriate therapy for typhoid fever. Indeed, the use of ceftriaxone is currently recommended as the first-line therapy [2, 15]; however, we should be cautious of the real possibility that strains resistant to third-generation cephalosporins will emerge in the future.

ACKNOWLEDGEMENTS

We thank all the municipal and prefectural public health institutes in Japan for providing us with isolates. This work was supported in part by a Grant-in-aid from Ministry of Health, Labour, and Welfare (H17-Shinko-Ippan-019, H20-Shinko-Ippan-013, H20-Shinko-Ippan-015) and International Cooperation Research Grant (18C5).

DECLARATION OF INTEREST

None.

REFERENCES

1. **Bhan MK, Bahl R, Bhatnagar S.** Typhoid and paratyphoid fever. *Lancet* 2005; **366**: 749–762.
2. **Connor BA, Schwartz E.** Typhoid and paratyphoid fever in travellers. *Lancet Infectious Diseases* 2005; **5**: 623–628.
3. **Asperilla MO, Smego Jr. RA, Scott LK.** Quinolone antibiotics in the treatment of *Salmonella* infections. *Reviews in Infectious Diseases* 1990; **12**: 873–889.
4. **Cao XT, *et al.*** A comparative study of ofloxacin and cefixime for treatment of typhoid fever in children. *Pediatric Infectious Disease Journal* 1999; **18**: 245–248.
5. **Eykyn SJ, Williams H.** Treatment of multiresistant *Salmonella typhi* with oral ciprofloxacin. *Lancet* 1987; **330**: 1407–1408.
6. **Rowe B, Ward LR, Threlfall EJ.** Ciprofloxacin-resistant *Salmonella typhi* in the UK. *Lancet* 1995; **346**: 1302.
7. **Threlfall EJ, *et al.*** Ciprofloxacin-resistant *Salmonella typhi* and treatment failure. *Lancet* 1999; **353**: 1590–1591.
8. **Gaind R, *et al.*** Molecular characterization of ciprofloxacin-resistant *Salmonella enterica* serovar Typhi and Paratyphi A causing enteric fever in India. *Journal of Antimicrobial Chemotherrapy* 2006; **58**: 1139–1144.
9. **Bernstein A, Wilson EMJ.** An analysis of the Vi-phage typing scheme for *Salmonella typhi*. *Journal of General Microbiology* 1963; **32**: 349–373.
10. **Clinical and Laboratory Standards Institute.** Performance Standards for Antimicrobial Susceptibility Testing: Eighteenth Informational Supplement M100-S18. Wayne: CLSI, 2008.
11. **Hunter SB, *et al.*** Establishment of a universal size standard strain for use with the PulseNet standardised pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. *Journal of Clinical Microbiology* 2005; **43**: 1045–1050.
12. **Hirose K, *et al.*** DNA sequence analysis of DNA gyrase and DNA topoisomerase IV quinolone resistance-determining regions of *Salmonella enterica* serovar Typhi and serovar Paratyphi A. *Antimicrobial Agents and Chemotherapy* 2002; **46**: 3249–3252.
13. **Hirose K, *et al.*** Antibiotic susceptibilities of *Salmonella enterica* serovar Typhi and *S. enterica* serovar Paratyphi A isolated from patients in Japan. *Antimicrobial Agents and Chemotherapy* 2001; **45**: 956–958.
14. **Hopkins KL, Davies RH, Threlfall EJ.** Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. *International Journal of Antimicrobial Agents* 2005; **25**: 358–373.
15. **Frenck Jr. RW, *et al.*** Azithromycin versus ceftriaxone for the treatment of uncomplicated typhoid fever in children. *Clinical Infectious Diseases* 2000; **31**: 1134–1138.