Epidemiol. Infect. (2009), **137**, 266–269. © 2008 Cambridge University Press
doi:10.1017/S0950268808000745 Printed in the United Kingdom

SHORT REPORT
Surveillance of antimicrobial resistance of *Salmonella enterica* serotype Typhi in seven Asian countries

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(*Accepted 9 April 2008; first published online 12 May 2008*)

SUMMARY

Two hundred and four *Salmonella enterica* serotype Typhi (*S.* Typhi) isolates were collected from seven Asian countries during 2002–2004. Multidrug-resistant *S.* Typhi (resistant to $\geqslant 3$ antibiotics) was detected in 84 (41·2%) isolates and 142 (69·6%) showed reduced susceptibility to ciprofloxacin (minimum inhibitory concentration = 0·125–1·0 mg/l). This study highlights the worsening situation of antimicrobial resistance of *S.* Typhi in Asia.

Typhoid fever is a systemic febrile illness caused by *Salmonella enterica* serovar Typhi (*S.* Typhi). The incidence of typhoid fever has declined greatly in developed countries, but remains an important cause of illness and death in developing countries. In the pre-antibiotic era, the case-fatality rate was about 20%. After the introduction of chloramphenicol for the treatment of typhoid fever in 1948, the fatality rate decreased to <1% [1]. Outbreaks of chloramphenicol-resistant *S.* Typhi were first reported in 1972 [2]. In the late 1980s, multidrug-resistant strains of *S.* Typhi that were resistant to ampicillin, chloramphenicol and trimethoprim–sulfamethoxazole (TMP–SMX) emerged and became endemic in South Asia, Southeast Asia and South Africa [3]. Strains of *S.* Typhi with reduced susceptibility to fluoroquinolones also emerged in Asia in the 1990s [4].

Most typhoid fever cases in developed countries are associated with travel. Knowledge of the geographic trends in antimicrobial susceptibility is crucial to health-care professionals when treating infected patients. There has been no coordinated global or regional effort regarding surveillance for antimicrobial resistance in *S.* Typhi. The actual geographic distribution of antimicrobial resistance is therefore unknown. In the present study, we collected isolates of *S.* Typhi from seven Asian countries for surveillance of the geographic distribution of antimicrobial resistance.

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Table 1. *Antimicrobial resistance in* Salmonella enterica *serotype Typhi from Asian countries*

Country	CIP No. (%)	TET No. (%)	CTR No. (%)	AMP No. (%)	CHL No. (%)	TMP–SMX No. (%)	MDR No. (%)	RCS No. (%)
Korea	2 (13·3)	1 (6·7)	0 (0)	2 (13·3)	4 (26·7)	2 (13·3)	1 (6·7)	7 (46·6)
Philippines	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	24 (85·7)
Singapore	0 (0)	3 (11·5)	0 (0)	3 (11·5)	3 (11·5)	4 (15·4)	3 (11·5)	10 (38·5)
Sri Lanka	0 (0)	3 (17·6)	0 (0)	3 (17·6)	2 (11·8)	3 (17·6)	1 (5·9)	3 (17·6)
Taiwan	0 (0)	4 (26·7)	0 (0)	5 (33·3)	4 (26·7)	4 (26·7)	4 (26·7)	6 (40·0)
Vietnam	0 (0)	76 (74·5)	0 (0)	76 (74·5)	80 (78·4)	75 (73·5)	75 (73·5)	92 (90·1)
Total	2 (1·0)	87 (42·6)	0 (0)	89 (43·6)	93 (45·6)	88 (43·1)	84 (41·2)	142 (69·6)

CIP, Ciprofloxacin; TET, tetracycline; CTR, ceftriaxone; AMP, ampicillin; CHL, chloramphenicol; TMP–SMX, trimethoprim–sulfamethoxazole; MDR, multidrug resistant; RCS, reduced ciprofloxacin susceptibility.
The only one strain from Hong Kong was susceptible to all antimicrobial agents listed above.

The nine centres in seven Asian countries that participated in this study are Samsung Medical Center in Korea, Chang Gung Childrens Hospital, Chang Gung Memorial Hospital and National Taiwan University Hospital in Taiwan, National University of Singapore in Singapore, University of Medicine and Pharmacy in Vietnam, Research Institute for Tropical Medicine in the Philippines, Princess Margaret Hospital in Hong Kong and University of Colombo in Sri Lanka. Clinical isolates of *S*. Typhi were collected during the period July 2002 to December 2004. All isolates were transported to the central laboratory at the Asian-Pacific Research Foundation for Infectious Diseases in Seoul, Korea and deposited at the Asian Bacterial Bank, Seoul.

The broth microdilution method was performed to determine the minimum inhibitory concentration (MIC) of the following antimicrobial agents: ciprofloxacin, tetracycline, ceftriaxone, ampicillin, chloramphenicol, and TMP–SMX [5]. *Staphylococcus aureus* ATCC29213 and *Escherichia coli* ATCC25922 were used as quality control strains. The isolates in the 'intermediate' category were deemed as 'resistant' in this report. Isolates concomitantly resistant to ampicillin, chloramphenicol, and TMP–SMX were defined as multidrug-resistant *S*. Typhi (MDRST). Isolates with a MIC of ciprofloxacin at 0·125–1·0 mg/l were defined as having reduced susceptibility to ciprofloxacin [6]. Forty-seven *S*. Typhi isolates were randomly selected for study of their clonal relationship by pulsed-field gel electrophoresis (PFGE). The DNA was digested with *Xba*I. Results of PFGE were interpreted as followings: isolates were classified as the same type if their restriction patterns had the same numbers of bands and the corresponding bands were of the same size, isolates were classified as a subtype if the PFGE patterns had two or three band differences, and isolates were considered as different types, if PFGE patterns had more than four band differences.

A total of 204 clinical isolates were collected in this study, including one from Hong Kong, 15 from Korea, 28 from the Philippines, 26 from Singapore, 17 from Sri Lanka, 15 from Taiwan and 102 from Vietnam. Antimicrobial resistance patterns of *S*. Typhi from each Asian country are summarized in Table 1. In total, 100 (49·0%) isolates were resistant to at least one antimicrobial agent. High resistance rates to ampicillin (43·6%), chloramphenicol (45·6%), TMP–SMX (43·1%), and tetracycline (42·6%) were detected. Eighty-four (41·2%) isolates were MDRST. All MDRST isolates were resistant to tetracycline. Eighty-two (97·6%) of MDRST isolates showed reduced susceptibility to ciprofloxacin. Only two isolates were resistant to ciprofloxacin; none was resistant to ceftriaxone. Although only two were resistant to ciprofloxacin, overall 142 (69·6%) isolates showed reduced susceptibility to ciprofloxacin.

When isolates from Vietnam were compared with those from other Asian countries, Vietnamese isolates had a significantly higher resistance rate to ampicillin (74·5% *vs*. 12·7%, $P<0·01$), chloramphenicol (78·4% *vs*. 9·8%, $P<0·01$), TMP–SMX (73·5% *vs*. 12·7%, $P<0·01$), and tetracycline (73·5% *vs*. 11·8%, $P<0·01$). Isolates from Vietnam also showed a significantly higher rate of MDRST (73·5% *vs*. 8·8%, $P<0·01$).

Eight PFGE types were identified. Forty isolates (85·1%) belonged to the same PFGE type A. There was only one isolate in each type other than type A. Fifteen subtypes were identified within type A. Sixteen isolates (34·0%) belonged to subtype A5

which is the most predominant subtype. Five of the eight (62·5%) isolates from Singapore and eight of the 16 (50%) from Vietnam belonged to subtype A5. The isolates from Singapore and Vietnam were significantly associated with subtype A5 ($P<0·01$), when compared with isolates from other Asian countries. PFGE types were diverse in countries other than Singapore and Vietnam. Thirteen of the 16 (81·5%) subtype A5 isolates were MDRST, compared to four of the 31 (12·9%) of other types or subtypes that were MDRST, the subtype A5 was significantly associated with multidrug resistance ($P<0·01$).

This study is the first regional surveillance of antimicrobial resistance of *S*. Typhi in East and Southeast Asia. Although half of the isolates were from Vietnam, the results indeed reflected the antimicrobial resistant patterns of *S*. Typhi in each country. Chloramphenicol-resistant *S*. Typhi emerged in Vietnam and Korea in the early 1970s. The proportion of chloramphenicol-resistant *S*. Typhi reached 80% in Vietnam in 1975 [7]. MDRST, first reported in Vietnam in the late 1980s, was found in almost 90% of the blood culture isolates in 1995 [8]. Although a decreasing trend in isolation of MDRST in South Asia has been reported [9], the rate of chloramphenicol-resistant *S*. Typhi and MDRST in Vietnam did not change in the present study. The proportion of MDRST in the other six Asian countries was <30%, which was much lower than that in Vietnam.

Fluoroquinolones became the drug of choice for the treatment of typhoid fever after the emergence of MDRST. Strains of *S*. Typhi fully susceptible to ciprofloxacin have a MIC $<0·03$ mg/l and are also susceptible to nalidixic acid [6]. Strains with reduced susceptibility to ciprofloxacin were first reported in 1992 [10]. Although strains with reduced susceptibility to ciprofloxacin are still sensitive to ciprofloxacin according to the Clinical and Laboratory Standards Institute (CLSI) antimicrobial susceptibility testing standards, they are, however, resistant to nalidixic acid. Nalidixic acid-resistant strains were associated with clinical failure and delayed response to ciprofloxacin treatment in patients with extraintestinal salmonellosis [11]. The proportion of reduced fluoroquinolone susceptibility of *S*. Typhi in Vietnam increased from 4% in 1993 to 76% in 1998 [12], and in this study, it was as high as 90·1%. On the other hand, the rate of reduced ciprofloxacin susceptibility surpassed the rate of multidrug resistance in most Asian countries, the Philippines in particular. Nearly 70% of *S*. Typhi isolates from this region showed reduced ciprofloxacin susceptibility. This indeed has become a serious problem in East and Southeast Asia, suggesting that ciprofloxacin is no longer a reliable drug to treat patients with typhoid fever in this region. Our data indicate that extended-spectrum cephalosporins are the drugs of choice for empirical therapy of typhoid fever.

Most isolates in our study belonged to PFGE type A, which suggested that most isolates from Asian countries were genetically related. Subtype A5 was predominant in this region and it was associated with multidrug resistance. Singapore and Vietnam showed a higher rate of isolation of subtype A5 strains, indicating dissemination of the resistant clone in Singapore and Vietnam. Typhoid fever currently remains an important public health problem in developing countries. With increasing international travel and trade, the spread of resistant strains become faster than ever and thus, could pose a serious threat to global public health.

ACKNOWLEDGEMENTS

The Enteric Pathogens Project of the Asian Network for Surveillance of Resistant Pathogens (ANSORP) was funded in part by the International Vaccine Institute, Seoul, Korea.

DECLARATION OF INTEREST

None.

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