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ORIGINAL ARTICLE

First isolation of ciprofloxacin-resistant *Salmonella enterica* serovar Typhi in Taiwan

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Background/Purpose: Typhoid fever is a major cause of disease burden in developing countries. The use of fluoroquinolones, once considered the drugs of choice, should be re-evaluated due to the emergence of quinolone-resistant *Salmonella enterica* serovar Typhi. In Taiwan, typhoid fever is rare but constitutes an important public health concern.
Methods: In August 2011, two ciprofloxacin-resistant S. Typhi isolates were identified from one patient who had recently travelled to India. The two isolates together with four other ciprofloxacin-susceptible S. Typhi isolates were subjected for molecular investigation. Polymerase chain reaction (PCR) and sequencing were used to analyze the resistance mechanisms. Pulsed-field gel electrophoresis (PFGE) was performed to delineate the genetic relatedness among the isolates.
Results: In 2011, a total of 49 typhoid fever cases were reported to the Center for Disease Control in Taiwan, with a significant increase in indigenous cases in northern Taiwan from August to November. In the two S. Typhi isolates with complete resistance to ciprofloxacin [minimum inhibitory concentration (MIC) >32 μg/mL], multiple point mutations in the quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* genes were identified. A unique PFGE pattern was found in the resistant isolates and was different from the other representative susceptible isolates.

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Conclusions: The first ciprofloxacin-resistant *S.* Typhi infection in Taiwan is reported. The emergence and spread of antimicrobial-resistant *S.* Typhi infection as a result of international travel may become a threat to public health in Taiwan. Clinicians should be well alert when treating patients who may have acquired resistant infections associated with international travel among endemic regions.

Introduction

Typhoid fever is a systemic infectious disease caused by *Salmonella enterica* serovar Typhi. It is a major cause of disease burden in developing countries. In 2000, typhoid fever resulted in approximately 21.7 million illnesses and 216.5 thousand deaths according to a report by the World Health Organization.[1]

Typhoid fever may lead to prolonged fever, severe diarrhea, gastrointestinal bleeding, bowel perforation, and death. A timely, appropriate antimicrobial treatment is crucial to improve the disease outcome. Effective antimicrobial regimens for typhoid fever may include ampicillin, chloramphenicol, and trimethoprim/sulfamethoxazole in the past. However, in the last 20 years, the worldwide emergence of multidrug-resistant (MDR) *S.* Typhi has become a cause of concern.[2,3] Third-generation cephalosporins and fluoroquinolones are hence considered the drugs of choice if typhoid fever caused by MDR strains is suspected.[4–7] However, nalidixic acid-resistant *S.* Typhi (NARST) with a reduced susceptibility to ciprofloxacin has become an endemic problem in south and southeast Asia for the past 10 years.[2,8–10] NARST has also been reported in the United Kingdom, United States, and other developed countries.[11–13] The drug resistance of *S.* Typhi makes treatment more difficult and costly.

Typhoid fever, although rare, is a notifiable disease in Taiwan. Once a diagnosis or suspicion of typhoid fever has been established, clinicians must report it to the public health authorities within 24 h. According to the Center for Disease Control in Taiwan (CDC, Taiwan), the annual number of typhoid fever cases, including indigenous and imported cases, has remained low at less than 50 in the past 5 years (http://www.cdc.gov.tw/). Antimicrobial resistance is also uncommon, and ciprofloxacin resistance has never been reported previously in *S.* Typhi.[14]

In this study, we present the first case of ciprofloxacin-resistant *S.* Typhi in Taiwan. Sequencing of the quinolone resistance-determining regions (QRDRs) and comparison of pulsed-field gel electrophoresis (PFGE) patterns between this resistant strain and other nonresistant isolates of *S.* Typhi were performed.

Methods

Setting

Chang Gung Memorial Hospital (CGMH) is a 3500-bed university-affiliated hospital located in northern Taiwan. The Clinical Microbiology Laboratory in CGMH is an accredited laboratory of the College of American Pathologists and is responsible for the microbial culture examination of all clinical specimens from this hospital.

Bacterial identification and antimicrobial susceptibility testing

All specimens submitted for microbial isolation and identification have been handled by standard methods. Serotyping of *Salmonella* isolates was further assessed by the O and H antisera (Difco Laboratories, Detroit, MI, USA) using standard methods and the results were interpreted according to the Kauffman–White scheme.[15] Antimicrobial susceptibility was investigated by the standard disc diffusion method. The antimicrobial agents examined included ampicillin, cefixime, ceftriaxone, chloramphenicol, ciprofloxacin, ertapenem, flomoxef, imipenem, and trimethoprim/sulfamethoxazole. Susceptibility and resistance were defined according to the guidelines described by the Clinical and Laboratory Standards Institute (CLSI).[16] The minimum inhibitory concentrations (MICs) of ciprofloxacin was determined by the E-test (AB BIODISK, Solna, Sweden). Ciprofloxacin resistance was defined as MIC >4 μg/mL.[16]

Characterization of ciprofloxacin-resistant isolates

Two ciprofloxacin-resistant *S.* Typhi isolates were identified from one patient in August 2011. The medical records of the patient were reviewed. The number of typhoid fever cases identified at CGMH and those published in the notifiable infectious diseases statistics system of the CDC, Taiwan (http://nidss.cdc.gov.tw/) in 2011 were analyzed for comparison.

To investigate the associated resistance mechanisms, the two resistant isolates together with other four ciprofloxacin-susceptible *S.* Typhi isolates identified from the same laboratory during July and September 2011 were subjected for molecular examination. Genetic relatedness of the isolates was investigated by PFGE as previously described.[17] The results were interpreted according to the recommendations of Tenover et al.[18]

Polymerase chain reaction (PCR) and sequencing were used to analyze the QRDRs of genes encoding the DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) subunits.[19] The results were compared with those of *Salmonella enterica* serovar Typhimurium LT2 published in the public domains. *Salmonella enterica* serovar Choleraesuis SCB-67, also a ciprofloxacin-resistant isolate previously published from this laboratory, was used as a control.[20]

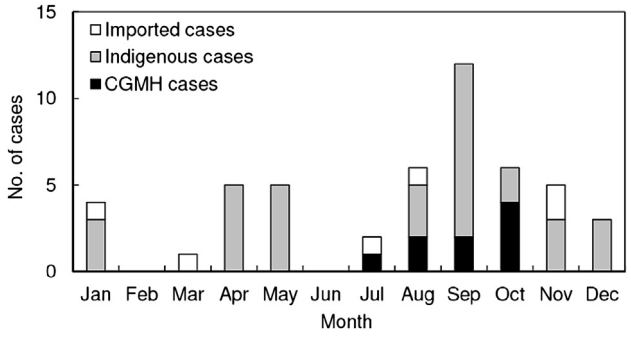

Figure 1. Typhoid fever (including indigenous and imported cases) in Taiwan, 2011 (http://nidss.cdc.gov.tw/). Cases reported from Chang Gung Memorial Hospital (CGMH) are indicated individually. One CGMH case in August was an imported case with ciprofloxacin resistance.

Results

A total of 49 typhoid fever cases, including 42 indigenous cases, were reported to CDC, Taiwan in 2011 (Fig. 1). Among them, 36 of the indigenous cases were from northern Taiwan. The number of typhoid fever cases that exceeded the epidemic threshold occurred from April to May and from August to November. A typhoid fever outbreak that occurred during April had been identified and reported by CDC (data not shown). From August to November, the indigenous cases increased significantly, especially in the northern areas of Taiwan; however, no outbreaks were confirmed.

At CGMH, 10 isolates from nine typhoid fever cases were identified in 2011 (Fig. 1). All of them were found between July and October. Although the majority of the *S.* Typhi isolates were susceptible to all antimicrobial agents tested, two ciprofloxacin-resistant *S.* Typhi isolates were identified from one of the typhoid fever cases in August (Fig. 1). The isolates were resistant to ciprofloxacin with MICs >32 μg/mL, but remained susceptible to all other antimicrobial agents tested (Table 1). The case was a 22-year-old female patient who had been in India for 2 weeks. Three days after returning to Taiwan, she developed fever and gastrointestinal symptoms. Because of persistent fever and diarrhea, which showed no response to symptomatic treatment, she visited our emergency department. After blood sampling and physical examination, which revealed nonspecific findings, the patient was discharged with oral form of ciprofloxacin under the suspicion of enteric fever. Her blood culture later revealed the presence of *S.* Typhi (ST-899). The patient was then admitted to the hospital and treated with ceftriaxone via intravenous route. During hospitalization, the same resistant isolate was found from her stool (ST-900). She recovered without complications after completing the antimicrobial treatment for 9 days.

The two ciprofloxacin-resistant isolates, ST-899 and ST-900, were subsequently proved to be the same strain by PFGE analysis (Fig. 2). Another two different PFGE patterns were found in the remaining four ciprofloxacin-susceptible *S.* Typhi isolates, with three of them (ST-904, ST-909, and ST-911) sharing one major PFGE pattern (Fig. 2). Analysis of the QRDRs of DNA gyrase and topoisomerase IV subunits revealed two point mutations in the *gyrA* gene and another mutation in *parC* from the two ciprofloxacin-resistant isolates, ST-899 and ST-900, identical to those previously found in *S.* Choleraesuis SC-B67 (Table 1). In *gyrA*, the first mutation consisted of a substitution of phenylalanine (Phe) for serine (Ser) at codon 83, and the second a substitution of asparagine (Asn) for aspartic acid (Asp) at codon 87. In *parC*, the point mutation was found at codon 80 with a substitution of isoleucine (Ile) for serine (Ser) (Table 1). No mutations were found in *gyrB* and *parE* genes. As expected, no mutations were detected in the four ciprofloxacin-susceptible isolates.

Table 1 Ciprofloxacin susceptibility and analysis of the quinolone resistance-determining regions (QRDRs) of DNA gyrase and topoisomerase IV subunits in six clinical isolates of *Salmonella enterica* serovar Typhi

Isolate	Specimen	MIC (μg/mL)	*gyrA*[a]		*parC*[a]
			Ser (83)	Asp (87)	Ser (80)
ST-898	Blood	0.016	Ser	Asp	Ser
ST-899	Blood	>32	Phe	Asn	Ile
ST-900	Stool	>32	Phe	Asn	Ile
ST-904	Blood	0.016	Ser	Asp	Ser
ST-909	Blood	0.016	Ser	Asp	Ser
ST-911	Blood	0.016	Ser	Asp	Ser

[a] Amino acid changes compared with the quinolone resistance-determining regions of *gyrA* (codons 83 and 87) and *parC* (codon 80) in *Salmonella enterica* serovar Typhimurium LT2. No mutation was found in *gyrB* and *parE*.
Asn = asparagine; Asp = aspartic acid; Ile = isoleucine; Phe = phenylalanine; Ser = serine; MIC = minimum inhibitory concentration.

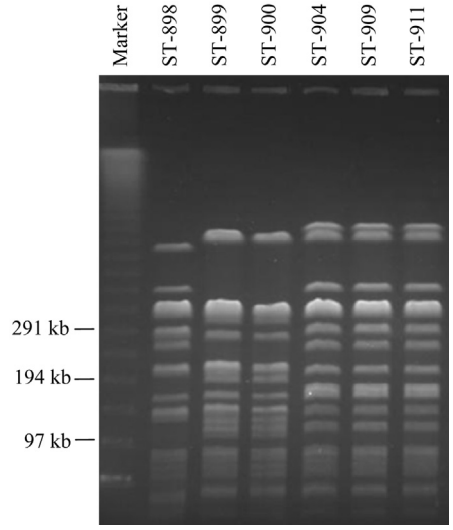

Figure 2. Pulsed-field gel electrophoresis patterns of ciprofloxacin-resistant *Salmonella enterica* serovar Typhi isolates (ST-899 and ST-900) and other representative non-resistant isolates from Chang Gung Memorial Hospital.

Discussion

In Taiwan, typhoid fever remains an uncommon disease, but sporadic cases do occur. Individuals who had a recent travel to an endemic area as well as expatriate employees were the main sources of such patients.[14] Unlike nontyphoid *Salmonella*, antibiotic resistance has not been an issue for *S.* Typhi in Taiwan.[21] Fluoroquinolones and third-generation cephalosporins are the most adequate choices in the treatment of typhoid fever. In this study, we reported the first case of ciprofloxacin-resistant *S.* Typhi infection in Taiwan. The resistance was due to multiple point mutations in the QRDRs of *gyrA* and *parC*, similar to those reported previously.[20,22] The patient had a recent travel history and ciprofloxacin was prescribed empirically. Due to the ciprofloxacin resistance expressed by the disease-causing strain, the patient had to re-admit to the hospital for further treatment.

In the past 10 years, the emergence of ciprofloxacin-resistant *S.* Typhi has been increasingly reported in developing countries, particularly in south and southeast Asia, and in developed countries, such as the United States and United Kingdom.[11,23–25] Almost all of the reported cases from these developed countries were associated with a prior travel history to an endemic area. Morita et al. reported the emergence of high-level fluoroquinolone-resistant *S.* Typhi during 2001 and 2006 in Japan and indicated that south Asia was deemed to be a particularly high-risk travel destination.[26] Our finding in the present study also coincided with these reports. India is in the typhoid fever endemic area and quinolone resistance is high among their local *S.* Typhi isolates.[3,10,22,27] Disease onset in our patient occurred 3 days after her return from India and so an infection caused by an antimicrobial-resistant strain should be well alerted. For patients who are associated with a recent travel history to areas with a high prevalence of drug-resistant *S.* Typhi, ciprofloxacin as the first-line empirical therapy for enteric fever should be reconsidered.

The PFGE pattern of our ciprofloxacin-resistant isolates was identical to that reported in a previous study conducted in the United States.[12] Medalla et al. reported nine ciprofloxacin-resistant *S.* Typhi isolates from 1999 to 2008.[12] In that study, nearly all of the cases had travelled to India before the infection. Three of their cases had identical PFGE patterns and the same antibiogram; two of them had traveled to India. The results suggest that the ciprofloxacin-resistant *S.* Typhi isolates studied herein were imported from India; it appears to have been prevalent in India for several years, thereby identified by both Medalla et al. and our group.

Vaccination is suggested by the World Health Organization for all travelers, regardless of the duration of their stay in typhoid-endemic countries. Steinberg et al. reported that among the travel-associated cases of typhoid fever diagnosed in the United States during 1994—1999, 37% of the cases occurred in travelers who stayed at their travel destination for less than 4 weeks, and 16% occurred in those who stayed for less than 2 weeks.[28] In the present study, the patient had traveled to India for only 2 weeks, but acquired the infection. Prevention of *S.* Typhi infection among travelers is crucial because international travel has become an important route for the transmission of the disease. Government and public health authorities should be more aggressive in promoting immunization for individuals, especially for those who plan to travel to endemic regions.

During the investigation, we incidentally found that one particular PFGE pattern was shared by three of the four ciprofloxacin-susceptible *S.* Typhi isolates that were recovered from clinical specimens between August and September. An apparent increase of typhoid fever cases, predominantly in northern Taiwan, was also noted from the notifiable infectious diseases statistics system of the CDC, Taiwan during the latter half of the year 2011. Whether or not the increase was due to the spread of an epidemic strain, which was coincidently demonstrated among our ciprofloxacin-susceptible *S.* Typhi control isolates, will need further laboratory evidence.

In conclusion, the first case of ciprofloxacin-resistant *S.* Typhi infection in Taiwan is reported herein. Although this may represent the start of the resistant typhoid fever history in Taiwan, fortunately, to the best of our knowledge, no other similar cases have been further reported to date. Personal hygiene education, adequate isolation precaution policy, and appropriate antimicrobial therapy are all effective strategies in preventing the transmission of the disease. The populations of expatriate employees and overseas travelers from endemic countries have increased in the past decades. Government and public health authorities should be well prepared for the prevention of communicable resistant infections, such as the one reported herein. Clinicians should also be more alert in the adjustment of therapeutic regimens for patients who may have acquired resistant infection from other highly endemic countries.

Conflicts of interest

All authors declare that they have no conflicts of interest relevant to this article.

Acknowledgments

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