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Original Article

Genomic surveillance reveals international circulation and local transmission of *Salmonella enterica* serovars Typhi and Paratyphi A in Taiwan

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Abstract *Background/purpose:* Morbidity and mortality from typhoid and paratyphoid fever remain an important problem for public health authorities in developing countries. In countries with lower incidences, most cases occur in travelers who visit regions in which typhoid and paratyphoid fever are highly endemic. The aim was to evaluate the source and transmission dynamics of typhoid and paratyphoid fever in Taiwan by using genomic analysis.
Methods: During 2012—2019, 15 clinical isolates of *Salmonella* Typhi and *S.* Paratyphi A were collected. Demographic and clinical information of the infections were analyzed. We performed whole genome sequencing and evolutionary analysis on these isolates.
Results: Clinical and microbiological data from 7 *S.* Typhi and 8 *S.* Paratyphi A isolates in Taiwan showed epidemiological and bacterial genomic link to the infection in South and Southeast Asia. The Taiwanese typhoidal isolates also share highly similar genomes with those collected from UK, indicating global circulation of the typhoidal clones. Local transmission of the imported but indigenized international clones was observed. Mutations occurring at *gyrA* 83 aa, including S83Y and S83F, were identified in the ciprofloxacin-resistant strains.
Conclusion: Due to the advance of global transportation and communication, the transmission

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mode of infectious disease has been modified. Domestic typhoid and paratyphoid fever caused by international resistant clones can occur in low-incidence countries. Genome analysis showed that the indigenous clone originally imported from other countries has been circulating in Taiwan for over a decade.

Introduction

Salmonella enterica can be classified into the nontyphoidal and typhoidal, which show distinct microbiological, clinical, and epidemiological characteristics. The former tends to exhibit a broad host range, and when infects human, usually causes self-limited gastroenteritis,[1–3] whereas the latter is mainly human-restricted and causes enteric fever.[4] Without effective treatment, the mortality of enteric fever can be up to 12—30%.[5] Globally, there are still 14 million enteric fever cases annually, most of which occur in low- and middle-income countries, such as those in South Asia, Southeast Asia and Africa.[6,7] In contrast, in developed countries two major changes have characterized the pattern of the disease: there is a marked decline in the incidence of the disease in the past half century and it has become predominantly a travel-associated disease.[6] The hygiene system and the availability of treatment are believed to lead to the different incidence between the two types of countries.[6]

Probably due to the humid climate as well as the swine industry, nontyphoidal infections are rampant in Taiwan.[8,9] In contrast, the enteric fever is an uncommon transmissible disease and is restricted in human only, indicating its different transmission mode from nontyphoidal infections. Amid the COVID-19 pandemic, understanding the molecular epidemiology of typhoid and paratyphoid fever is important because the clinical symptoms and signs are extremely similar between the two infections and in countries like Taiwan both are mainly imported from South and Southeast Asian countries.[10] Here we investigated enteric fever cases in a medical center in Taiwan from 2012 to 2019 and found that South and Southeast Asia were the main repertoire of the infection, supported with both epidemiological and genomic evidence. More importantly, these typhoidal *Salmonella* (including S. Typhi and S. Paratyphi A; the same below) have not only spread in Taiwan, but already to worldwide countries.

Methods

Sample collection

This study was conducted in Chang Gung Memorial Hospital (CGMH) and was approved by the Institutional Review Board of CGMH (IRB 201802237B0). All *Salmonella* isolates collected in CGMH during 2012—2019 were involved in this study. Bacterial identification was conducted by Bruker MALDI Biotyper (Bruker Daltonics, Billerica, MA).

Serovar identification and MIC analysis

All *Salmonella* isolates collected as described above were further serotyped using multiplex PCR-based method as described previously.[11] Minimum inhibitory concentration (MIC) of ceftriaxone and ciprofloxacin to these isolates were determined by E-test and interpreted according to the recommendations given by CLSI.[12]

Genomic sequencing and analysis

Genomic DNA of the typhoidal isolates was subjected to whole genome sequencing (WGS) using the Illumina Miseq platform (Illumina, CA, USA). The sequencing throughput for each of the investigated isolates was at least >1.0 Gb, i.e., >200x coverage. The short reads generated were de novo assembled into contigs using SPAdes v3.11.1 with the "–careful" option.[13] The genomic sequences were deposited into the GenBank database, with the accession numbers being listed in Table 1.

BacWGSTdb was searched for the genomes that showed <50 single nucleotide polymorphisms (SNPs) compared to those sequenced in this study.[14] Parsnp software v1.4 (https://github.com/marbl/parsnp) was used to find the backbone genomes of the compared isolates. Gubbins software was used to filter recombinant SNPs.[15] The neighbor-joining trees were built using MEGA X under the 'No. of differences' substitution model; 500 bootstraps were performed.[16] Antimicrobial resistance genes and chromosomal gene mutations mediating Antimicrobial resistance were predicted by the online service ResFinder (https://academic.oup.com/jac/article/75/12/3491/5890997).

Results

Taiwan is a high-income economy, as defined by World Bank, with flourishing international trade and travel. Chang Gung Memorial Hospital (CGMH) is a main referral hospital for cities in northern Taiwan, the population of which is approximately seven million. During 2012—2019, 7 infection cases caused by STY and 8 cases by SPA were admitted (Fig. 1; Table 1). Almost all isolates were cultured from the blood, and only two S. Typhi isolates were from stool. These cases were sporadic, and the average incidence for STY and SPA infections was both <0.1 per 100,000 population per year. The age of the patients ranged from 3.6 to 53 years old. Four STY patients and five SPA patients had travel history in India, Cambodia, Vietnam, and Indonesia within two

Table 1 Information of the *Salmonella* isolates sequenced.

Serovar[a]	Isolate	Accession No.	Sequence type	Collection time	Ciprofloxacin[b]	Patient's travel history
SPA	Sal-1543	JABUVB01	85	Aug-2012	S	Cambodia
SPA	Sal-1980	JABUVC01	129	Mar-2013	S	Indigenous
SPA	Sal-1986	JABUVD01	129	Apr-2013	S	Indigenous
SPA	Sal-3858	JABUVF01	85	Jun-2016	R	Indonesia
SPA	Sal-6647	JABUVG01	129	Jun-2016	S	Indigenous
SPA	Sal-4286	JABUVH01	85	Jan-2017	R	Cambodia
SPA	Sal-5887	JABUVL01	85	Jan-2019	R	Cambodia
SPA	Sal-6475	JABUVM01	129	Sep-2019	S	Indonesia
STY	ST33	PZLJ01	1	Mar-2013	S	Indigenous
STY	ST34	PZLK01	2	Apr-13	S	Indigenous
STY	ST35	PZLL01	2	May-14	S	Indigenous
STY	Sal-2894	JABUVE01	1	May-2015	S	Indonesia
STY	Sal-4874	JABUVJ01	1	Sep-2017	R	India
STY	Sal-5864	JABUVK01	2	Jan-2019	S	Vietnam
STY	Sal-5942	JACFYH01	1	Mar-2019	S	Indonesia

[a] SPA: *S.* Paratyphi A; STY, *S.* Typhi.
[b] Susceptible, S; Resistant, R.

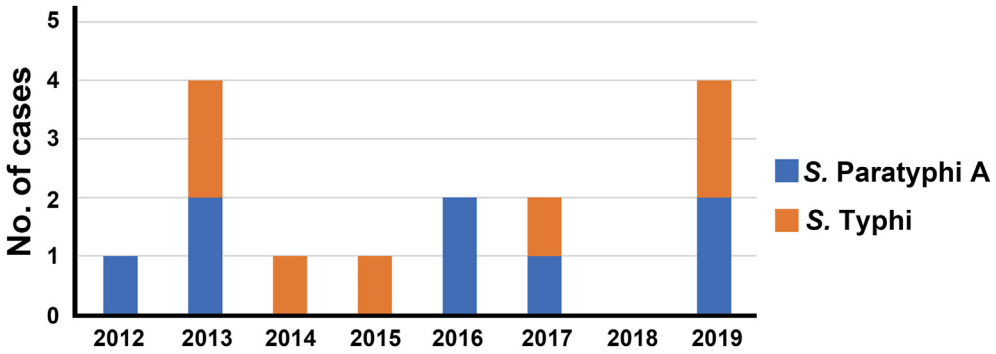

Figure 1. Number of the typhoidal infections in northern Taiwan during 2012—2019.

months before the onset of illness, which therefore belonged to imported cases. The remaining cases were indigenous.

The most common clinical manifestation was fever (100%), followed by diarrhea (40.0%) and abdominal pain (26.7%). There was no bloody stool, skin rash, relative bradycardia, bowel perforation among the 15 patients. Lack of these typical symptoms may be due to the patients' early medical attention. One STY and three SPA isolates were resistant to ciprofloxacin, but none were resistant to extended-spectrum cephalosporins. All patients were successfully treated with ceftriaxone.

The genomes of the isolates were all sequenced and compared. The core genome size was 4,516,084 bp for the Typhi isolates, and 4,169,917 bp for the Paratyphi A isolates. When 20 SNPs within the core genome was used as a threshold to determine whether the query isolates belonged to the same clone, we found that the STY isolates were scattered in 7 clones and SPA in 5 clones (Fig. 2). A few isolates, e.g. SPA Sal-1980 and Sal-1986, belonged to the same clone; the patients, however, had no direct contact with each other, indicating they were both infected independently by an indigenous clone. Further comparison of the typhoidal genomes sequenced in this study against those deposited in the public database confirmed this phenomenon: the STY isolate ST34 collected in 2012 was highly similar to the Taiwanese isolates collected in 2001, 2005, 2006, demonstrating that this indigenous clone had been circulating in Taiwan over a decade.

Comparison against the public database also revealed that the Taiwanese typhoidal isolates were genetically close to a few international isolates, majority of which were collected from Southeast Asia and South Asia (Fig. 2).

The mutations occurring at *gyrA* 83 aa, including S83Y and S83F, were identified in the ciprofloxacin-resistant strains (Supplementary Table 1; Fig. 2). Obviously, the mutations did not occur independently but cluster in a few clones, suggesting that ciprofloxacin resistance had spread worldwide along with the clonal dissemination.

Discussion

Typhoid and paratyphoid fever continue to be a serious public health problem throughout the world.[17] The infection is most often acquired by ingestion of food or water contaminated by the feces of patients and carriers. Transmission related to contamination with infected urine can occur occasionally. In the countries with low incidence of

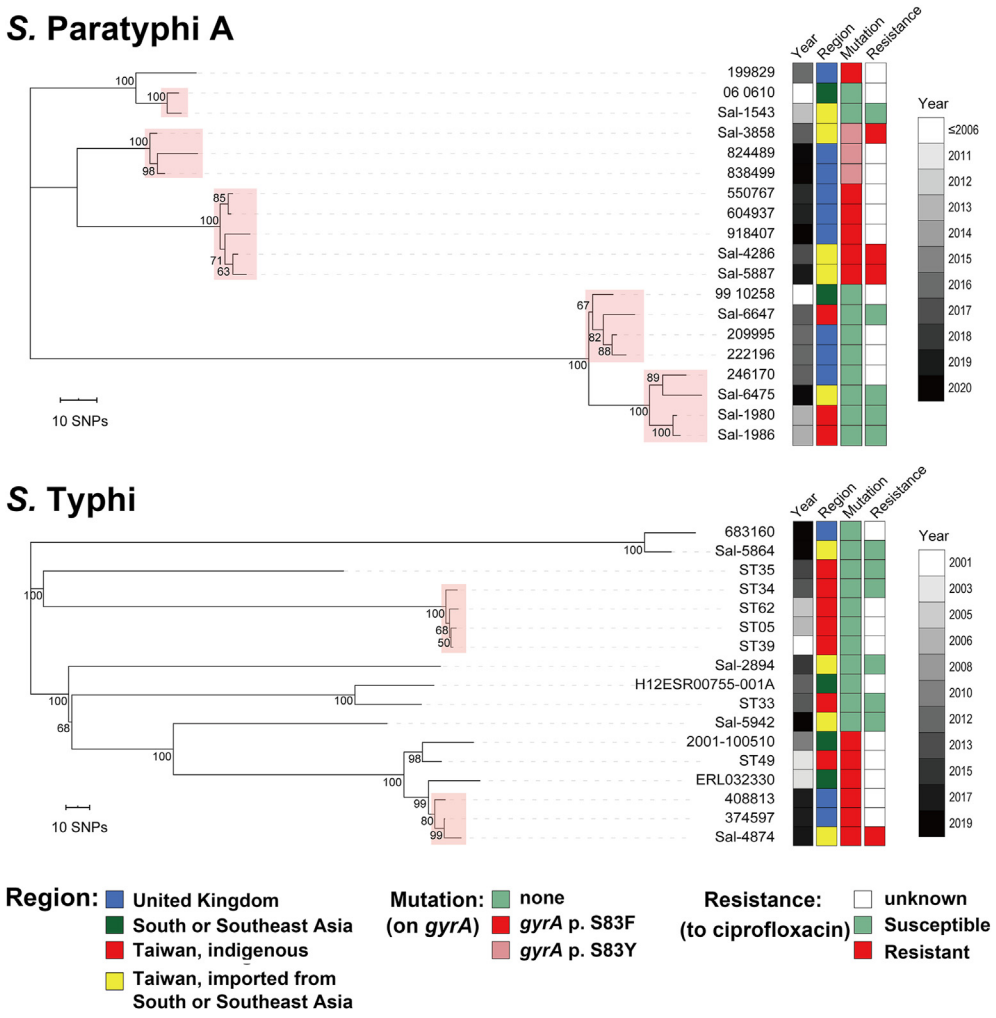

Figure 2. Genome comparison of the Taiwanese typhoidal isolates and their genetically similar international isolates. The left neighbor-joining trees were built based on whole-genome SNPs under the 'No. of differences' substitution model. Genomes with less than 20 SNPs are marked within red boxes. The numbers on the nodes are the bootstrap values.

typhoid fever such as Taiwan, most cases occur in travelers who visit regions in which typhoid fever is highly endemic. For the Taiwanese patients who traveled to Southeast Asian countries, the genomic and epidemiological features were very consistent, both supporting these cases being imported from those endemic countries. For the patients with no travel history, there were two hypotheses explaining why their isolates were genetically homogenous to the South and Southeast Asian typhoidal strains: 1) the clone was initially imported from foreign countries and later circulated in Taiwan and formed an indigenous clone; 2) the patients may contact some asymptomatic patients who recently returned from foreign countries.

Intriguingly, for both STY and SPA infections, we found that many of the international strains being close to the Taiwanese typhoidal strains were from the UK (Fig. 2). Given the fact that none of the patients traveled to UK as well as the low incidence of enteric fever in UK, the related cases were hardly attributed to direct transmissions between Taiwan and the UK. Instead, this phenomenon may result from the bias that UK has much higher sequencing density so that a considerable part (16.2%) of *Salmonella* genomes in the public database is derived from UK.[14] According to the sequence information, the source of the isolates instead of the sequence submitter from UK could be derived from Southeast Asia, especially Vietnam because the *Salmonella* studies from United Kingdom investigated *Salmonella* in Vietnam before. Denying the direct transmission between UK and Taiwan, however, indicated transmission between Taiwan and an unknown region where the typhoidal clone had also prevailed, which further indicated this clone's global dissemination.

In this study, we found that mutations occurred invariably at *gyrA* 83 aa, including S83Y and S83F, in the ciprofloxacin-resistant isolates. The mutations did not occur independently but cluster in a few clones, suggesting that ciprofloxacin resistance had spread worldwide along with the clonal dissemination. The first imported case of extensively drug-resistant (XDR) typhoid fever in Taiwan who contracted with a bacterial strain, which was most closely related to the $bla_{CTX\text{-}M\text{-}15}$-carrying strains linked to Pakistan was reported in Taiwan in 2019.[18] This XDR *S.* Typhi isolate contained a 4.8-Mbp chromosome (GenBank accession number CP046429) and an 83.4-Kbp plasmid (GenBank

accession number CP046430). The chromosome carried an S83F mutation in the *gyrA* to mediate fluoroquinolone resistance, same as our isolates. Additionally, the isolate harbored a cryptic resistance gene *aac(6')-laa*, and 7 additional resistance genes, *catA1*, *dfrA7*, *sul1*, bla_{TEM-1B}, *strA*, *strB*, and *sul2*.[18] However, the plasmid, unlike a resistance plasmid commonly present in multidrug-resistant *S. enterica*,[19,20] did not carry any drug resistance genes. Our study again highlighted the critical issue of global dissemination of antimicrobial-resistant *S.* Typhi clones.

In this study, the Taiwanese typhoidal pathogens mainly originate from South and Southeast Asia, which were brought in by either travelers or imported food. However, there are clones originating from UK without epidemiological link as well. The typhoidal clones reported in this study not only may have been brought to Taiwan but already spread all over the world. This phenomenon suggests that, when there is no epidemiological link, it should be more prudent to trace the source of infection and reconstruct the transmission routes by using genome sequencing and comparison.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2021.06.004.