Molecular and Clinical Epidemiology of *Salmonella* Paratyphi A Isolated from Patients with Bacteremia in Nepal

Jatan Bahadur Sherchan,¹ Masatomo Morita,² Takashi Matono,² Hidemasa Izumiya,² Makoto Ohnishi,² Jeevan B. Sherchand,³ Sarmila Tandukar,³ Ujjwal Laghu,³ Maki Nagamatsu,⁴ Yasuyuki Kato,⁴ Norio Ohmagari,⁴ and Kayoko Hayakawa^{4*}

¹Department of Clinical Microbiology, Kathmandu University School of Medical Sciences, Dhulikhel, Nepal; ²Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan; ³Public Health Research Laboratory, Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu, Nepal; ⁴Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan

Abstract. Little is known about the epidemiology of typhoid and paratyphoid fever in Nepal. We aimed to elucidate the molecular and clinical epidemiology of *Salmonella* Paratyphi A in Nepal. Isolates were collected from 23 cases of bacteremia due to *S. Paratyphi* A between December 2014 and October 2015. Thirteen patients (57%) were male, and the median age was 21 years. None of the patients had an underlying chronic disease. All *S. Paratyphi* A isolates were sensitive to ampicillin, trimethoprim/sulfamethoxazole, ceftriaxone, and chloramphenicol. All isolates were resistant to nalidixic acid and were categorized as intermediately susceptible to levofloxacin. Phylogenetic analysis revealed close relatedness among the isolates, including several clonal groups, suggesting local spread. Patients with bacteremia due to *S. Paratyphi* A in Kathmandu, Nepal, were relatively young and nondebilitated. Improving control of *S. Paratyphi* infections should focus on effective infection control measures and selection of empirical therapy based on current resistance patterns.

INTRODUCTION

Salmonella enterica serotype Typhi or *S. enterica* serotype Paratyphi cause an estimated 22 million new cases of enteric fever (typhoid or paratyphoid) annually, and 200,000 deaths.¹ Nepal is located in South Asia where typhoid and paratyphoid fever are most prevalent.² Especially, Kathmandu is known to have the significant burden of enteric fever caused by *S. Typhi* and *S. Paratyphi* A.³ The recent spread of multidrug resistant *S. Typhi* and *S. Paratyphi* A is a serious threat to public health; however, little is known about the epidemiology of typhoid and paratyphoid fever in Nepal. Previous reports have suggested an increase in the incidence of paratyphoid fever, against which currently available typhoid vaccines provide little to no protection.^{2,3} This study aimed to elucidate the molecular and clinical epidemiology including the prevalence of drug-resistant strains and phylogenetic analyses of *S. Paratyphi* A in Nepal.

METHODS

From December 2014 to October 2015, *S. Paratyphi* A isolates were collected from patients with bacteremia at Tribhuvan University, Kathmandu, Nepal. For patients from whom more than one *S. Paratyphi* A strain was isolated during the study period, only the first episode was analyzed (i.e., unique patient episodes). Institutional review boards at the Tribhuvan University approved the study before its initiation. Parameters retrieved from the patient records included demographics, background conditions and clinical symptoms, duration of hospital stay (for patients who were hospitalized), empiric antimicrobial treatment; information on occupation and exposure (e.g., animal contact, contact to similar cases), and clinical outcome.

Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method using the Dry Plate Eiken (Eiken Chemical, Tokyo, Japan), in accordance with the Clinical and Laboratory Standard Institutions (CLSI) criteria (M100-S26) at the National Center for Global Health and Medicine. Whole-genome sequencing and phylogenetic analysis were conducted at the National Institute of Infectious Diseases. Genomic DNA was prepared using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genomic DNA libraries were prepared using the Nextera XT DNA sample prep kit (Illumina, San Diego, CA) and paired-end (300 × 2 bp) short reads for each library were sequenced on a MiSeq instrument (Illumina). Sufficient DNA sequence reads were generated to cover the genome at least 60 folds. Sequence reads were assembled with the de novo genome assembly program CLC Genomics Workbench v.8.5.1 (CLC Bio, Aarhus, Denmark) to generate a multicontig draft genome for each sample. All contigs were compared with the reference genome for *S. Paratyphi* A strain ATCC 9150 (CP000026.1) to detect mutations on *gyrA*, *gyrB*, *parC*, and *parE* genes. To compare short-read mapping data for all strains with the reference chromosomal sequence of *S. Paratyphi* A strain ATCC 9150 (CP000026.1), *bwasw*⁴ and *samtools*⁵ software were used with default parameters. Single nucleotide polymorphisms (SNPs) were extracted with VarScan v.2.3.4⁶ using default parameters. The SNPs in repetitive and recombination regions were excluded for further analyses. Exact and inexact repeat regions were detected using the MUMmer v.3.23.⁷ RecHMM was used to identify recombination regions.⁸ The remaining 254 SNPs were concatenated to generate a pseudosequence for phylogenetic analysis; maximum likelihood phylogenetic analysis was performed using RAXML v.8.2.0⁹ with 1,000 bootstrap iterations.

RESULTS

During the study period, 23 cases of bacteremia due to *S. Paratyphi* A, and 86 cases of bacteremia due to *S. Typhi*

* Address correspondence to Kayoko Hayakawa, Disease Control and Prevention Center, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan. E-mail: kayokohayakawa@gmail.com

TABLE 1

Clinical characteristics of patients with bacteremia due to *Salmonella* Paratyphi A (*N* = 23)

	Number of patients (%)
Demographics	
Age, median (IQR)	21 (17–23)
Male patients	13 (56.5)
Inpatients/out patients	12 (52.2)/11 (47.8)
Occupation	
Student	12 (52.2)
House wife	4 (17.4)
Farmer/gardener	3 (13)
Driver	2 (8.7)
Nurse	1 (4.3)
Pastry shop worker	1 (4.3)
Clinical symptom	
Fever	23 (100)
Abdominal pain	10 (43.5)
Diarrhea	3 (13)
Vomiting	2 (8.7)
Empiric antimicrobial treatment	
Fluoroquinolone*	12 (52.2)
Cefixime	4 (17.4)
Ceftriaxone	2 (8.7)
Azithromycin	1 (4.3)
Duration of hospitalization, median (IQR), days	5 (3–6)

IQR = interquartile range. One patient received cefixime, azithromycin, ceftriaxone, and another patient received ceftriaxone, azithromycin, and levofloxacin.

* Fluoroquinolones include ciprofloxacin (*N* = 10), ofloxacin (*N* = 1), and levofloxacin (*N* = 1).

were identified. Among the 23 cases of bacteremia due to *S. Paratyphi* A, 13 patients (56.5%) were male, the median age was 21 years (interquartile range [IQR]: 17–23 years, range: 5–68 years). Among the 86 cases of bacteremia due to *S. Typhi*, 51 patients (59.3%) were male, the median age was 21 years (IQR: 17–26 years, range: 4–70 years). Clinical characteristics are summarized in Table 1. Twelve (52.2%) patients were hospitalized, the other were outpatients. None of the patients had an underlying chronic disease or immunosuppressive status. Twelve patients (52.2%) were students,

four (17.4%) were housewives, three were farmers or gardeners, two (8.7%) were drivers, one (4.3%) was a nurse, and one (4.3%) worked at a pastry shop. The most common clinical symptom was fever (*N* = 23, 100%), followed by abdominal pain (*N* = 10, 43.5%), diarrhea (*N* = 3, 13%), and vomiting (*N* = 2, 8.7%). Most commonly prescribed empiric antimicrobial treatment was fluoroquinolone (*N* = 12, 52.2%), followed by cephalosporins (cefixime [*N* = 4, 17.4%], ceftriaxone [*N* = 2, 8.7%]), and azithromycin (*N* = 1, 4.3%). The median duration of hospitalization was 5 (IQR: 3–6) days, and no patient had complications or died.

Antibiotic susceptibility and MICs of the *S. Paratyphi* A isolates are listed in Table 2. All isolates were sensitive to ampicillin, trimethoprim/sulfamethoxazole, ceftriaxone, and chloramphenicol, and had an azithromycin MIC of less than 16 µg/mL. All isolates were resistant to nalidixic acid. The susceptibilities to fluoroquinolones differed, i.e., all isolates were resistant to ofloxacin based on CLSI criteria (M100-S26), four (17.4%) were resistant to ciprofloxacin, and all isolates were categorized as intermediately susceptible to levofloxacin.

Phylogenetic analyses showed that except for one strain (151006PA), all isolates clustered together, with SNP distances of 0–9 SNPs (Figure 1). Clonal isolates (genetically closest isolates with 0 SNPs) were detected in five groups, whereas definitive outbreak from food handlers (the housewives and pastry shop worker) and family infection were not found. Among the five groups of clonal isolates, each isolate was predominantly detected 1 month or more apart, and only three paired isolates had a near sampling dates: 151080PA (on July 1, 2015) and 151086PA (on July 16, 2015); 151029PA (on March 5, 2015) and 151032PA (on March 12, 2015); and 151033PA (March 15, 2015) and 151031PA (March 8, 2015). All *S. Paratyphi* A isolates had a same single mutation C248T in *gyrA* encoding Ser83Phe; however, no mutation was found in *gyrB*, *parC*, and *parE*.

TABLE 2
Antibiotic susceptibility and minimal inhibitory concentration (MIC) of *Salmonella* Paratyphi A (*N* = 23)

Antibiotics	Antibiotic susceptibility, number of resistant* isolates (%)			MIC (µg/mL)	
	S	I	R	MIC ₅₀	MIC ₉₀
Nalidixic acid	–	–	23 (100)	> 128	> 128
Ciprofloxacin	–	19 (82.6)	4 (17.4)	0.5	1
Levofloxacin	–	23 (100)	–	1	1
Ofloxacin	–	–	23 (100)	2	2
Norfloxacin†	–	–	–	4	4
Gatifloxacin†	–	–	–	0.5	1
Prulifloxacin‡	–	–	–	0.5	0.5
Tosufloxacin‡	–	–	–	0.5	0.5
Gentamicin	23 (100)	–	–	0.12	0.25
Kanamycin	23 (100)	–	–	0.5	1
Trimethoprim- sulfamethoxazole	23 (100)	–	–	0.12/2.38	0.25/4.75
Tetracycline	23 (100)	–	–	2	4
Minocycline	23 (100)	–	–	4	4
Azithromycin‡	23 (100)	–	–	8	8
Cefotaxime	23 (100)	–	–	0.12	0.25
Ceftriaxone	23 (100)	–	–	0.12	0.25
Ampicillin	23 (100)	–	–	4	4
Aztreonam‡	–	–	–	0.12	0.12
Imipenem	23 (100)	–	–	0.25	0.25
Panipenem‡	–	–	–	0.12	0.12
Biapenem‡	–	–	–	0.25	0.25
Chloramphenicol	23 (100)	–	–	8	8

* Based on Clinical and Laboratory Standard Institutions (CLSI) criteria 2016 (M100-S26) unless otherwise noted.

† CLSI criteria 2016 (M100-S26) did not specify *Salmonella* spp. as they did for other fluoroquinolones, and thus, antibiotic susceptibility was left blank.

‡ No breakpoint is available for *S. Paratyphi* based on CLSI criteria 2016 (M100-S26).

FIGURE 1. Maximum-likelihood tree generated from single nucleotide polymorphisms in the core genome. (A) Phylogenetic tree of *Salmonella Paratyphi A* isolates from Tribhuvan University, Kathmandu, Nepal. (B) Phylogenetic sub-tree of major endemic *S. Paratyphi A* isolates.

DISCUSSION

In this study, we identified the molecular and clinical epidemiology of *S. Paratyphi A* in Kathmandu, Nepal. As we included sequential patients with bacteremia due to *S. Paratyphi A*, our findings would reflect the epidemiologic characteristics of this endemic region. It was revealed that *S. Paratyphi A* mainly affects relatively young subjects. The high prevalence of fever is consistent with recent reports on *S. Typhi* and *S. Paratyphi* bacteremia.¹⁰ In our cohort, the prevalence of abdominal pain was slightly higher, whereas diarrhea and vomiting were less prevalent than in previous reports.¹⁰ This might be due to differences in baseline characteristics, including age, of the tested population.

In this study, the antibiotic susceptibility and MICs revealed patterns of re-emergence of susceptibility to conventional antibiotics (e.g., chloramphenicol, trimethoprim/sulfamethoxazole, and ampicillin) similar to those described previously.¹¹ On the other hand, MICs for quinolones were elevated. Importantly, *S. Paratyphi A* isolates of 10 patients who received ciprofloxacin as empiric therapy showed ciprofloxacin MICs ≥ 0.5 $\mu\text{g/mL}$, and one patient who received ofloxacin carried *S. Paratyphi A* with an ofloxacin MIC of 2 $\mu\text{g/mL}$. Along with recent trial results on the use of gatifloxacin,¹⁰ reconsideration is warranted in terms of empiric antimicrobial treatment against *S. Paratyphi A* bacteremia in Nepal.

Based on phylogenetic analyses, there seem to be two distinct endemic strains of *S. Paratyphi A* in the study region. The major endemic strain was genetically diverged as compared with outbreak-associated isolates in China.¹² The relatively high prevalence of patients who handle food (e.g., food-handling business, housewives) and patients who have close contact with other people (e.g., students) in this study suggests a potential point of intervention to reduce the

transmission of *S. Paratyphi*, for which no vaccine is readily available.

High relatedness, coupled with high prevalence of reduced susceptibility to fluoroquinolones, was observed in *S. Paratyphi* isolated from patients with bacteremia in Nepal, Kathmandu. Further studies are warranted in terms of appropriate effective therapy and effective infection control approaches in this region.

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Authors' addresses: Jatan Bahadur Sherchan, Department of Clinical Microbiology, Kathmandu University School of Medical Sciences, Dhulikhel, Nepal, E-mail: jatansherchan@gmail.com. Masatomo Morita, Takashi Matono, Hidemasa Izumiya, and Makoto Ohnishi, Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan, E-mails: mmorita@niid.go.jp, tmatono@niid.go.jp, izumiya@nih.go.jp, and ohnishi7@nih.go.jp. Jeevan B. Sherchand, Sarmila Tandukar, and Ujjwal Laghu, Department of Microbiology and Parasitology, Tribhuvan University Teaching Hospital, Kathmandu, Nepal, E-mails: jeevansherchand@hotmail.com, sar1234tan@gmail.com, and ujjwal111@iom.edu.np. Maki Nagamatsu, Yasuyuki Kato, Norio Ohmagari, and Kayoko Hayakawa, Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan, E-mails: dfrmm217@yahoo.co.jp, ykato@hosp.ncgm.go.jp, nohmagari@hosp.ncgm.go.jp, and kayokohayakawa@gmail.com.

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