Salmonella enterica serovar Paratyphi A: an emerging cause of febrile illness in Nepal

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ABSTRACT

With an aim to evaluate the isolation rate and antibiotic susceptibility pattern in *Salmonella enterica* serovar Typhi and *S.* Paratyphi A, 656 blood samples collected from clinically diagnosed enteric fever patients at National Public Health Laboratory, Nepal during January through December 2008 were processed. Isolates were identified by standard microbiological procedures including serotyping. Antibiotic susceptibility testing was performed by disc diffusion method and minimum inhibitory concentration (MIC) to ciprofloxacin, ofloxacin and nalidixic acid was determined by agar dilution method following CLSI guidelines. Altogether 59 isolates of *S.* Typhi (49.15%) and *S.* Paratyphi A (50.85%) were recovered. A total of 80% isolates were resistant to nalidixic acid with *S.* Paratyphi A (93%) showing significantly higher resistance ($P<0.05$) compared to *S.* Typhi (66%). The nalidixic acid resistant *S.* Paratyphi A strains required significantly higher MICs ($P<0.001$) to quinolone (MIC expressed as mean±SD for nalidixic acid 477.87±87.02µg/mL, ofloxacin 1.8±0.63µg/mL, ciprofloxacin 0.62±0.3µg/mL) compared with that of *S.* Typhi (nalidixic acid 173.18±72.03µg/mL, ofloxacin 0.43±0.11µg/mL, ciprofloxacin 0.25µg/mL). Increased MIC of fluoroquinolone (FQ) is of particular concern in emerging strains of *S.* Paratyphi A as exposure to these drugs fuels up further development of full FQ resistant populations. Use of FQs as the first-line drugs for empirical therapy and management of enteric fever in areas where these strains are prevalent is questionable and requires an urgent review.

Keywords: Salmonella Paratyphi A, enteric fever, fluoroquinolone resistance.

INTRODUCTION

Paratyphoid fever, caused by *Salmonella enterica* serovar Paratyphi A, was estimated to cause 5·4 million cases and previously considered as the subtle febrile illness compared to typhoid fever with an estimated 21.6 million cases and 2,20,000 deaths worldwide.[1,2] Studies from China, Pakistan, India, Vietnam, Indonesia and Nepal report that *S.* Paratyphi A can contribute up to half of all the enteric fever cases in some settings and times and is emerging as a major cause of febrile illness.[3-7] Moreover, reports from developed countries also found an increasing incidence of *S.* Paratyphi A among travelers returned from endemic regions.[8] Furthermore, *S.* Paratyphi A although previously believed to cause a milder disease than *S.* Typhi, several recent studies showed that it produces indistinguishable clinical features[9,10] and possibly greater complication with localized infections.[11,12]

High prevalence of multidrug-resistant (MDR) *S.* Typhi and *S.* Paratyphi A strains resistant to most of the traditional drugs during 1990s brought fluoroquinolones (FQs) into picture for the management of enteric fever.[1,13] Subsequently, increased rates of *Salmonella enterica* strains with reduced susceptibility to FQs[14-16] progressing into high-level resistance to these drugs[17-19] have been reported from different parts of world.

Enteric fever still remains a major diagnosis among febrile patients with yearly increase in Nalidixic resistance and reduced susceptibility to FQs in *S.* Typhi and *S.* Paratyphi A.[20-22] Reduced susceptibility to FQs in *S.* Paratyphi A is even more striking with dramatic increase in nalidixic acid resistance compared to *S.* Typhi.[23,24] More importantly, currently available typhoid vaccines and diagnostics targeting *S.* Typhi but having no efficacy against *S.* Paratyphi A may interfere the use of these products in regions with a high incidence of *S.* Paratyphi A cases.[25,26] This study aimed to analyze the rate of isolation and antimicrobial susceptibility pattern of *Salmonella* serotypes from enteric fever cases attending to a national reference laboratory of Nepal.

MATERIALS AND METHODS

Study setting: Study was conducted prospectively at National Public Health Laboratory (NPHL) on clinically defined enteric fever patients requesting for blood culture and susceptibility testing during January through December 2008**.** A total of 656 blood samples from suspected enteric fever patients were included in the study.

Table-1: Monthwise distribution of *S.* Typhi and *S.* Paratyphi A

	Months												Total
	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	
Febrile cases	37	31	45	29	44	48	104	103	88	40	52	23	644
S. Typhi	1	0	1	1	0	0	15	6	2	2	0	1	29
S. Paratyphi A	6	2	2	1	4	0	2	3	5	1	2	2	30
Total isolates	7	2	3	2	4	0	17	9	7	3	2	3	59

Isolation and identification of *Salmonella*: Blood samples were processed by selective enrichment in bile broth followed by daily subculture on MacConkey agar after overnight incubation at 37ÚC. Isolates were further identified by standard microbiological procedures including serotyping using specific antisera (Denka Seiken Co. Ltd., Tokyo, Japan). Samples were considered negative for *Salmonella* if no growth was observed until 10 days incubation.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing of the isolates was performed by Kirby Bauer disc diffusion method and CLSI recommended interpretive criteria. The following antibiotics were tested for all the conformed isolates: Ampicillin (10ìg), ciprofloxacin (5ìg), ofloxacin (5ìg), nalidixic acid (30ìg), cotrimoxazole (1.25/23.75ìg), tetracycline (30µg), chloramphenicol (30µg) and ceftriaxone (30µg). *Escherichia coli* ATCC 25922 was used for quality control.

Determination of MIC: MICs of ciprofloxacin, ofloxacin and nalidixic acid were determined by agar dilution method following CLSI guidelines.[27] *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains and each test and measurement were carried out twice to ensure reproducibility of the results. The MIC breakpoints used were those defined by the CLSI for *Enterobacteriaceae*.[27]

Statistical analysis: Statistical analysis was performed using SPSS 11.6 and WHONET 5.4 software. Student t-test and chi-square test were used to determine the significance difference.

RESULTS

Demographic findings: Among the 656 patients investigated throughout the year, 59 (8.99%) were confirmed as enteric fever cases. The age of patients under investigation ranged 1-91 years (mean 26.37 years) with the mean age of enteric fever cases being 22 years (range 3-71 years). In our study, enteric fever was more common among males (69%), though the sex-wise difference is statistically insignificant (P>0.05). Despite the majority of suspected cases (57.77%) fell in age group 19-45 years, higher percentage of enteric fever cases was observed among 5-18 years (Fig.1). This difference in enteric fever cases by age group was also statistically significant (P<0.05).

Distribution of *S.* Typhi and *S.* Paratyphi A: Of the 59 isolates, 29(49.15%) were *S.* Typhi and 30(50.85%) were *S.* Paratyphi A. The distribution of these serotypes also varied among different age groups (Fig. 1). The distribution of both febrile illness and enteric fever cases varied monthwise with more than half of the confirmed cases reported during July to September. Among enteric fever cases, distribution of *S.* Typhi isolates was more common during July to October while that of *S.* Paratyphi A was throughout the year (Table-1).

Antibitic resistance pattern: Altogether 80% isolates were resistant to nalidixic acid with 5.08% of the total isolates being MDR. Resistance to nalidixic acid in *S.* Typhi and *S.* Paratyphi A was 93% and 66%, respectively. The difference in nalidixic acid resistance in *S.* Typhi and *S.* Paratyphi A was statistically significant (P<0.05). Only 3% of total isolates were resistant to both chloramphenicol and tetracycline while 8% of the isolates were resistant towards ampicillin (Fig. 2). The difference in resistance towards other antibiotic among *S.* Typhi and *S.* Paratyphi A however was statistically insignificant (P>0.05). In overall, 86.44% of the isolates were susceptible to all ampicillin, chloramphenicol, cotrimoxazole and tetracycline (ACCoT). All the isolates were susceptible towards ceftriaxone.

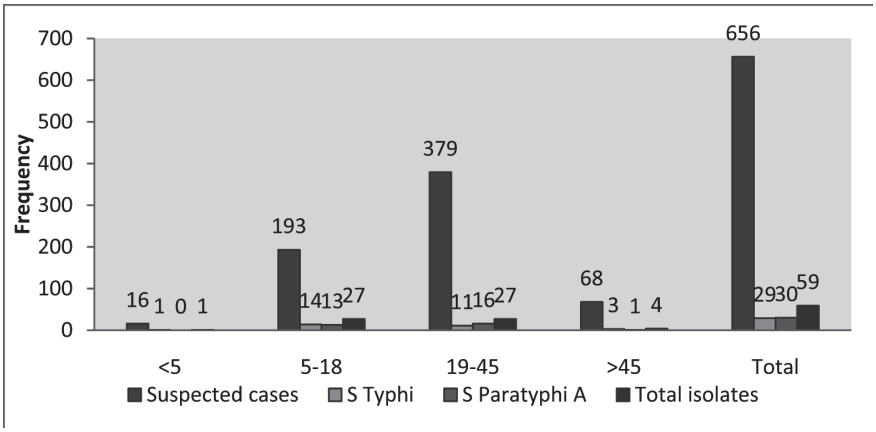

Fig. 1. Distribution of the suspected cases and enteric fever in different age group

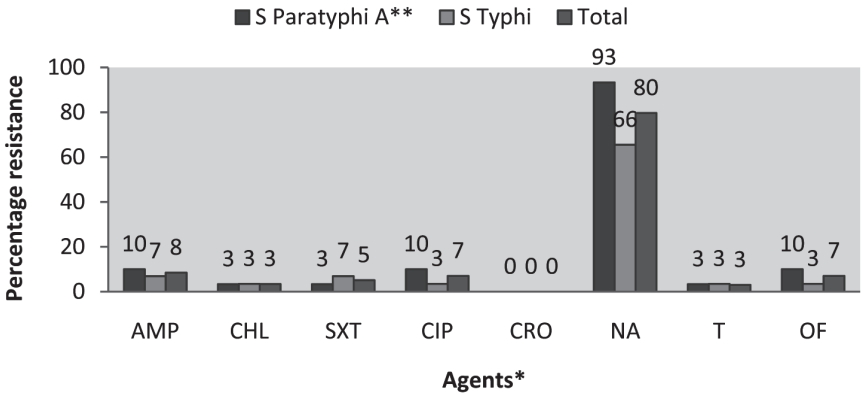

Fig. 2. Antibiotic resistance pattern in *S.* Typhi and *S.* Paratyphi A

*AMP, Ampicillin; CHL, Chloramphenicol; SXT, Cotrimoxazole; CIP, Ciprofloxacin; CRO, Ceftriaxone; NA, Nalidixic acid; T, Tetracycline; OF, Ofloxacin. **Intermediate resistance (10%) observed with both ciprofloxacin and ofloxacin exclusively in *S.* Paratyphi A has been illustrated as resistance in the figure.

Quinolone MIC in nalidixic acid resistant isolates: Quinolone MIC in nalidixic acid resistant *S.* Typhi and *S.* Paratyphi A with reduced susceptibility to these drugs is further analysed to observe the difference. The nalidixic acid resistant *S.* Paratyphi A strains required higher MICs to quinolones compared with that of *S.* Typhi (Table-2). The difference in mean quinolone MIC in nalidixic acid resistant *S.* Typhi and *S.* Paratyphi A was statistically significant (P<0.001).

In the present study, difference in both MIC and zone diameter in nalidixic acid sensitive and nalidixic acid resistant isolates was found to be stastistically significant (P<0.001) and decreased susceptibility to FQs was strongly correlated (sensitivity and specificity of 100%) with resistance to nalidixic acid (data not shown).

DISCUSSION

The proportion of *S.* Paratyphi A and *S.* Typhi among enteric fever cases we found was 50.85% and 49.15%, respectively. Based on the previous reports, an estimated one case of paratyphoid fever occurs for every four cases of typhoid fever.[2] In contrast to this estimate, here we report higher proportion of paratyphoid fever. The past trend in Nepal showed the gradual increase in the proportion of *S.* Paratyphi A isolates among enteric fever cases from 23% in 1993-1998 to 34% during 1999-2003.[21] A possible reason proposed for the higher incidence for paratyphoid is more likely due to the large inocula achieved through food borne transmission as waterborne transmission of *S.* Typhi usually involves small inocula.[4] Higher risk for transmission of typhoid fever occur within household while paratyphoid fever seemed to be transmitted more frequently outside the household[28,29] further supports food borne transmission of paratyphoid fever.

We found that more than half of the confirmed enteric fever cases prevailed during July to September. This may be possibly due to the sewage-mediated contamination of drinking water[30,31] during the rainy seasons contributing higher cases of typhoid fever. Among enteric fever cases, *S.* Typhi isolates were more common during July to October while *S.* Paratyphi A isolate were throughout the year. Although different factors may play role in seasonal distribution and transmission[4,28,29], being focused in the laboratory aspects, the possible involvement of different risk factors in the transmission of typhoid and paratyphoid fever in Nepalese context is difficult to elucidate.

In our study, overall nalidixic acid resistance rate was quite high (80%). *S.* Paratyphi A strains showed even higher (93%) resistance towards nalidixic acid than *S.* Typhi (66%). A hospital based study from Nepal reports rapid increase in nalidixic acid resistance among *S.* Paratyphi A compared to *S.* Typhi.[21] In another study carried out in Nepal in 2005, 73.3% and 94.9% of *S.* Typhi and *S.* Paratyphi A strains showed the resistance to nalidixic acid.[32] Furthermore, rising nalidixic acid resistant strains of *S.* Paratyphi A have also been reported in travelers returning from the endemic areas.[8] The emergence of these strains is worrying, as FQ treatment for strains with elevated MIC values have been associated with increased rates of clinical failure.[1,33,34] These observations may have important clinical significance, given that ciprofloxacin and ofloxacin are the most common drugs for enteric fever treatment, and irrational use without prescription **and misuse of antibiotics even for milder cases is common in Nepal**.[35]

Table-2: Quinolone MIC in nalidixic acid (NA) resistant S. Typhi and S. Paratyphi A

Agents	MIC (µg/mL)				P value
	NA Resistant S. Typhi		NA Resistant S. Paratyphi A		
	Mean ±SD	Range	Mean ±SD	Range	
Nalidixic acid	173.18±72.03	64-256	477.87±87.02	256-512	<0.001
Ciprofloxacin	0.25	0.25	0.62±0.3	0.25-1	<0.001
Ofloxacin	0.43±0.11	0.25-0.5	1.8±0.63	1-2	<0.001

In this report, the reduced FQs susceptibility was correlated to nalidixic acid resistance with significant difference (P<0.001) in the MIC values and inhibition zone diameters of FQs. Interestingly, the nalidixic acid resistant *S.* Paratyphi A required increased MICs of the FQs compared to *S.* Typhi (P<0.001). In *S.* Paratyphi A, the increase in isolates with reduced susceptibility to FQs was more striking compared to that of *S.* Typhi, with 84% of isolates in 2004 showing such resistance.[24] Recent report from India showed that 91.1% *S.* Typhi and 97.5% *S.* Paratyphi A showed resistance to nalidixic acid.[23] This may imply that development of full FQ resistance could be more aggressive in near future among *S.* Paratyphi A populations compared to *S.* Typhi.

In contrast to the nalidixic acid resistance, 86.44% of the isolates were susceptible to all ampicillin, chloramphenicol, cotrimoxazole and tetracycline (ACCoT) showing re-emergance of susceptibility to these antibiotics with marked reduction in MDR in *S. enterica* compared to previous reports from Nepal.[21,22,36] In Nepal, ciprofloxacin replaced chloramphenicol in 1994, but from 2000, increasing failures of treatment with this drug were reported.[37] Since 1994, the proportion of MDR *S.* Typhi has decreased and there has been a dramatic increase in nalidixic acid resistance.[21] Studies from most of the countries claim that the incidence of MDR isolates appears to have decreased with significant increase in the isolates with reduced susceptibility to FQs.[21,38-40] Over the last 10 years, there have been reports of return in susceptibility to first line drugs (ACCoT) in most of the regions.[38,41,42]

To conclude, *S.* Paratyphi A with elevated FQ MIC and alarming nalidixic acid resistance contributes increased proportion of enteric fever cases in our settings irrespective of the seasonal distribution. As isolates with reduced susceptibility to FQs may further develop full resistance, the use of these drugs for empirical therapy and management of enteric fever in areas where these strains are endemic is questionable and requires an urgent review. Moreover, low exposure to FQs reducing the selective pressure and use of conventional drugs when susceptible in treatment may definitely lessen the likelihood of selecting FQ resistant mutants. This problem of FQ resistance in enteric fever management should be well addressed while formulating treatment policy.

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