Brief Original Article

Re-emergence of susceptibility to conventional first line drugs in *Salmonella* isolates from enteric fever patients in Nepal

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Abstract

Introduction: Enteric fever is endemic in Nepal and poses a significant public health burden. The first-line drugs ampicillin, chloramphenicol, and cotrimoxazole have not been part of empirical therapy for two decades due to the development of multidrug-resistant *Salmonella* strains. The objective of this study was to determine the antibiogram pattern of *Salmonella* serovars isolated from the blood of clinically suspected enteric fever patients.

Methodology: A cross sectional study was carried out in a tertiary care hospital in Lalitpur, Nepal, between July 2011 and February 2012. Standard microbiological procedures were followed during collection and processing of blood samples, isolation and identification of *Salmonella* serotypes. The antimicrobial sensitivity of ampicillin, chloramphenicol, cotrimoxazole, nalidixic acid, and ciprofloxacin was determined using a modified Kirby-Bauer disk diffusion method as per the guidelines of the Clinical and Laboratory Standards Institute.

Results: Out of 86 *Salmonella* isolates, 56 (65.1%) were *Salmonella* Typhi and 30 (34.9%) were *Salmonella* Paratyphi A. *Salmonella* Typhi were 100% sensitive to chloramphenicol, cotrimoxazole, and ciprofloxacin and 98.2% sensitive to ampicillin. Similarly, *Salmonella* Paratyphi A isolates were 100% sensitive to ampicillin and cotrimoxazole and 96.7% sensitive to chloramphenicol and ciprofloxacin. More than 90.0% of isolates were nalidixic acid resistant and none of the *Salmonella* isolates were multi-drug resistant.

Conclusions: This study revealed the increasing frequency of nalidixic acid-resistant *Salmonella* isolates, indicating the possibility of fluoroquinolone resistance in near future. Furthermore, re-emergence of susceptibility to conventional first-line drugs ampicillin, chloramphenicol, and cotrimoxazole supports the possibility of using these drugs in empirical therapy.

Key words: enteric fever; *Salmonella*; susceptibility; re-emergence; first-line drugs; Nepal.

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Introduction

Enteric fever, an acute generalized infection affecting the reticuloendothelial system, intestinal lymphoid tissue, and the gallbladder, includes typhoid fever caused by *Salmonella enterica* serovar Typhi (*Salmonella* Typhi) and paratyphoid fever caused by *Salmonella* Paratyphi A or *Salmonella* Paratyphi B (or rarely, *Salmonella* Paratyphi C) [1]. The disease is a major public health problem in developing countries such as Nepal where water supply and sanitation are substandard [2].

Despite the use of antibiotics and the development of newer antimicrobial agents, enteric fever continues to be a major public health problem causing high morbidity and mortality in developing countries [3]. The first-line drugs ampicillin, chloramphenicol, and cotrimoxazole were used as a standard treatment regimen for enteric fever until the mid-1980s [4]. However, the indiscriminate use of these drugs and acquisition of plasmid-mediated resistance led to the development of typhoid resistant to ampicillin, chloramphenicol, and cotrimoxazole – multi-drug resistant (MDR) typhoid – in 1989 [5,6]. The first report of MDR *Salmonella enterica* serovar Typhi in Nepal was published in 1991 [7]. Due to the emergence of multi-drug resistant *Salmonella*, quinolones such as ofloxacin and ciprofloxacin have now become the drug of choice in the treatment of typhoid fever [8,9]. However, since the early 1990s, several reports of nalidixic acid-resistant salmonellae and decreased susceptibility to ciprofloxacin or ciprofloxacin resistance leading to treatment failure from Bangladesh, India, Nepal, Pakistan, Thailand, Tajikistan, United States, Vietnam, and other parts of the world have been documented [9-14]. Consequently, third-generation cephalosporins

(ceftriaxone, cefixime, and cefotaxime) and azithromycin are increasingly being used [15]. Third-generation cephalosporin-resistant *S*. Paratyphi A and *S.* Typhi strains have also been reported infrequently from South Asian developing countries such as Nepal, India, and Bangladesh [16,17].

In recent years, the re-emergence of susceptibility to ampicillin, chloramphenicol, and trimethoprim has been reported in India and Nepal [3,18,19,20]. In this realm where therapeutic options for treating enteric fever have been reduced, the re-emergence of susceptibility to ampicillin, chloramphenicol, and cotrimoxazole needs to be evaluated to determine the therapeutic importance of these drugs. The knowledge of the prevalence of *Salmonella* serovars and determination of their antimicrobial resistance pattern are very important for the guidance of clinical management of enteric fever [21]. This study was undertaken to determine antibiotic susceptibility patterns of the *Salmonella* serovars isolated from blood so that appropriate strategies could be adopted in the management of enteric fever.

Methodology

A cross-sectional study was carried out in clinically suspected enteric fever patients between July 2011 and February 2012 at Alka Hospital Jawalakhel, Lalitpur, Nepal. The cases defined by physicians as a probable cases of enteric fever with fever (38°C and above) for at least three days and showing clinical signs and symptoms of enteric fever were included in this study. Patients who had already started antibiotic therapy prior to samples being taken were excluded from the study.

About 5 mL of blood from adults and 2 mL of blood from children were collected either by nurses or laboratory technicians using standard aseptic techniques, transferred in culture bottles containing about 50 mL and 20 mL of BHI broth (HiMedia Laboratories, Mumbai, India) for adults and children, respectively, and incubated at 37°C for seven days. The samples showing signs of growth such as turbidity and gas formation on BHI broth at 24, 48, and 72 hours were subcultured in blood agar and MacConkey agar (HiMedia Laboratories, Mumbai, India) and incubated at 37°C for 24 hours. On day seven, all bottles were subcultured before being discarded as negative. Non-lactose-fermenting colonies from MacConkey agar were further processed and identified by biochemical reactions and confirmed by a serological agglutination test with group and type specific *Salmonella* antisera (Murex Biotech, Dartford, UK).

In vitro antimicrobial susceptibility testing of isolated *Salmonella* serovars was determined by the modified Kirby-Bauer disk diffusion method in Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) against antibiotic disks of ampicillin (10 µg), chloramphenicol (30 µg), cotrimoxazole (25 µg), nalidixic acid (30 µg), and ciprofloxacin (5µg) (HiMedia Laboratories, Mumbai, India) as specified in the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (2007). The results were interpreted as sensitive, intermediate, or resistant. Reference strain *E. coli* ATCC 25922 was used as quality control. SPSS version 17.0 was used as the statistical tool for data analysis.

Ethics statement

Ethical approval was obtained from the Ethical Review Committee, Alka Hospital, Jawalakhel, Lalitpur, Nepal, and informed consent was obtained from the patients or their legal representatives. The research was fully compliant with the Helsinki Declaration.

Results

Of the 1,273 febrile patients who visited the Alka hospital during the study period, 1,256 patients showed clinical signs and symptoms of enteric fever; of these, 1,202 patients were included in this study. Out of 1,202 blood samples cultured, only 86 (7.2%) showed positive culture results for *Salmonella* isolates, among which 56 (65.1%) were *Salmonella* Typhi and 30 (34.9%) were *Salmonella* Paratyphi A. *Salmonella* Typhi isolates were 100% sensitive to chloramphenicol, cotrimoxazole, and ciprofloxacin, and 98.2% sensitive to ampicillin. Similarly, *S.* Paratyphi A isolates were 100% sensitive to ampicillin and cotrimoxazole, and 96.7% sensitive to chloramphenicol and ciprofloxacin (Table 1). None of the *Salmonella* isolates were MDR. Nalidixic acid resistance was found among 51 (91.1%) *S*. Typhi isolates and 27 (90.0%) *S*. Paratyphi A isolates. All nalidixic acid-resistant *S*. Typhi isolates were sensitive to ciprofloxacin, but only one (3.7%) nalidixic acid-resistant *S.* Paratyphi A isolate was resistant to ciprofloxacin (Table 2).

Table 1. Antibiotic susceptibility pattern of *Salmonella* isolates

Antibiotics	Susceptibility of *Salmonella* Typhi		Susceptibility of *Salmonella* Paratyphi A	
	Sensitive N (%)	Resistant N (%)	Sensitive N (%)	Resistant N (%)
Ampicillin	55 (98.2)	1 (1.8)	30 (100)	-
Chloramphenicol	56 (100)	-	29 (96.7)	1 (3.3)
Cotrimoxazole	56 (100)	-	30 (100)	-
Nalidixic acid	5 (8.9)	51 (91.1)	3 (10)	27 (90)
Ciprofloxacin	56 (100)	-	29 (96.7)	1 (3.3)

Table 2. Ciprofloxacin susceptibility pattern of nalidixic acid-resistant *Salmonella* isolates

Nalidixic acid-resistant *Salmonella* isolates	Ciprofloxacin susceptibility		
	Sensitive N (%)	Resistant N (%)	Total N (%)
Salmonella Typhi	51 (100)	-	51 (91.1)
Salmonella Paratyphi A	26 (96.3)	1 (3.7)	27 (90.0)
Total	77 (98.7)	1 (3.7)	78 (90.7)

Discussion

Enteric fever is a disease of public health concern in developing countries such as Nepal and remains endemic in the capital city Kathmandu due to lack of clean drinking water, poor sanitary conditions, and cross-contamination of water supply with sewerage [22]. Because of wide variation in the sensitivity patterns of various *Salmonella* strains circulating in different geographic regions, it is imperative to assess the sensitivity of *Salmonella* serotypes to antibiotics before instituting empirical therapy [3]. We attempted to evaluate antibiotic susceptibility patterns in blood isolates of *Salmonella* serotypes from a tertiary care hospital in Kathmandu with a view to understanding current trends in antibiotic sensitivity patterns that would be helpful in institutionalizing proper treatment regimens.

In our study, more enteric fever cases were caused by *S.* Typhi than *S.* Paratyphi A. This is not in accordance with the results of other studies in different parts of Nepal, where *S.* Paratyphi A outnumbered *S.* Typhi among enteric fever cases (8.96% and 13.17% overall, respectively) [11,16,22]. Both *S.* Typhi and *S.* Paratyphi A isolates were found to be highly susceptible to the conventionally used drugs ampicillin, chloramphenicol, and cotrimoxazole. Our study revealed a re-emergence of susceptibility to these drugs in greater proportion than reported by other similar studies conducted in different parts of Nepal at different times [16,20,23], except for one study that also reported 100% susceptibility to chloramphenicol [18]. In a retrospective study conducted at a tertiary hospital in Kathmandu between 1993 and 2003, Maskey *et al.* reported a significant decrease in MDR strains of *S.* Typhi and *S.* Paratyphi A as a result of the re-emergence of susceptibility to the conventionally used drugs chloramphenicol, cotrimoxazole, and amoxicillin [24]. Similarly, a study conducted in eastern Nepal between 2000 and 2004 showed a decreasing trend of MDR *Salmonella* Typhi [25]. Consistent with our findings, high susceptibility to chloramphenicol, cotrimoxazole, and ampicillin were also reported from different parts of India such as Bangalore, Chennai, Chandigarh, central west, and northern India [3,4,26-29]. Re-emergence of chloramphenicol sensitivity was reported in northern India, with 96% sensitivity in *Salmonella enterica* serovar Typhi and 100% sensitivity in *Salmonella enterica* serovar Paratyphi A [19]. Our findings strongly contrast the reports published in Bangladesh, Cambodia, Kuwait, and the United Arab Emirates that reported MDR with reduced susceptibility to fluoroquinolones [30-32]. Interestingly, in our study, none of the *Salmonella* isolates were MDR, even in the era of antibiotic resistance. Furthermore, the higher frequency of nalidixic acid-resistant *Salmonella* isolates found in our study indicates the possibility of fluoroquinolone resistance occurring in near future as a consequence of the rampant use of fluoroquinolones.

Because conventional first-line drugs have been restricted for therapeutic use for almost two decades due to the development of MDR strains, fluoroquinolones such as ciprofloxacin and ofloxacin are currently used to treat MDR cases, whereas third-generation cephalosporins such as ceftriaxone, cefixime, and cefotaxime are used to treat cases with nalidixic acid resistance and decreased susceptibility to fluoroquinolones. The discontinuation of ampicillin,

chloramphenicol, and co-trimoxazole in clinical therapy for long periods of time may be the cause for the re-emergence of *S.* Typhi and *S.* Paratyphi A isolates sensitive to these drugs. In addition, the revival of sensitivity may be due to the loss of resistance plasmids to chloramphenicol and other first-line drugs such ampicillin and co-trimoxazole or may be due to the emergence of susceptible *de novo* strains [33].

The major limitations of the study were the small sample size and short duration of time. We collected the samples from a tertiary care centre, so we could have missed the cases that preferred to seek care in local health settings. We excluded patients who had already started antibiotic therapy prior to the samples being taken, which limited our sample size. The inclusion of patients from different geographic areas would have been helpful for more specific results. Furthermore, we did not calculate the minimum inhibitory concentration (MIC) value of the antibiotics that could support our findings to confirm the lower susceptibility among the local isolates. Due to the unavailability of equipment and resources in our setting, we could not correlate our study to molecular analysis.

It is obvious from our study that the high frequency of nalidixic acid-resistant *Salmonella* strains is coupled with the re-emergence of susceptibility to old drugs. These findings would be helpful to concerned health authorities to rationalize the policy of empirical therapy of enteric fever. Ciprofloxacin can no longer be considered the drug of choice for nalidixic acid-resistant *Salmonella* serovars infections. Furthermore, though the third-generation cephalosporin ceftriaxone is effective in clinical therapy, the cost and route of administration make ceftriaxone less appropriate for therapeutic use in developing countries such as Nepal. Thus, the use of ampicillin, chloramphenicol, and cotrimoxazole, which are economically affordable due to their low cost, in empirical therapy would be useful and cost effective in such countries.

Conclusions

This study revealed the increasing frequency of nalidixic acid-resistant *Salmonella* isolates and the re-emergence of susceptibility to conventional first-line drugs ampicillin, chloramphenicol, and cotrimoxazole. Since the study was confined to a single hospital, a multi-center surveillance study comprising a wide geographic area could be more promising. These findings might be useful to revise current empirical therapy policies for enteric fever caused by nalidixic acid-resistante *Salmonella* isolates to include these drugs in treatment regimens as an alternative to third-generation cephalosporins.

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Authors' contributions

HJC, the main researcher, designed and carried out all research works, analyzed data, and prepared the manuscript. KRR contributed substantially in the designing of research, analysis of the data, interpretation of data, and drafting of the manuscript. VKS and BJ supervised the laboratory work and helped in preparing the manuscript. BN helped in data analysis and drafting the manuscript. All authors read and approved the final manuscript.

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