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Antibiogram pattern of *Salmonella* in blood samples of enteric fever patients at Lalitpur, Nepal

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ABSTRACT

Objective: To determine the status of isolation blood stream serotypes of enteric fever pathogens and their antibiotic susceptibility patterns and to guide clinicians for appropriate therapy.
Methods: Samples were examined by microbiological techniques to identify the causative agent and determine their antimicrobial susceptibility patterns by Kirby-Bauer disk diffusion methods and interpreted as per Clinical and Laboratory Standards Institute guidelines.
Results: Among 403 blood samples, 76 (18.85%) showed growth for *Salmonella* isolates. Distribution of *Salmonella typhi* and *Salmonella paratyphi* A isolates were found to be 54% and 46% respectively. Among 76 *Salmonella* isolates, 28 (36.84%) were from male and remaining 48 (63.15%) were from female belonging to all age-groups. Multidrug-resistance was found to be 17% among the *Salmonella* isolates. Nalidixic acid resistance was 73.68% in *Salmonella* with higher proportion in *Salmonella paratyphi* A (85.7%) in comparison to *Salmonella typhi* (63.42%). *Salmonella* isolates demonstrated 100% susceptibility to azithromycin, ceftriazone, ciprofloxacin, ofloxacin and imipenem.
Conclusions: The need of continual surveillance of resistance levels to guide clinicians for appropriate therapy based on the antibiotic susceptibility pattern for *Salmonella* isolates is sustained with discouragement in misuse of antibiotics prior to prescription as multidrug-resistance-nalidixic acid resistant strains.

1. Introduction

Febrile disease with multiple important causative agents is common. A confirmative diagnosis ensures appropriate and effective treatment. Enteric fever is defined as a syndrome of systemic illness associated with *Salmonella* infection with an estimated 12–33 million cases and 600 000 deaths occurring worldwide each year[1,2]. Enteric fever is endemic in many developing countries including Nepal and other Southeast Asian countries, where 80% of the world's typhoid fever cases occur[3]. *Salmonella* species are

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important causes of febrile illness in crowded and impoverished populations with poor drinking water system, and unhygienic condition. Ingestion of food and water contaminated with faeces or urine increases the risk of being infected with *Salmonella* not only to the inhabitants, but also to the travelers visiting endemic region of the country[4]. *Salmonella typhi* (*S. typhi*) is the most common etiological agent, while *Salmonella paratyphi* A (*S. paratyphi* A) is responsible for only a minority of enteric fever cases and is traditionally associated with asymptomatic and/or mild clinical illnesses[5]. Since all the signs and symptoms of typhoid fever are nonspecific, a definitive diagnosis of the disease depending on the clinical presentation alone is very difficult. An accurate diagnosis of enteric fever is important in clinical settings where antimicrobial therapy prevent serious complications and deaths and reduces inappropriate antimicrobial usage[6].

Blood culture is a definitive diagnostic method for isolation of *S. typhi* and *S. paratyphi* which has quite variable yield result[7]. With standard broth culture, *Salmonellae* have been found in the blood of 30%–90% of patients with clinically suspected cases of enteric fever. The proportion of typhoid fever patients with positive blood

cultures decreases with increasing duration of illness[7]. The volume of blood taken and the laboratory methods used for isolation are also important factors determining the yields from blood culture.

In Nepal, *S. typhi* and *S. paratyphi* A are common causative organisms for typhoid and paratyphoid fevers respectively, whereas serotype paratyphi B is rare[8]. However, studies propose that sustained higher incidences of *S. paratyphi* A cases have been observed in recent years[9,10]. The disease may occur in all ages, with the highest incidence found particularly in children[11,12]. *S. paratyphi* A is the most commonly identified cause of febrile illness among adults in urban[13]. For the treatment of enteric fever, traditional drugs such as chloramphenicol, ampicillin and cotrimoxazole were most effectively used as first-line drugs[12,13]. However, during the late 1980s and early 1990s the occurrence of multidrug-resistant (MDR) *S. typhi* and *S. paratyphi* A strains resistant to chloramphenicol, ampicillin and cotrimoxazole led to the use of fluoroquinolones, in particularly ciprofloxacin, and third generation cephalosporin for the treatment of enteric fever[14]. The wide spread use of fluoroquinolones led to increased occurrence of *Salmonella enterica* (*S. enterica*) strains having reduced susceptibility to fluoroquinolones that were being reported more frequently, particularly in Europe, Asia and Africa[15]. Isolates resistant to nalidixic acid are less responsive to fluoroquinolones antibiotics. Nalidixic acid susceptible *Salmonella* isolates with reduced susceptibility to fluoroquinolones are also increasingly being recognized. Thus, the use of nalidixic acid disc diffusion test during the antibiotic sensitivity test has been recommended by Clinical And Laboratory Standards Institute to screen reduced susceptibility to fluoroquinolones[16]. Besides, occurrence of nalidixic acid resistant (NAR) strain, the increase in use of cephalosporin and azithromycin for the treatment of typhoid and paratyphoid fever also resulted in increasing rates of cephalosporin and azithromycin resistance among *S. enterica*[17]. Susceptibility to chloramphenicol and ampicillin in South Asia is soaring[18].

In our study, we are determining and monitoring the altering antimicrobial resistance pattern with MDR *Salmonella* serotypes, re-outgrowth of *Salmonellae* susceptible to first line antibiotics (ampicillin, chloramphenicol and cotrimoxazole), increasing resistant serotypes to fluoroquinolones and the third-generation cephalosporin. Currently used antibiotics for the treatment of *Salmonella* infection are in urgency for their determination of resistance and re-evaluation. New approaches to the treatment and the control strategies in *Salmonella* infections are emerging issues. Thus, our study can be the part of continual surveillance of resistance levels for clinicians to guide appropriate therapy based on the antibiotic susceptibility patterns of *S. typhi* and *S. paratyphi*.

2. Materials and methods

2.1. Sample collection

Blood samples were collected aseptically from 403 patients from the Outpatient and Inpatient Department of Medicine in Kist Medical College, Teaching Hospital in Kathmandu, Nepal, from September 2014 to January 2015. The patients were categorized in four age groups 0–28 days, 29 days to 20 years, 21–40 years and > 41 years. Study was conducted on patients who were referred for blood culture in the laboratory.

This study was approved by Institutional Review Committee of KIST Medical Teaching Hospital. Official letter was submitted to the Kist Medical College Imadole, Lalitpur. Informed verbal agreement was taken from Patients. The intent and the importance of the study were explained and approval was received from the Head of Department of Medical Microbiology at Nobel College, Pokhara University.

2.2. Isolation of bacterial colonies

The aseptically collected blood samples were inoculated on culture bottle with Brain-heart infusion broth with sodium polyanethol sulfonate and were incubated at 37 °C. Broth cultures exhibiting turbidity were then inoculated on blood agar plate and MacConkey agar plate, at 37 °C. The isolates were identified by following standard microbiological procedure which includes colony morphology characters, Gram stain reactions and biochemical reactions such as oxidase test, triple sugar iron test, urease test, citrate test and Indole test. If growth is not observed, the blind subcultures in MacConkey agar and blood agar were done after the 5 days of incubation before discarding. In positive cases, colonies characters were noted down. The isolates of *Salmonella* were further confirmed by slide agglutination with specific antisera O-antigens and H-antigens.

2.3. Sensitivity test

Susceptibility and resistance of all isolates were checked by performing the Kirby-Bauer disc diffusion method as per the guidelines of Clinical and Laboratory Institute; formerly National Committee for Clinical Laboratory Standards[19,20]. Following 24-h incubation of the antibiotic incorporated plates at 37 °C, zone of inhibition around the antibiotic disc was measured. The antibiotics used were amikacin, ampicillin, azetonam, azithromycin, cefipime, cefixime,cefotaxime, chloramphenicol, ciprofloxacin, cotrimoxazole, gentamycin, imipenem, nalidixic acid, ofloxacin, tetracycline, which were tested *in-vitro* (Clinical And Laboratory Standards Institute, 2013). The isolates were considered as MDR if they were resistant to at least two classes of the antibiotics.

2.4. Data collection and analysis

Data were first recorded in notebook and then entered computer based MS-Excel version 2013 and appropriate statistical analysis was done using SPSS version 20.0.0.

3. Results

Among 403 blood culture, 327 (81%) showed no growth for *Salmonella* while 76 (19%) were culture positive for *Salmonella* isolates. Distribution of *S. typhi* and *S. paratyphi* A isolates were found to be 41 (54%) and 35 (46%) respectively. Among 76 *Salmonella* isolates, 28 (36.84%) were from male and remaining 48 (63.15%) were from female. Out of 28 *Salmonella* isolates in male, the presence of 11 *S. typhi* and 17 *S. paratyphi* A were observed. Likewise, out of 48 *Salmonella* isolates in female, 26 in *S. typhi* and 22 in *S. paratyphi* A were present (Figure 1). Based on age group, the highest *Salmonella* isolates were in adult (21–40) age group followed by 28 days to 20 years and more than 41 years. Neonatal

salmonellosis was 6.66% caused by *S. paratyphi*. All the 35 isolates of *S. typhi* were tested for antimicrobial susceptibility patterns using 13 antibiotics. All the isolates were fully sensitive (100%) to azithromycin, cefixime, cefotaxime, ceftriazone, ciprofloxacin, gentamicin, imipenem, and ofloxacin. Antibiotic susceptibility pattern of *S. typhi* showed that it was 75% sensitive to ampicillin, chloramphenicol and cotrimoxazole severally, followed by 37.5% sensitive and 25% intermediate to cefipime. *S. typhi* showed 62.5% resistance to nalidixic acid (Figure 2). All the 41 isolates of *S. paratyphi* A were tested for antimicrobial susceptibility patterns using 13 antibiotics. All the isolates were fully sensitive (100%) to azithromycin, ampicillin, cefixime, cefotaxime, ceftriazone, chloramphenicol, ciprofloxacin, cotrimoxazole, gentamicin, imipenem, and ofloxacin (Figure 3). *S. paratyphi* A showed 57.1% sensitivity and 14.3% intermediate to cefipime. *S. paratyphi* A was 85.7% resistance to nalidixic acid.
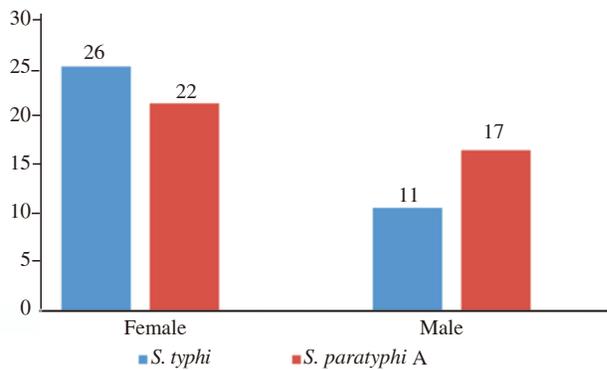

Figure 1. Sex wise Distribution of *Salmonella* Isolates
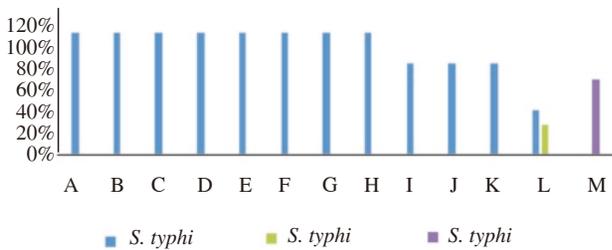

Figure 2. Antibiotic susceptibility pattern of *S. typhi*.
A: Azithromycin; B: Cefixime; C: Cefotaxime; D: Ceftriazone; E: Ciprofloxacin; F: Gentamicin; G: Imipenem; H: Ofloxacin; I: Ampicillin; J: Chloramphenicol; K: Cotrimoxazole; L: Cefipime; M: Nalidixic acid.

Figure 3. Antibiotic susceptibility pattern of *S. paratyphi* A.
A: Azithromycin; B: Cefixime; C: Cefotaxime; D: Ceftriazone; E: Ciprofloxacin; F: Gentamicin; G: Imipenem; H: Ofloxacin; I: Ampicillin; J: Chloramphenicol; K: Cotrimoxazole; L: Cefipime; M: Nalidixic acid.
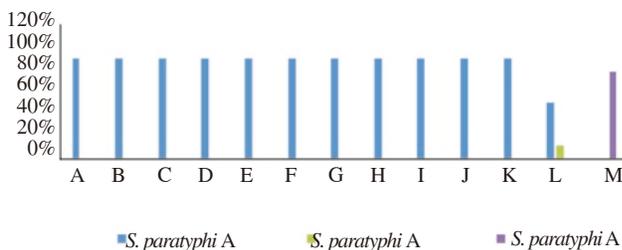
Among the total cases, 13 (17.10%) isolates of *Salmonella* were MDR and 63 (83%) were non-MDR. Both MDR-isolates were resistant to ampicillin, chloramphenicol, cotrimoxazole, nalidixic acid (MDR-NAR) and cefepime. Among 76 isolates, 56 NAR strain with 26 in *S. typhi* and 30 *S. paratyphi* A, while 20 isolates belonged to non-NAR group (Figure 4).
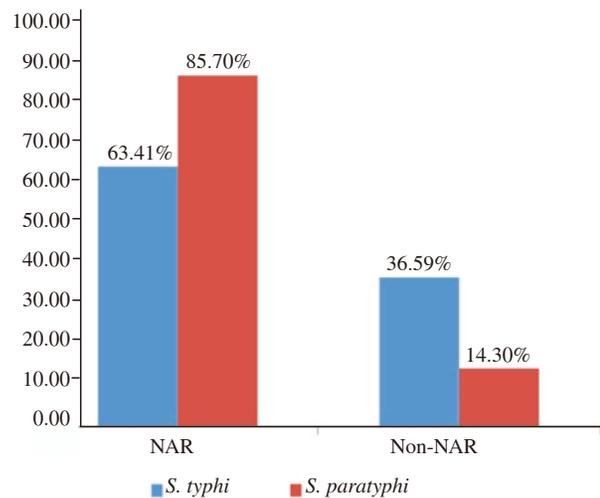

Figure 4. Proportion of NAR *S. typhi* and *S. paratyphi* A.

4. Discussion

Enteric fever cases have occurred throughout the study period which implied that safety of drinking water hygienic practice and sanitation have not improved much over that period. In our study period, only 18.85% showed growth which is similar to the results done by Masoumi *et al*. and Sultana *et al*.[21,22]. There is direct relationship between the volume of blood obtained and the yield of a blood culture (University of Pennsylvania Medical Center Guidelines for Antibiotic Use, 2010). Lack of growth in blood culture during isolation of pathogen is common, most probably, due to the use of antibiotics prior to blood collection for culture. Availability and misuse of antibiotics even for mild cases of fever is common in Nepal[22]. A relation between antibiotic use and its resistance in clinical isolates had been proven in more studies. However, a straight quantitative relationship between the amount of antibiotic used and the frequency of resistance is still lacking[21,22]. This study showed the higher prevalence of enteric fever in female. On the blood culture, the growth was 46.0% and 53.94% for male and female respectively. Similar results were found by Adabara *et al*.[20], Masoumi *et al*.[21], Sultana *et al*.[22]. We isolated single *S. paratyphi* A as a neonatal septicemia. Sepsis due to *S. enterica* serovar typhi and paratyphi A is rare in neonates and described as a mild and unrecognized sickness in infants, life-threatening hindrance and even deaths have been reported[22,23]. *Salmonella* infections should be considered in the differential diagnosis of neonatal septicemia, especially in endemic areas. All the 76 isolates were tested for antimicrobial susceptibility patterns using 13 antibiotics. All the isolates were fully sensitive (100%) to azithromycin, cefixime, cefotaxime, ciprofloxacin, gentamicin, imipenem, and ofloxacin.

About 75% *Salmonella* isolates were sensitive to ampicillin, chloramphenicol and cotrimoxazole. In the study of Emary *et al*.[23], the proportion of *S. typhi* isolates showed high susceptibility to first line antibiotics ampicillin, chloramphenicol and cotrimoxazole and suggested that reconsidering the addition of these antimicrobial drugs into the treatment regime of enteric fever.

High resistance to cefepime was encountered in this study. Only 37.30% were sensitive to cefepime in *S. typhi* and 57.10% in *S. paratyphi* A. As a general principle of antimicrobial intervention, intermediate susceptibility should be regarded as equivalent to resistance Lynch *et al*.[24]. So, we included intermediate cases of

cefipime, as resistant. In this study 17.10% of isolates were MDR. There has been increasing concern about the outgrowth of MDR *S. typhi* and *S. paratyphi* A in Nepal[23,25,26]. The emergence of these strains is worrying due to the malpractice of use of antibiotics from pharmacies without prescription by physician in Nepal. But overall there is a general trend of decline in MDR strains. The reasons for this could be due to discontinuation of traditional drugs in the treatment of typhoid fever, thus relieving the selection pressure. We have found only MDR *S. typhi* but not in *S. paratyphi* A and our results coincided with Acharya et al.[24] and Gaind *et al.*[26]. In this study, all the MDR isolates showed resistance to ampicillin, chloramphenicol, cotrimoxazoleand nalidixic acid (MDR-NAR). This finding is similar to the Acharya *et al.*[25]. Further in present study all MDR were resistant to cefipime. The combination of MDR and NAR (MDR-NAR) is a particular problem, because it severely restricts the therapeutic options for patients with typhoid fever. The increased prevalence of MDR-NAR strains of *S. typhi* and *S. paratyphi* A has posed a therapeutic challenge.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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