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**ANTIBIOTIC SUSCEPTIBILITY PATTERN OF NALIDIXIC ACID
RESISTANT *SALMONELLA* ISOLATES IN NEPAL**

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ABSTRACT

Typhoid fever is a major health problem, due to lack of culture facilities and other definitive diagnosis methods at most of district hospital in Nepal. This study was aimed to know the prevalence of Nalidixic acid resistant *Salmonella* isolates and their antibiotic susceptibility pattern. A total of 4619 febrile patients suspecting the cases of typhoid fever by clinician, attending at Shree Birendra hospital during May- November 2013 were subjected to culture. Blood sample (5ml) was collected from the suspected cases and inoculated immediately into 45ml of Brain heart infusion broth (BHI) and further processed for the identification of *S. Typhi* and *S. Paratyphi*. Antimicrobial susceptibility pattern of *S. Typhi* and *S. Paratyphi* isolates were determined by the modified Kirby-Bauer disc diffusion method. Out of 4619 blood sample, 8.7% (n= 403) sample were culture positive. Among culture positive, 66.3% (n=267) cases were *S. Typhi*, 26.1% (n=105) cases were *S. Paratyphi* and 7.7% (n=31) were other than *Salmonella* isolates respectively. Out of 372 *Salmonella* isolates, most of the *S. Typhi* isolates i.e. 95.51% (n=255) and *S. Paratyphi* isolates i.e. 97.14% (n=102) are highly resistant to nalidixic acid. Most of these isolates were also found resistant to ciprofloxacin and ofloxacin. Therefore, screening of nalidixic acid susceptibility might be done prior to prescribe the drug for the treatment of enteric fever.

Key Words: Blood culture, Nalidixic acid, *Salmonella*, enteric fever.

INTRODUCTION

The term enteric fever consists of both typhoid and paratyphoid fevers [1]. Typhoid and paratyphoid fever remain important public health problems globally and major causes of morbidity in the developing world including Nepal [2,3]. Enteric fever caused by *S. Typhi* and *S. Paratyphi A* is the most common clinical diagnosis among febrile patients presenting to hospital in Nepal [3]. Although a wide range of *Salmonella* serotypes may cause human disease, broadly grouped into several typhoidal species that are specific human pathogens and includes serotypes *S. Typhi* and *S. Paratyphi*, and other serotypes that are primarily spread to humans from animal

sources are non-typhoidal [4]. However, non-typhoidal *Salmonella* can also cause a variety of life-threatening extra-intestinal infections. Typhoid is unique to human, characterized by malaise, fever, abdominal discomfort, transient rash, splenomegaly, hepatomegaly, bradycardia, and leucopenia, the most prominent major complications are intestinal hemorrhage, and perforation. The real impact of typhoid fever is difficult to estimate because the clinical picture is confused with other febrile infections [5]. Therefore this study was aimed to know the prevalence of Nalidixic acid resistant *Salmonella* isolates and their antibiotic susceptibility pattern.

MATERIALS AND METHODOLOGY

This study was conducted at Shree Birendra Hospital, Chauni, Kathmandu during May to November, 2013. A total of 4619 blood samples were collected from

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the patients suspected of enteric fever. The blood samples were collected by veni-puncture under aseptic condition and then collected sample was transferred in BHI broth (3ml or 5 ml blood in 45 ml of brain heart infusion broth). It was then subjected to culture for *Salmonella* at 37°C and sub-cultured on Mac Conkey agar (MA) after every 24 hours of incubation. On the next day, tiny non-lactose fermenting colonies on MA was then processed for identification according to standard microbiological methods (microscopic examination, biochemical tests). The isolates were then subjected to antimicrobial susceptibility testing by Modified Kirby-Bauer disk diffusion method following clinical and laboratory standard institute (CLSI) guideline on Muller- Hinton agar plates [6,7]. The antibiotics used are: nalidixic acid, amoxycilin(10µg), ceftriaxone (30µg), cephotaxime (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), co-trimoxazole (25µg), and ofloxacin (5µg) (Hi Media., Mumbai, India) [7].

RESULT

Out of 4619 blood specimens cultured, only 403

(8.7%) samples had shown bacterial growth. Out of 403, 372 (92.31%) were identified as *Salmonella* isolates. Among the *Salmonella* isolates, 267(66.2%) were *Salmonella* Typhi and 105(26.1%) were *Salmonella* Paratyphi A.(Table-1).

Out of total *Salmonella* Typhi isolated, nalidixic acid 255 (95.5%), ciprofloxacin 257 (96.3%), and ofloxacin 257 (96.3%) were found to be resistant to respective drugs where as commonly used drug chloramphenicol 265 (99.3%) still found to be effective. (Table-2).

Out of 105 *Salmonella* Paratyphi A isolates, cotrimoxazole 104 (99%), ceftriaxone 104 (99%), and chloramphenicol 104 (99%) were found to be sensitive whereas nalidixic acid 102(97.1), ciprofloxacin 102 (97.1%) and ofloxacin 102 (97.1%) were found to be highly resistant.(Table-3).

Out of the total *Salmonella* isolates, 357(95.97 %) were NARS isolates which included both *S. Typhi* 255 isolates and *S. Paratyphi A* 102 isolates respectively. Out of total *S. Typhi* isolates 95.51% and *S. Paratyphi A*, 97.14% were NARS. (Table-4).

Table 1. Month wise distribution of *Salmonella* isolates

Month	S.Typhi		S. Paratyphi A		Total <i>Salmonella</i> isolates	
	Number	%	Number	%	Number	%
May	22	8.2	13	12.4	35	9.4
June	98	36.7	23	21.9	121	32.5
July	52	19.5	22	21.0	74	19.9
August	54	20.2	12	11.4	66	17.7
September	20	7.5	22	21.0	42	11.3
November	21	7.9	13	12.3	34	9.2
Total	267	100	105	100	372	100

Table 2. Antibiotic susceptibility pattern of *S. Typhi*

Antibiotics	Sensitive		Resistance		Total
	Number	%	Number	%	
Nalidixic Acid	12	4.5	255	95.5	267
Amoxycilin	261	97.8	6	2.2	
Cotrimoxazole	264	98.9	3	1.1	
Ceftriaxone	261	97.8	6	2.2	
Cephotaxime	263	98.5	4	1.5	
Chloramphenicol	265	99.3	2	0.7	
Azithromycin	263	98.5	4	1.5	
Ciprofloxacin	10	3.7	257	96.3	
Ofloxacin	10	3.7	257	96.3	

Table 3. Antibiotic susceptibility pattern of *S. Paratyphi A*.

Antibiotics	Sensitive		Resistance		Total
	Number	%	Number	%	
Nalidixic Acid	3	2.9	102	97.1	
Amoxycilin	97	92.4	8	7.6	
Cotrimoxazole	104	99	1	1	
Ceftriaxone	104	99	1	1	
Cephotaxime	102	97.1	3	2.9	

Chloramphenicol	104	99	1	1	105
Azithromycin	97	92.4	8	7.6	
Ciprofloxacin	3	2.9	102	97.1	
Ofloxacin	3	2.9	102	97.1	

Table 4. Nalidixic acid susceptibility pattern *Salmonella* isolates.

Bacterial isolates	Antibiotic Susceptibility Pattern of Nalidixic acid						Total
	Resistance			Sensitive			
	Male	Female	Total	Male	Female	Total	
<i>S. Typhi</i>	191	64	255 (95.51%)	8	4	12(4.49%)	267
<i>S. Paratyphi</i>	78	24	102 (97.14%)	3	0	3(2.86%)	105
Total	269	88	357(95.97%)	11	4	15(4.03%)	372

DISCUSSION

Enteric fever is a disease of concern in developing countries like Nepal and remains endemic in the capital city Kathmandu due to lack of supply of clean drinking water, poor sanitation, and cross-contamination of water supply with sewerage [8]. Various researchers reported wide variation in the sensitivity patterns of various *Salmonella* strains circulating in different geographic regions of Nepal, so it is essential to assess the sensitivity of *Salmonella* serotypes to antibiotics before instituting empirical therapy [9]. We attempted to evaluate antibiotic susceptibility patterns in blood isolates of *Salmonella* serotypes from Shree Birendra Army hospital in Kathmandu with a view to understanding current trends in antibiotic sensitivity patterns.

In our study, out of 4,619 specimens processed for culture, only 403 (8.7%) isolates had shown growth, i.e. 267(66.2%) were *Salmonella* Typhi and 105(26.1%) were *Salmonella* Paratyphi. This is not in similar with the results of other studies in different parts of Nepal, where *S. Paratyphi* A had reported main causative organism for enteric fever. *S. Typhi* (8.96%) and *S. Paratyphi* A (13.17%) [10,11]. In our study, most of the febrile cases and diagnosed enteric fever cases were from month between June-July (121), July-August (74), and August-September (66). Similar results also have shown by Malla *et al.* (2005) [12] and Acharya *et al.*, (2012) [3] i.e. the peak occurrence of enteric fever in summer and rainy season. The reason behind such result may be at this time; temperature and rainfall are relatively high and higher chance of mixing sewage to water supply pipelines due to unmanaged water supply system in Kathmandu Valley. In our study, most of *S. Typhi* isolates were highly sensitive with amoxicillin, co-trimoxazole, ceftriaxone, cephotaxime, chloramphenicol and azithromycin but they are highly resistant to nalidixic acid, ciprofloxacin and ofloxacin. Amoxicillin, Chloramphenicol and Co-trimoxazole (ACCo) were found to be effective having efficacy rate of 97.8%, 98.9% and 99.3% respectively. Similarly, most of *Salmonella* Paratyphi A isolates were highly sensitive with Amoxicillin, Co-trimoxazole, Ceftriaxone, Cephotaxime, Chloramphenicol and

Azithromycin. Our study revealed a re-emergence of susceptibility to amoxicillin, chloramphenicol and co-trimoxazole in greater proportion than reported by other similar studies conducted in different parts of Nepal at different times [11,13,14]. In this study, the nalidixic acid resistance in *S. Typhi* was found to be 95.5% and in *S. Paratyphi* A was found to be 97.1% which is in agreement with the findings of Prajapati *et al.*, (2009) [15]. Furthermore, isolation of 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 haphazard use of fluoroquinolones without antibiotic susceptibility test.

The major limitations of our study were limited sample size and short duration of time. Furthermore, the samples were collected from a tertiary care centre, so cases that preferred to seek health care in local settings were missed. The inclusion of patients from different geographic areas would have been helpful for more specific results. Furthermore, minimum inhibitory concentration (MIC) value of the antibiotics was not calculated. Molecular identification and characterization of isolates were not performed due to the unavailability of equipment and resources in our setting.

CONCLUSION

The higher sensitivity of third generation cephalosporins (ceftriaxone and cephotaxime) and macrolide (azithromycin) indicates that these drugs along with chloramphenicol and cotrimoxazole may still be considered as better options for the treatment of enteric fever. Hence cephalosporins remain the alternative drugs against infections with ciprofloxacin resistant *Salmonella* isolates. Therefore, the use of cephalosporins in the empirical therapy, misuse and over use should be discouraged. Resistance to nalidixic acid as a screening test for detecting reduced susceptibility to the fluoroquinolones helps in early diagnosis and substitution of appropriate antibiotic therapy which is very important in the management of enteric fever.

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