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Higher Nalidixic acid resistance pattern of *Salmonella* isolates from enteric fever patients in Kathmandu Model Hospital, Nepal

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ABSTRACT

In Nepal, enteric fever remains endemic due to consumption of unsafe drinking water, poor sanitation practice, lack of proper diagnostic facilities and treatment failure. The *Salmonella* isolates, resistant to mostly used drugs and, also, emergence of multidrug resistance patterns are public health threat. This study carried out at Kathmandu Model Hospital (KMH), Kathmandu, Nepal to find antibiotic susceptibility patterns of *Salmonella* isolates from suspected enteric fever patient. A total 1549 blood samples were subjected for culture following standard microbiological procedure and serotyping for isolation, identification and characterization. Antibiotic susceptibility patterns were tested by using the modified Kirby Bauer disc diffusion methods with nine commonly used drugs. Culture positive rate was found to be 6.6% (102/1549) [age group 15-30 (9.1%) and <15 years (6.8%)] with no significant differences between male and female (p>0.05). Isolates were characterized as *S.* Typhi 54.0% (55/102) and *S.* Paratyphi A 46.0% (47/102). Ceftriaxone, Cephotaxime and Ofloxacin were found to be most effective drugs (efficacy rate 100%) against the *Salmonella* isolates. No isolates were resistant towards mostly used drug Ceprofloxacin, however, 44(93.6%) of *S.* Paratyphi A and 34(61.8%) of *S.* Typhi isolates were resistant to Nalidixic acid indicating the reduced susceptibility of quinolone drugs. One multidrug resistant isolate (*S.* Typhi) was resistant towards Amoxycillin, Chloramphenicol, Cotrimoxazole and Nalidixic acid. Third line Cephalosporins found effective and Flouroquinolones showed reduced efficacy rate.
Keywords: Enteric fever, *Salmonella*, Multidrug resistant, Nepal

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INTRODUCTION

Enteric fever caused by *Salmonella enterica* serotype Typhi and Paratyphi A, B and C causes 21.7 million cases with 217,000 deaths annually worldwide [1,2]. Among them, recently *S*. Paratyphi infection is on the increase however actual burden of enteric fever is poorly quantified due to limited diagnostic approaches [1,3]. Enteric fever remains a threat for developing countries like Nepal where contamination of foods and drinking water is common [1,4-7]. Most of patients are being diagnosed with clinical signs which are also observed in other infectious diseases leading the diagnostic dilemma [8]. The use of self prescribed drugs, unauthorized dispensing by drug retailers without much knowledge of disease is causing the unsuccessful treatment and isolation of multidrug resistant strains [9-11].

Flouroquinolones are mostly recommended drugs for treatment and also the third line Cephalosporins and Azithromycin for quinolone resistant cases [12, 13]. But the decreased sensitivity and increased MIC value of Fluroquinolones is a rising problem in recent years [3, 14-19]. The resistance towards third line Cephalosporins and Azithromycin are also reported [1, 13, 20]. For control strategies and appropriate therapy, continual surveillance of resistance level and antimicrobial susceptibility testing is very important. In this paper we attempted to show the prevalence of enteric fever and antibiotic sensitivity patterns of *Salmonella* isolates reported at Kathmandu Model Hospital.

MATERIALS AND METHODS

This study was carried out during April 2009 to September 2009 at *Kathmandu Model Hospital* located in centre of Kathmandu Valley. Aseptically drawn venous blood from 1549 suspected enteric fever patients visiting OPD section were cultured for *Salmonella* (5 ml blood in 45 ml Brain heart infusion media) and incubated at 37ºc till 120 hours. BHI incubates were sub-cultured on blood agar, Mac-Conkey agar and *Salmonella-Shigella* agar in every 24 hours interval till 120 hours. No bacterial growth even after 120 hours was considered as negative culture. Standard bacteriological procedures including serotyping (using antisera from Denka Seiken Co.Ltd, Tokyo, Japan) were employed for identification.

Antibiotic susceptibility test of isolates was done using modified Kirby–Bauer disc diffusion Method. Commonly prescribed drugs in Nepal namely Amoxycillin (30 mcg), Chloramphenicol (30 mcg), Cotrimoxazole (1.25/23.75 mcg), Cephotaxime (30 mcg), Cefixime (5mcg), Nalidixic acid (30 mcg), Ciprofloxacin (5 mcg), Ofloxacin (5 mcg) and Ceftriaxone (30 mcg) were employed. The result of antibiotic susceptibility testing was interpreted as per the criteria given by the antibiotic disc manufacturer. The statistical analysis of data was done using SPSS version 11.5 software.

RESULT

In this study, only 102 (6.6%) cases were culture positive out of 1549 blood samples processed for *Salmonella* species. The highest growth positive rate was found in age group 15-

30 (9.1%) followed by age group <15 years (6.8%) which was statistically significant (p<0.05) (Table-1). There was no significant difference in culture positivity between male (6.4%) and female (6.8%) (p>0.05). Of total 102 isolates, 55 (54%) isolates were *S.* Typhi and 47 (46%) were *S.* Paratyphi A.

All *Salmonella* isolates were sensitive to Ceftriaxone, Cephotaxime ,Ciprofloxacin and Ofloxacin (100%). All isolates were sensitive to Chloramphenicol and Cotrimoxazole except one *S.* Typhi isolate being resistant to both drugs. *S.* Typhi has sensitivity 96.4% for both Cefixime and Amoxycillin whereas *S.* Paratyphi A isolates were found sensitive for Amoxycillin (93.6%) and Cefixime (91.5%) respectively. For Nalidixic acid , 34(61.8%) *S.* Typhi isolates and 44(93.6%) *S.* Paratyphi A isolates were found resistant. One of the isolates of *S.* Typhi was found to be multidrug resistant towards Amoxycillin, Cotrimoxazole, Chloramphenicol and Nalidixic acid. (Table-2) (Table-3)

Table -1: Growth positive rate in different age groups

Age group	Cases	Positive	%	
<15	147	10	6.8	
15-30	868	79	9.1	P<0.001
>30	487	13	2.7	

Table -2: Drug sensitivity of *Salmonella* Typhi isolates (n=55)

Antibiotic used	Sensitive	Intermediate	Resistant
Ceftriaxone	55(100%)	0	0
Cephotaxime	55(100%)	0	0
Ofloxacin	55(100%)	0	0
Chloramphenicol	54(98.2%)	0	1(1.8%)
Cotrimoxazole	53(96.4%)	1(1.8%)	1(1.8%)
Cefixime	52(94.5%)	1(1.8%)	2(3.6%)
Amoxycillin	52(94.5%)	1(1.8%)	2(3.6%)
Ciprofloxaxin	46(83.6%)	9(16.4%)	0
Nalidixic acid	21(38.2%)	0	34(61.8%)

Table -3: Drug sensitivity of *Salmonella* Paratyphi A isolates (n=47)

Antibiotic used	Sensitive	Intermediate	Resistant
Cephotaxime	47(100%)	0	0
Ceftriaxone	47(100%)	0	0
Ofloxacin	47(100%)	0	0
Cotrimoxazole	47(100%)	0	0
Chloramphenicol	47(100%)	0	0
Amoxycillin	44(93.6%)	1(2.1%)	2(4.2%)
Cefixime	43(91.5%)	2(4.2%)	2(4.2%)
Ciprofloxaxin	38(80.8%)	9(19.1%)	0
Nalidixic acid	3(6.4%)	0	44(93.6%)

DISCUSSION

Outbreak of enteric fever remains endemic in developing countries, which is associated with poor sanitation, lack of safe drinking water and proper treatment. In Nepal, there are possibilities of enteric fever outbreak at any time due to poor environmental sanitation resulting to contamination of water and foods [1, 3, 4, 6, 7,11, 21-23].

The overall growth positive rate for *Salmonella* spp in our study is found relatively less (6.6%) than reported by many previous researches [3, 21] which shows the declining prevalence of enteric fever though the growth positive rate in developing countries like Nepal is higher than the developed countries [13] and it might be associated with improvement in sanitary condition or by use of self prescribed drugs before consulting the physician. Some reports had the similar overall growth positive rate with our report [11, 23]. Unlike many researches, showing high culture positivity in children below age of 15 and higher complication and hospitalization rate in children below age of [5, 10-11,13,20], we found highest culture positive rate in age group 15-30 year (9.1%) followed by age group below 15 years (6.8%) (P<0.05). This higher infection rate might be due to physically active age group (15-30 year), likely to consume contaminated food/street food, water in schools and work place without parental care. There was no statistically different growth positive rate among male (6.8%) and female (6.4%) (>0.05), however, higher growth positivity were found in male [20, 23-26] and also in female [11] in other studies. This might be explained by public concentration on gender equality of awareness and health facilities.

In this study, prevalence of *S.* Typhi was more than *S.* Paratyphi A, in agreement with some workers [20, 21, 26-27]. However, many studies had shown the increasing proportion of *Salmonella* Paratyphi A [3, 11, 15, 28-29]. Dissimilarity rate in isolation of these *Salmonella* sero-var, with being *S.* Typhi (waterborne infection) more prevalent, is an important indication of higher drinking water contamination.

The use of fluoroquinolones drugs as treatment of enteric fever was widely used after 1990s and became the choice drug but the resistance towards the quinolones is the major problem for treatment strategies in today medicine [13]. Here in our study all the *Salmonella* isolates were found sensitive towards the ciprofloxacin (as widely used fluoroquinolone drug) and ofloxacin. Though, various reports from Nepal have reported ciprofloxacin found effective [11, 28-29] similar to our report, many researches showed resistance pattern against ciprofloxacin [3] and Ofloxacin [11, 23, 24]. The alarming report was the resistance toward Nalidixic acid, 76.47% of total *Salmonella* isolates were found resistant. Risistance to Nalidixic acid with ciprofloxacin susceptibility had been used as a reliable indicator of decreased ciprofloxacin susceptibility [1, 30] hence this result indicated the reduced susceptibility of quinolones and full fluoroquinolones drug resistance pattern may arise in near future. Similar results were found in many reports with elevated MIC value of flurorquinolones [14, 15, 18, 30]. Various results having high resistance towards Nalidixic acid were reported advising the review for the use of quinolone drugs as effective treatment [14, 17, 20, 21, 24]. *S.* Paratyphi A (93.6%) was found to showing higher resistance to Nalidixic acid compared to *S.* Typhi (61.8%) which is similar to the report of Acharya *et al* 2009 [also showed higher MIC value to quinolones in *S.* Paratyphi A compared to *S.* Typhi isolates] and others [29,31]. This showed the acquired rate of resistance pattern of S. Paratyphi A is higher and may become a burden in near future.

The reemergence of susceptibility towards the first line drugs like Chloramphenicol and Cotrimoxazole has been reported in many reports [15, 21, 24, 29] and 99.02% of *Salmonella* isolates in this study were found sensitive to these drugs with only one MDR isolates of *S.* Typhi being resistant. Ceftriaxone a third line cephalosporin was found most effective to all isolates. Many studies have also reported the 100% effectiveness of Ceftriaxone [11, 20, 21, 32] however Prajapati *et al.*, 2008 reported reduced efficacy which might become new burden for treatment strategies.

As concluding statements, enteric fever remains endemic in Kathmandu Valley with the emergence of drug resistant strains of *Salmonellae*. For the treatment, third line cephalosporin are effective, however, this study indicated, decreased susceptibility of quinolone drugs. The re-emergence of susceptibility towards first line drugs should also be reviewed to develop treatment strategies and also to reduce the increased resistance towards the newer drugs. So, environmental sanitation, personal hygiene and drug resistant patterns of *Salmonella* isolates should be kept in view for successful prevention, control and to develop public health strategies.

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