Prevalence of *Salmonella typhi* and *paratyphi* infection in children: a hospital based study

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ABSTRACT

The purpose of this study was to find out the prevalence and antibiotic sensitivity pattern of *Salmonella typhi* and *paratyphi* isolated from children presenting with fever at Kanti Children's Hospital in Kathmandu, Nepal. A total of 9,856 blood samples collected for culture during one year period (April 2007 to March 2008) were included in the study. Out of total, 235 (2.0%) were positive for *S. typhi* and *paratyphi A.* Of the total positive, 195 (83.0%) were *S. typhi* and 40 (17.0%) were *S. paratyphi A.* The growth positive rate in two genders (M: 53.2% and F: 46.8%) was not significant (P>0.05). Over two-third of cases were clustered in the age-group of 1-10 years. The occurrence of infections was common in summer months (rainy season). *S. typhi* was found to be most sensitive to cefotaxime (100.0%) followed by ceftriaxone (98.9%), ofloxacin (93.5%), cotrimoxazole (93.5%) and chloramphenicol (93.2%) and was least sensitive to amoxyccilin (66.7%) followed by ciprofloxacin (86.6%). *S. paratyphi* also was found to be most sensitive to cefotaxime (100.0%), followed by ceftriaxone (97.4%), cotrimoxazole (97.1%) and chloramphenicol (92.5%) and was least sensitive to amoxycillin (15.0%) followed by ciprofloxacin (51.3%) and ofloxacin (70.3%).

Keywords: *S. typhi, S. paratyphi*, prevalence, antibiotic sensitivity, children, Nepal.

INTRODUCTION

Enteric fever is an important public health problem in many underdeveloped and developing countries[1] including Nepal.[2] Though it occurs also in developed countries, the incidence and case fatality rate is very low.[3] The global incidence is estimated to be 21 million with 700,000 deaths each year[1] primarily in South East Asia, Africa and Latin America[4] attributed to rapid population growth and unplanned urbanization, inadequate and improper waste disposal, lack of potable water supply. Outbreaks are more common in summer season[5] affecting mainly children.[6] Infected and healthy carriers are the source of infection and "five Fs" (food, fingers, flies, fomites and faeces)[7] play an important role in the spread of the disease.

Enteric fever is predominantly caused by *S. typhi* followed by *S. paratyphi* A.[6] Though antimicrobial therapy markedly reduces the morbidity and mortality, emergence of resistance to first line antibiotics poses challenge in its management.[8] Resistance to chloramphenical, "gold standard of therapy" since its introduction in 1948,[8] appeared in 1970 leading to many outbreaks in countries in Latin America and Asia.[9] It then resulted into multi drug resistance (MDR: resistant to ampicillin, cotrimoxazole and chloramphenical) in 1989.[10] Fluoroquinolones were the drug of choice for MDR *S. typli* in Asia during past decade.[11] However, emergence of resistance even to these drugs has become a major setback in the management. Moreover, third generation cephalosporins used as alternative agents are also becoming effectiveless.[12] However, there are reports of re-emergence of sensitivity of *S. typhi* to chloramphenicol.[13] Therefore, it is essential to reappraise the antibiotic sensitivity pattern of the isolates periodically.

Enteric fever in Nepal is more common during the summer months[2] with outbreaks in some areas.[14] As the disease mainly affects children, this paper reports the prevalence and antibiogram of *S. typhi* and *paratyphi A* isolates isolated from children presenting with fever.

MATERIALS AND METHODS

A total of 9,856 children (neonates and children aged up to 14 years) presenting with fever at Kanti Children's Hospital in Kathmandu, Nepal in one year period (April 2007 to March 2008) were included in this study. Blood samples collected by aseptic venipuncture were subjected for bacteriological culture (1 ml of blood was put in 10 ml brain heart infusion broth) and incubated at 37^{o}C over night followed by sub-culture on MacConkey agar. Growth negative culture was continued incubation for five days sub-culturing in between at the interval of 48, 72 and 96 hours. Growth negative even after 120 hrs incubation were regarded as negative. The non-

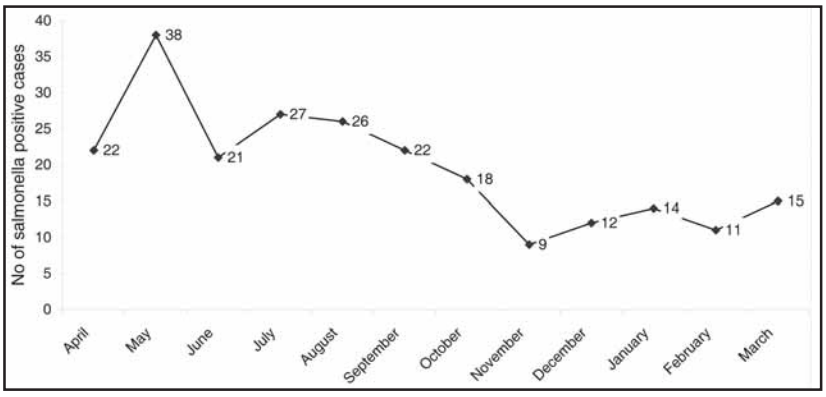

Fig.-1: Distribution of salmonella positive cases by month

lactose fermenting colonies MacConkey agar resembling *Salmonella* were subjected for serotyping using polyvalent and monovalent antisera (Denka-Seiken Company Ltd, Japan).

S. typhi and *paratyphi A* isolates were subjected to antimicrobial susceptibility testing following Kirby-Bauer's disk diffusion technique using Muller Hinton Agar. Antibiotic discs used in this study included amoxycillin (10mcg), chloramphenicol (30mcg), cotrimoxazole (25mcg), ciproflxacin (5mcg), ofloxacin (5mcg), ceftriaxone (5mcg) and cefotaxime (5mcg) (Oxoid, Hampshire, UK). The sensitivity and/or resistance results of the tested isolates were recorded following the instructions of the disc manufacturer.

RESULTS

Of the total 9,856 blood samples cultured, 235 (2.0%) were positive for *S. typhi* and *paratyphi A.* Out of the total positive samples, 195 (83.0%) were *S. typhi* and 40 (17.0%) were *S. paratyphi A. S paratyphi B* and *C* were not isolated. The overall growth positive rate was relatively higher in male (53.2%) compared to female (46.8%) child but the difference was not significant statistically (P>0.05) (Table-1). Similar trend in growth positivity of *S. typhi* (M: 52.8% and F: 47.2%) and *paratyphi A* (M: 55.0% and F: 45.0%) was observed in both sexes. Over two third of growth positive cases were seen in the age-group of 1-10 years. The occurrence of enteric fever was higher in summer months (rainy season) (Fig.-1).

Table-1: Distribution of growth positive cases by age and sex

Age group (year)	Sex					
	Male		Female		Total	
	n	%	n	%	n	%
<1	2	40.0	3	60.0	5	2.1
1-5	50	54.3	42	45.7	92	39.2
6-10	50	55.6	40	44.4	90	38.3
11-14	23	47.9	25	52.1	48	20.4
Total	125	53.2	110	46.8	235	100.0

S. typhi was found to be most sensitive to cefotaxime (100.0%) followed by ceftriaxone (98.9%), ofloxacin (93.5%), cotrimoxazole (93.5%) and chloramphenicol (93.2%). It was least sensitive to amoxyccilin (66.7%) followed by ciprofloxacin (86.6%) (Table-2). *S. paratyphi A* also was found to be most sensitive to cefotaxime (100.0%), followed by ceftriaxone (97.4%), cotrimoxazole (97.1%) and chloramphenicol (92.5%) and was least sensitive to amoxycillin (15.0%) followed by ciprofloxacin (51.3%) and ofloxacin (70.3%) (Table-3).

DISCUSSION

Enteric fever is common in developing countries[1] including Nepal.[2] It is attributed to rapid population growth and unplanned urbanization, inadequate and improper waste disposal, lack of potable water supply.[4] Emergence of drug resistance in the organism pose great challenge.[15] Physicians, therefore, should always be aware of the antibiotic sensitivity/resistance profile of the organism in a given community[16] for the rational use of antibiotics.[17]

In this study, children aged 1-10 years were mostly affected (over two-third of the growth positive cases). However, Ansari *et al*[18] have reported two-third of the cases in the age group of 4-9 years. However, under-five children were found to be mostly affected in India.[19] Though enteric fever is rare among under-one year of age,[20] five cases (2.1%) were found in this study. Interestingly, *S. typhi* was isolated also from a one-month-old infant. Reed *et al*[21] also reported similar finding and postulated that the bacteria might have transmitted through vertical route.

Though males had marginally higher incidence, it was not significant (male to female ratio of 1.1:1). Similar finding has been reported from neighboring country India.[22] A slightly different male to female ratio has been

Table-2: Antibiotic sensitivity pattern of S. typhi (n=235)

Antibiotics	Isolates (n)	Sensitive	Intermediate	Resistance
Amoxycillin	195	130 (66.7%)	29 (14.8%)	36 (18.5%)
Ciprofloxacin	194	168 (86.6%)	22 (11.3%)	4 (2.1%)
Chloramphenicol	192	179 (93.2%)	1 (0.5%)	12 (6.3%)
Ceftriaxone	183	181 (98.9%)	0	2 (1.1%)
Ofloxacin	169	158 (93.5%)	0	11 (6.5%)
Cotrimoxazole	169	158 (93.5%)	0	11 (6.5%)
Cefotaxime	20	20 (100.0%)	0	0

reported by Bhattarai *et al* (1.2:1)[14] and Ansari *et al* (1.3:1)[18] in the valley. The male preponderance seen could be due to their relatively more outdoor activities exposing them to the source of infection.

As reported by Malla *et al*,[2] this study also showed higher incidence of cases during summer season. This appeared to be due to contamination of drinking water and environment with fecal matter. The streets in the city become flooded by rainwater mixed with bad smelling safety tank overflow attributed to virtually non-existent or insufficient sewerage system. This situation has been explained to be associated with the re-emergence of hookworm (parasite) recently.[23] Water samples (collected from natural spout, well and hand pump) and from different points of distribution system (tap water) in Kathmandu Valley have shown fecal contamination.[24] Similar findings have also been found in other parts of the country (unpublished data).

The incidence of *S. typhi* and *S. paratyphi A* seen in this study was similar to finding reported by Mubeena *et al*[25] from Pakistan. In the contrary, Malla *et al*[2] have reported lower percentage of *S. typhi* cases from Nepal. This difference could be due to the inclusion of adult and elderly population by them.

S. typhi was most sensitive to cefotaxime followed by ceftriaxone, ofloxacin, cotrimoxazole and chloramphenicol in this study. This was in agreement to previous findings.[14,18] However, much lower sensitivity (69.1%) has also been reported.[26] Very small percentage (1.1%) of *S. typhi* was resistance to ceftriaxone as was in agreement with the findings reported from Pakistan.[12,25] One hundred percent sensitivity to ceftriaxone has been reported by other investigators also[2,8,15,18] The resistance of *S. paratyphi* to ceftriaxone seen in this study was slightly higher and was in agreement with report from Pakistan.[25] However, Bhatia *et al*, [8] from India has reported 100.0% sensitivity.

The sensitivity rate of isolates against ciprofloxacin in this study was lower compared with previous reports from Nepal[2,14,26,27] and India.[15] Nair *et al*[15] have shown an increasing trend of resistance to ciprofloxacin (from 0.0% in 1995 to 12.5% in 2003) indicating wide use of the drug in treating different kinds of infections. In this study, 93.5% of *S. typhi* strains were sensitive to ofloxacin. This observation was slightly lower than those reported by other workers.[8,12,25,26]

In this study, sensitivity to chloramphenicol was high enough (over 92.0%). This observation was similar to the findings reported by other investigators from Nepal.[26] Bhatia *et al*[8] in India have reported 96.0% and 100.0% sensitivity of *S. typhi* and *S. paratyphi*, respectively. Nair *et al*[15] have shown decreasing trend in resistance to chloramphenicol (50.0% in 1995 to 12.5% in 2003). Malla *et al*[2] have also reported decreasing resistance pattern to chloramphenicol (30.0% in 2002 to 5.0% in 2004). On

Table-3: Antibiotic sensitivity pattern of S. paratyphi (n= 40)

Antibiotics	Isolates (n)	Sensitive	Intermediate	Resistance
Amoxycillin	40	6 (15.0%)	27 (67.5%)	7 (17.5%)
Chloramphenicol	40	37 (92.5%)	1 (2.5%)	2 (5.0%)
Ciprofloxacin	39	20 (51.3%)	12 (30.8%)	7 (17.9%)
Ceftriaxone	39	38 (97.4%)	0	1 (2.6%)
Ofloxacin	37	26 (70.3%)	8 (21.6)	3 (8.1%)
Cotrimoxazole	34	33 (97.1%)	0	1 (2.9%)
Cefotaxime	9	9 (100.0%)	0	0

the other hand, Mubeena *et al*[25] in Pakistan found very low sensitivity to this drug (27.0%). Low sensitivity (60.0%) has also been reported in eastern Nepal.[27]

In this study, it was worth noting of higher sensitivity to cotrimoxazole (over 93%). This was higher than those reported earlier (64.3% by Bhattarai *et al*[14] and 74.4% by Mubeena *et al*[25]). The resistance pattern of the isolates to cotrimoxazole during recent years is decreasing (from 80.0% in 1995 to 37.7% in 2003 in India)[15] and (from 30.0% in 2002 to 5% in 2004 in Nepal).[2]

Two-third of *S. typhi* were sensitive to amoxycillin. It was similar to the finding of Bhattarai *et al*.[14] On the contrary, Rauniar *et al*[27] observed higher sensitivity rate to this drug (80.0%). On the other hand, another study done in India[22] showed very low sensitivity (40%). *S. paratyphi* was found to be most sensitive to cefotaxime, followed by ceftriaxone, cotrimoxazole and chloramphenicol. Fifteen percent of *S. paratyphi* was sensitive to amoxycillin.

Although the culture positive rate for *S. typhi* and *S. paratyphi A* was only 2.0% of total blood culture samples collected from feverish child, it still indicates the major public health importance of this disease in Nepal. Despite the use of only seven antibiotics for sensitivity testing, these findings help know the present status of enteric fever among Nepalese children. It is advisable to do the sensitivity test of cefixime - the recommended drug in the treatment of enteric fever. Considering the changing drug sensitivity pattern (sensitive to resistance and vice-versa) it is advisable to do continuous evaluation of sensitivity-resistance pattern of isolates as to make rational use of antibiotics in the management of enteric fever cases in future.

REFERENCES

1. Shanahan PM, Jesudason MV, Thomson CJ, Amyes SG. Molecular analysis and identification of antibiotic resistance genes in clinical isolates of *Salmonella typhi* from India. *J Clin Microbiol* 1998; 36: 1595-600.
2. Malla S, Kansakar P, Serichantalergs, Rahman M, Basnet S. Epidemiology of typhoid and paratyphi fever in Kathmandu: two years study and trends of antimicrobial resistance. *J Nepal Med Assoc* 2005; 44: 18-22.
3. Ackers ML, Puhr ND, Tauxe RV, Mintz ED. Laboratory-based surveillance of *Salmonella* serotype typhi infections in the United States: antimicrobial resistence on the rise. *J Amer Med Assoc* 2000; 283: 2668-73.
4. Edelman R, Levine MM. Summery of an international workshop on typhoid fever. *Rev Infect Dis*1986; 8: 329-49
5. Fjaerli HO, Heger B, Gundersen SG, Hoet T, Espinoza R. Outbreak of typhoid in family. *Tidsskr nor Leageforen* 1993; 113: 3022-4.
6. Ratish KC, Chandrashekhar MR, Nagesha CN. An outbreak of multidrug resistant typhoid fever in Bangalore. *Indian J Pediar* 1995; 62: 445-8.
7. Old DC, Threlfal EJ. Salmonella In: Topley and Wilson's Microbiology and Microbial Infections, 9th edition, volume 2 (Systemic Bacteriology), Arnold, London 1998, 969-97.
8. Bhatia JK, Mathur AD, Arora MM. Reemergence of chloramphenicol sensitivity in enteric fever. *Med J Armed Forces India* 2007; 63: 212-14.
9. Olarte J, Galindo E. *S. typhi* resistant to chloramphenicol, ampicillin and other antimicrobial agents: strains isolated in extensive typhoid fever epidemic in Mexico. *Antimicrob Agents Chemother* 1973; 4: 597-601.
10. Jesudason MV, Malathy B, John TJ. Trend of increasing levels of MIC of ciprofloxacin to S. typhi. *Indian J Med Res* 1996; 103: 247-9.
11. Gulati PD, Saxena SN, Gupta PS, Chuttani HK. Changing pattern of typhoid fever. *Amer J Med* 1968; 45: 544-8.
12. Jamil B, Lashari I, Bhatti S, Hasan RS. Enteric fever in adult patients: clinical features, outcome and antibiotics susceptibility patterns. *Infect Dis J Pakistan* 2004; 13: 68-72.
13. Sood S, Kapil A, Das B, Jain Y, Kabra SK. Reemergence of chloramphenicol sensitive *Salmonella typhi*. *Lancet* 1999; 353: 1241-2.
14. Bhattarai PM, Bista KP, Dhakwa JR e*t al.* A clinical profile of enteric fever at Kanti Children's Hospital. *J Nepal Paediatr Soc* 2003; 21: 50-3.
15. Nair L, Sudarsana J. Changing sensitivity pattern of *Salmonella typhi* in Calicut. *Calicut Med J* 2004; 2: 141-4.
16. Oreskovic NM, Sembrano EU. Repeat urine cultures in children who are admitted with urinary tract infections. *Pediatr* 2007; 119: 325-9.
17. Jones RN, Thornsberry C. Cefotaxime: a review of in vitro antimicrobial properties and spectrum of activity. *Rev Infect Dis* 1982; 4: 5300-15.
18. Ansari I, Adhikari N, Pandey R, Dangal MM, Karanjit R. Enteric fever: is ciprofloxacin failing? *J Nepal Paediatr Soc* 2002; 20: 6-16.
19. Prakash P, Mishra OP, Singh AK, Gulati AK, Nath G. Evaluation of nested PCR in diagnosis of typhoid fever. *J Clin Microbiol* 2005; 43: 431-2.m
20. Bhutta ZA. Typhoid Fever. In: Nelson Textbook of Pediatrics; 18th ed, Philadelphia, WB Saunders 2007; 1186-91.
21. Reed RP, Klugman KP. Neonatal typhoid fever. *Pediatr Infect Dis J* 1994; 13: 774-7.
22. Khan M, Coovadia YM, Connolly C, Sturm AW. Influence of sex on clinical features, laboratory findings and complications of typhoid fever. *Amer J Trop Med Hyg* 1999; 61: 41-6.
23. Rai SK, Shrestha HG, Nakanishi M *et al*. Hookworm infection recorded at an university teaching hospital in Kathmandu, Nepal over one decade period. *Japanese J Trop Med Hyg* 1997; 25: 81-4.
24. Adhikari RK, Rai SK, Pokhrel BM, Khadka JB. Bacterial study of drinking water of Kathmandu Valley. *J Inst Med (Nepal)* 1986; 8: 313-6.
25. Mubeena RS, Saleem AK, Ameena RS. Prevalence of enteric fever in Karachi. *Infect Dis J Pakistan* 2006: 15: 103-5.
26. Sharma N, Koju R, Karmacharya B *et al*. Typhoid fever in Dhulikhel Hospital, Nepal. *Kathmandu Univ Med J* 2003; 2: 188-92.
27. Rauniar GP, Das BP, Baral DD, Nagarani MA. Treatment pattern of typhoid fever at a tertiary care teaching hospital in Eastern Nepal. *J Nepal Med Assoc* 2000; 39: 218-21.