# EPIDEMIOLOGY OF TYPHOID AND PARATYPHOID FEVER IN KATHMANDU : TWO YEARS STUDY AND TRENDS OF ANTIMICROBIAL RESISTANCE

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#### ABSTRACT

Enteric fever is prevalent in developing countries including Nepal, where it still remains as a major health problem. Appropriate antibiotics are essential for the treatment of typhoid and paratyphoid fever. A prospective study was carried out to characterize the epidemiological features of enteric fever in Kathmandu, Nepal and to analyse the recent trend of antimicrobial resistance pattern of the Salmonella isolated from the cases of enteric fever from different hospitals in Kathmandu during June, 2002 to June, 2004. A total of 1469 Salmonella typhi and Salmonella paratyphi 'A' isolates collected during this period from five different hospital laboratories situated in Kathmandu were studied. The antimicrobial susceptibilities of the isolates towards Ampicillin (10mcg), Chloramphenicol (30mcg), Cotrimoxazole (25mcg), Ciprofloxacin (5mcg) and Ceftriaxone (5mcg) were determined by standard disc diffusion technique and Agar dilution technique were used to determine the minimum inhibitory concentration (MIC) for Ampicillin, Ciprofloxacin, Chloramphenicol and Ceftriaxone. All the isolates tested were found to be sensitive to Ceftriaxone and Ciprofloxacin, the most commonly used antibiotic for treatment of enteric fever in Nepal. Of the total isolates studied, 15.5% from 2002, 8% from 2003 and 3.45% from 2004 were found to be multidrug resistant (exhibiting resistance towards Ampicillin, Chloramphenicol and Cotrimoxazole). Of the total multi drug resistant Salmonella isolates, 92% were Salmonella typhi. All the multidrug resistant isolates were also further tested for susceptibilities towards Tetracycline (30mcg), Nalidixic acid (30 mcg), Streptomycin (10units), Gentamycin (25mcg), Azithromycin (15mcg), Kanamycin (30mcg), Neomycin (30mcg). 50% of the multi drug resistant Salmonella typhi were also resistant to Tetracycline. Plasmid analysis revealed that all of the Mutidrug resistant Salmonella typhi isolates with Tetracycline resistance harbored a large molecular weight (147 Kb) plasmid.

#### Key Words: Enteric fever, Salmonella, Antibiotic, Plasmid, Multidrugresistant.

## INTRODUCTION

Enteric fever is prevalent in developing countries including Nepal, where it still remains as a major health problem. The annual global incidence of this disease has been estimated to be 21 million cases with more than 700.000 deaths.<sup>1</sup> Areas with high incidence of typhoid include South East Asia, Africa and

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Received Date : 12<sup>th</sup> Jan., 2005 Accepted Date : 28<sup>th</sup> June, 2005 Latin America. Countries like Indonesia, India and Nigeria report high mortality rate due to typhoid fever ranging from 12-32% in different studies.<sup>2,3,4</sup> These countries seem to share several characteristics including rapid population growth, increased urbanization, inadequate human waste disposal, decreased water supply and overburdened health care system.<sup>5</sup> Chloramphenicol was the drug of choice for enteric fever since its introduction in 1948.<sup>6</sup> But in 1970s, plasmid mediated resistance to Chloramphenicol appeared and has been associated with outrbreaks in Latin America<sup>7</sup> and Asia.<sup>8,9,10</sup> With the emergence of chloramphenicol resistant strains, ampicillin and trimithoprim were considered suitable alternatives.<sup>11</sup> Since 1989, however, muti drug resistant (MDR) S typhi strains that are no longer susceptible to these three first line antibiotics have emerged.<sup>12</sup> In response to the emergence of muti-antibiotic resistant S. typhi , a number of studies have investigated the efficacies of newer compounds including expanded spectrum cephalosporins and fluoroquinolones.<sup>13,14</sup> Ciprofloxacin is recommended as the drug of choice for typhoid fever since 1990s, but there are reports of S typhi resistant to or with increased minimum inhibitory concentration (MIC) to ciprofloxacin from various parts including India,<sup>5</sup> Korea,<sup>15</sup> Vietnam.<sup>16</sup>

Antibiotic resistance in Salmonella typhi is often plasmid mediated.<sup>1</sup> In particular, resistance to ampicillin, chloramphenicol, cotrimoxazole and tetracycline is often encoded by large molecular weight plasmid (about 180 Kb) belonging to H1 incompatibility group. These plasmids are large (~180 Kb) and conjugating and originate from Southeast Asia.<sup>1</sup>

This study aims at characterizing the recent epidemiological features of enteric fever in Kathmandu, Nepal and to analyze the recent trends of antimicrobial susceptibility of S. typhi and S. paratyphi A for the last two years.

## MATERIALS AND METHODS

#### Bacteria strains:

Salmonella typhi and Salmonella paratyphi A strains isolated from cases of enteric fever at four major hospitals in Kathmandu-Bir Hospital, Tribhuvan University Teaching Hospital (TUTH), Kanti Children's Hospital (KCH), and Maternity Hospital and the isolates from National Public Health Laboratory were included for the study. These strains included for analysis were isolated between June 2002 to July 2004. Bacterial identification were confirmed by biochemical testing on the following media: Kliger iron agar slants, Urea broth , Simmons citrate agar, Sulphide Indole Motility (SIM) medium (all from Oxoid) and serological agglutination with specific antisera (Denka Seiken, Japan). MDR Salmonella strains were defined as those strains possessing Chloramphenicol, Ampicillin and Cotrimoxazole resistance.

#### Sensitivity Test:

The antimicrobial susceptibility of the isolates towards Ampicillin (10 mcg), Chloramphenicol (30 mcg), Cotrimoxazole (25 mcg), Ciprofloxacin (5 mcg) and Ceftriaxone (5 mcg) all from Oxoid, Hampshire were tested by Kirby Bauer's Disk diffusion technique. Agar dilution techniques were also used for determination of the minimum inhibitory concentration (MIC) for Ampicillin, Chloramphenicol, Ciprofloxacin and Ceftriaxone. All the multidrug resistant isolates were also further tested for susceptibilities towards Tetracycline (30mcg), Nalidixic acid (30mcg), Streptomycin (10units), Gentamicin (25mcg) , Azithromycin (15 mcg), Kanamycin (30mcg) and Neomycin (30mcg).

#### Plasmid study:

Extraction of plasmid DNA was carried out for all the multidrug resistant Salmonella typhi by rapid alkaline lysis technique<sup>17</sup> and the molecular weight of plasmid isolated was determined by agarose gel electrophoresis.<sup>18</sup>

#### RESULTS

#### Epidemiological characteristics:

A total of 1469 Salmonella (typhi and paratyphi A) isolates were collected during June 2002-June 2004 from four different hospitals and National Public Health Laboratory in Kathmandu. Distribution of the isolates collected is shown in Table I.

Table I : Distribution of total Salmonella isolates collected							
Isolate	June-Dec, 2002	Jan-June, 2003	July-Dec, 2003	Jan-June, 2004	Total		
S. typhi	106	427	319	187	1039 (70.7%)		
S paratyphi A	23	161	141	105	430 (29.3%)		
Total Salmonella	129	588	460	292	1469		

Table II : Age and	Genderwise	distribution	of Salmonella	analysed
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Age (yrs)	males	females	Total	
0-9	20%	15%	35%	
10-19	20%	11.8%	31.8%	
20-29	20%	7%	27%	
30-39	3%	1.6%	4.6%	
40-49	0.8%	0.63%	1.43%	
50-59	0.3%	-	0.3%	
>60	0.1%	0.22%	0.23%	
Total	64%	36%	100%	

The distribution of isolates by age, gender and seasons were also analysed. The infection occurred most frequently in the age group of 0-9 yrs followed by 10-19 and 20-29 years. Of the total Salmonella isolates analysed, 64 % were from males and 36% were from females, indicating higher incidence of infection in males than in females (Table II).

The distribution of Salmonella isolates collected showed a clear seasonal clustering. The incidence rate was low in winter (from November to January), increased gradually from March and reached its peak in May-July (fig.1). However the number of isolates reported decreased from 2003 to 2004.



Fig. 1 : Monthly distribution of isolates analysed.

#### Antimicrobial susceptibilities:

All 1469 Salmonella typhi and paratyphi A included in the analysis were blood isolates. Of the total 1469 isolates, 70.7%% were S. typhi and 29.3% were S. paratyphi A and 8.8% were multidrug resistant (MDR) strains, defined as resistant to ampicillin, chloramphenicol and cotrimoxazole. Resistance to Ciprofloxacin and Ceftriaxone were not seen. Of the total MDR Salmonella, 92% were S. typhi. Distribution of multi-drug resistant strains in the past two years is shown in fig.2.



Fig. 2 : Percentage of Multi-drug resistant Salmonella typhi and paratyphi 'A'.

Antimicrobial susceptibility test revealed that all the isolates tested were sensitive to Ciprofloxacin and Ceftriaxone. The overall resistance rate to ampicillin, chloramphenicol and cotrimoxazole were 32%, 30% and 30% in late half of 2002, 32%,13% and 15% in 2003 and 13%, 5% and 5% in the first half of 2004 respectively. (fig.3,4,5) For MDR strains, resistance



Fig. 3 : Antimicrobial susceptibility of Salmonella isolated in 2002



Fig. 4 : Antimicrobial susceptibility of Salmonella isolated in 2003



Fig. 5 : Antimicrobial susceptibility of Salmonella isolated in 2004



Fig. 6 : Antibiotic susceptibility pattern of multi drug resistant Salmonella

rate to Azithromycin, Nalidixic acid, Gentamicin, Neomycin, Streptomycin, Tetracycline and Kanamycin were 0%, 98%, 0%, 0%, 51%, 50% and 0% respectively. (fig. 6)

#### Plasmid size:

All the MDR Salmonella typhi strains were further processed for plasmid DNA extraction and analysed by agarose gel electrophoresis for the presence of large molecular weight plasmid. It was found that all the MDR S. typhi strains with



Fig. 7: 147 KB plasmid from MDR, tetracycline resistant S typhi

tetracycline resistance harbored a large molecular weight plasmid of 147 Kb. (Fig.7).

## DISCUSSION

This study provides comprehensive information on the epidemiology of enteric fever in Kathmandu, Nepal and the recent trends in antimicrobial resistance of the causative agents. The study shows that majority of enteric fever in Kathmandu is caused by S typhi (70.7%), follwed by S paratyphi-A (29.3%). While no cases of enteric fever caused due to S. paratyphi-B and/or S. paratyphi-C was reported from any of the hospitals/laboratory included in the study. The infection was prevalent in the age group of 0-9 yrs age followed by 10-19 yrs and the incidence was higher in the male population (64%) in comparison to female population (36%). The number of isolates reported each month showed that enteric fever in Kathmandu occurred sporadically throughout the year with marked peaks in the summer and rainy seasons (May-July).

The increase in multi-drug resistant Salmonella is a huge problem in developing countries. The incidence rates of MDR strains were 26% in UK and 17% in USA, but infections have been in patients with a recent history of travel to India or Pakistan.<sup>19,20</sup> In India 64.5% of S typhi was reported as being MDR in 1993.<sup>21</sup> MDR S. typhi strains were first reported in Nepal in 2002 during Bharatpur outbreak of enteric fever in Chitwan, Nepal in May-June, 2002. This outbreak of enteric fever in Bharatpur, 2002 was reported to be a large single source outbreak due to multidrug resistant S. typhi.<sup>22</sup> Since then the necessity of monitoring the antimicrobial resistance in Salmonella initiated this study. In this study, the overall percentage of multi-drug resistant strains was 8.8%, however the yearly breakdown of the MDR strains showed a gradual decrease, 2002 (15.5%), 2003 (7.5%) and 2004 (3.5%). Ciprofloxacin is the drug of choice for multi drug resistant S.

typhi in Asia for the past decade. But acquired drug resistance to this drug has posed a serious problem for the treatment of enteric fever. Single point mutation in the quinolone resistance– determining region (QRDR) of the topoisomerase gene Gyr A in Salmonella usually leads simultaneously to resistance against Nalidixic acid and decreased Ciprofloxacin susceptibility (5).However ,in our study all the isolates were sensitive to Ciprofloxacin (MIC<1mcg/ml) although Nalidixic acid resistance among MDR Salmonella was very high (98%).

Multi-drug resistance was known to be mediated by a large sized conjugable plasmids and several reports suggested the international transfer of R-plasmids. Reports suggest that these plasmids, which belong to the Inc HI incompatibility group, frequently encode resistance to Chloramphenicol, Cotrimoxazole, Ampicillin and Tetracycline.<sup>23,24,25,26</sup> A recent study on MDR S typhi strains in Vellore, India has reported plasmids belonging to IncHI group, specifically IncHI1 and calculated as being between 140 and 170 Kb.1 The plasmids detailed in the current study were also estimated as being 147 Kb. Although, conjugational study for transfer of drug resistance and pulsed field gel electrophoresis (PFGE) based analysis couldnot be carried out in this investigation, recent studies indicate multi drug resistant S. typhi with plasmid mediated block resistance to chloramphenicol, ampicillin and cotrimoxazole. However, further genotypic studies are needed to analyze the spread of MDR strains in Kathmandu.

## CONCLUSION

In conclusion, we have noticed MDR Salmonella isolated from cases of enteric fever in Kathmandu showing resistance to all three first line antibiotics used for treatment-Ampicillin. Chloramphenicol and Cotrimoxazole. Although there are reports of gradual increase in the incidence of resistance to newer drugs like quinolones,<sup>5,15,16,26,27</sup> all the isolates tested in our study are still sensitive to Ciprofloxacin (MIC value <= 1 mcg/ml) and Ceftriaxone (MIC value <= 8 mcg/ml). However, there are reports that when the sensitivity pattern indicates resistance to nalidixic acid which is a marker for delayed clinical response to fluoroquinolones, it is necessary to increase the oral dose of ciprofloxacin or treat the patient with third generation cephalosporin like Ceftriaxone.<sup>28,29</sup> In the present study, all the S. typhi strains were sensitive to ciprofloxacin and Ceftriaxone. Since strains resistant to Ciprofloxacin and Ceftriaxone werenot identified, surveillance for resistance to first line antibiotics and fluoroquinolones should be continued.

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