Original Article

Drug resistance patterns of Salmonella in enteric fever in the medicine inpatient of a tertiary care hospital

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Abstract: *Enteric fever, caused by serovars typhi and paratyphi of Salmonella enterica, showed recent upsurge in cases caused by multidrug resistant strains. This has raised a major public health concern in developing countries including Bangladesh. To explore the drug resistance and also the antimicrobial sensitivity pattern of Salmonella among enteric fever patients with a view to encourage good antibiotic stewardship and also to guide empiric therapy in enteric fever. This was a cross-sectional observational study, carried out in the medicine inpatient of a tertiary care hospital, from January to December, 2014. Sixty patients with culture positive enteric fever aged 18 years and above were selected. Antibiotic susceptibility test for all isolates was performed by NCCLS recommended Karby-Bauer disc diffusion method. The used antibiotics were ampicillin, co-trimoxazole, chloramphenicol, ciprofloxacin, azithromycin, cefixime, ceftriaxone, and imipenem. The isolates were considered as multidrug resistant if they were resistant to at least two classes of antibiotics. Resistance pattern of Salmonella such as resistant strains, epidemic strains and multidrug resistant strains was assessed. The mean age of patients was 34.6 ± 14.8 years with a male to female ratio of 1.22:1. Sub-culture isolates of Salmonella typhi showed sensitivity pattern to different antibiotics as follows- ampicillin (51.7%), cotrimoxazole (36.7%), chloramphenicol (65.0%), azithromycin (70.0%), ciprofloxacin (55.0%), cefixime (78.4%), ceftriaxone (85.0%) and imipenem (100.0%). The isolates were resistant, epidemic and multidrug resistant strains in 58.3%, 11.7% and 20.0% cases respectively. Complications were found in 6.7% of the cases but no mortality occurred. Continuous surveillance and antibiotic susceptibility testing is required in different parts of the country to know the sensitivity pattern of the enteric fever pathogens, which will be helpful for clinicians for rational use of antibiotics against Salmonella in enteric fever.*

Key words: *Enteric fever, Salmonella, resistance, Multidrug resistance*

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Introduction

Though enteric fever is a common infectious disease in all parts of the globe it is endemic in certain parts of Asia, Africa and Latin America.[1] This water borne infection is especially prevalent in developing countries because of poor standards of water supply and sanitation. Over 22 million new cases and 200000 deaths occur worldwide every year.[2] Developing countries, particularly Asian countries bear 93% of the global typhoid burden.[3]It is a common cause of febrile illness in Bangladesh also.[4-7] But there is a rising concern about the resistance of *Salmonella typhi* to different microbial agents like ampicillin, chloramphenicol, cortrimoxazole and

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ciprofloxacin which have been used over the past few decades. Simultaneous resistance of *Salmonella typhi* to three or more different groups of antimicrobial drugs, which is defined as multidrug resistance (MDR) Salmonella, has emerged worldwide, especially in South America, Africa and South East Asia.[8,9] In India, drug resistance has been reported since 1960 following the first outbreak of MDR Salmonella typhi in Calicut. MDR *Salmonella typhi* is still common in many areas of the globe, whereas in some regions highly sensitive strains have re-emerged.[10,11] So, efforts for learning about the recent regional trends of sensitivity and resistance of this organism is a worthy field of research.

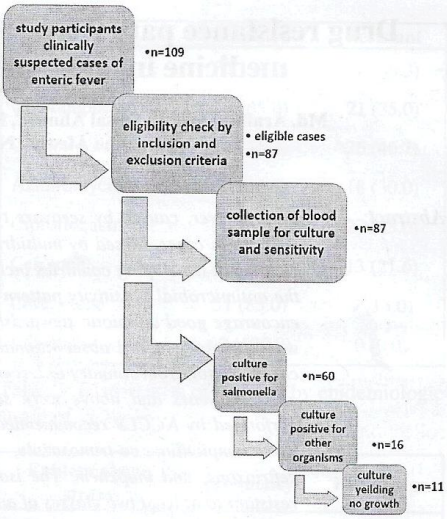
2. Materials and Methods

This was a cross-sectional observational study, carried out in the department of medicine, Sylhet Women's Medical College Hospital, Sylhet, from January to December, 2014. All febrile patients in the medicine inpatient admitted during this period, who were 18 years or older, with a clinical diagnosis of enteric fever and subsequently yielding growth of *Salmonella* in blood culture, were the study population. Patients with fever for more than 5 weeks and less than 5 days were excluded from the study. Patients having a co-existing diagnosis of febrile illness of other etiology and those unwilling to participate, were excluded from the study. After exclusion, a total of 60 patients with culture positive enteric fever were finally selected. (Figure 1)

Sample size: Sample size was calculated using Cochran's formula considering 5% level of significance, 3% precision level (marginal error) and incidence of typhoid fever of 1,402 cases per 100,000 persons in 2004 (1.4%).[12]

Data collection tool: Predesigned semi-structured data collection sheet was used for data collection.

Figure 1: Flow chart of study procedure



Procedure of data collection: All enrolled patients were assessed by detailed history, physical examination and relevant investigations and were recorded in the standard predesigned semi-structured data collection form.

Samples were collected from peripheral venous blood (antecubital vein) after proper disinfection of skin site with iodine and 70% isopropyl alcohol. About 5 ml sample was drawn in a sterile disposable syringe using 20-gauge needle and poured immediately into blood culture bottle containing 40 ml Brain Heart Infusion (BHI) broth (CONDA Spain) with 0.025% of SPS as anticoagulant. The culture bottle was sent to the microbiology laboratory where it was incubated at 37^0C for up to 4 days and was examined daily for any bacterial growth. Bacterial growth was indicated by the presence of turbidity in blood culture bottle. It was then sub-cultured on solid medium (MacConkey agar). Growth of Salmonella was marked by development of tiny non-lactose fermenting colonies on the culture plate. Identification of the organism was confirmed by microscopic examination and

further biochemical studies. No growth on sub-culture for up to 120 hours (five days) was regarded as culture negative.

Antibiotic susceptibility test of each isolate was performed by NCCLS recommended Karby-Bauer disc diffusion method. The antibiotic incorporated plates were incubated at 37^0C and zone of inhibition around the antibiotic were measured after 18 hours and within 24 hours of incubation. The used antibiotics were ampicillin, co-trimoxazole, chloramphenicol, ciprofloxacin, azithromycin, cefixime, ceftriaxone, and imipenem. Microbiological culture was done in the same laboratory to maintain uniformity. Resistance pattern of Salmonella such as resistant strain, epidemic strain and multidrug resistant strain was assessed. All relevant data were then recorded in the previously mentioned data collection sheet.

Primary outcome variables

Frequency and pattern (resistant, epidemic or multidrug resistant strain) of drug resistance of Salmonella in enteric fever.

Secondary outcome variables

Age, sex, clinical presentation and susceptibility pattern of Salmonella infection.

Data analysis:

Data were processed manually and analyzed with the help of SPSS (Statistical package for social sciences) Version 21.Quantitative data were expressed as mean and standard deviation and comparison was done using one way ANOVA. Qualitative data were expressed as frequency and percentage.A probability value of less than 0.05 ($p<0.05$) was considered statistically significant.

Ethical issue:

Informed written consent was taken from each of the patients before enrollment. The consent form clearly described the purpose and methods of the study, confidentiality of the interview, risks and benefits of participating in the study, the participant's rights to participate voluntarily and to refuse at any point in time without consequences. An approval of the protocol was obtained from the Institutional Ethical Committee of Sylhet Women's Medical College, Sylhet.

Operational Definition

Resistant Strain: Resistant strains of salmonella are those who are resistant to at least one of the first line drugs (chloramphenicol, cotrimoxazole and ampicillin).[13]

Epidemic strain: Epidemic strains of salmonella are those who are resistant to chloramphenicol and cotrimoxazole but susceptible to ampicillin.[13]

Multidrug Resistant strain: Multidrug resistant strains (MDR) are those resistant to all of the three first line drugs or at least two classes of drugs besides chloramphenicol, cotrimoxazole and ampicillin.[13]

Results

In this study, the drug resistance pattern of Salmonella in 60 culture positive patients admitted in the medicine inpatient of a tertiary medical college hospital in 1 year, was observed. The age of the patients ranged from 18-67 years with a mean of 34.6 ± 14.8 years. The highest percentage (30%) of patients was in 21-30 year age group. Out of 60, 33 (55%) patients were male and 27 (45%) were female with a male to female ratio 1.22:1. Besides fever, cough (35%), abdominal pain (56.7%), anorexia (76.7%), nausea/vomiting (60%), diarrhea (36.7%), constipation (31.7%) and rash (25.0%) were common associated symptoms and coated tongue (55.5%), relative bradycardia (35%), hepatomegaly (38.3%), splenomegaly (46.7%) and rose spot (21.7%) were common signs among the patients. Low blood hemoglobin level was found in 33% of the patients. Leucopenia and thrombocytopenia was found in 26% and 16% respectively. Mean ESR was 41.5 mm in the first hour(Table 1). *Salmonella typhi*isolates were susceptible to ampicillin in 51.7%, cotrimoxazole in 36.7%, chloramphenicol in 65.0%, azithromycin in70.0%, ciprofloxacin in 55.0%, cefixime in 78.4%, ceftriaxone in 85.0% and

imipenem in100.0% of the patients (Table 2). The *S. typhi* isolates were 35 (58.3%) resistant strains, 7 (11.7%) epidemic strains and 12 (20.0%) multidrug resistant strains (Table 3). The duration of hospital stay was 6.14 ± 0.88 days in the patients having infection with resistant strains, 7.71 ± 0.95 days with epidemic strains, 8.83 ± 0.58 days with multidrug resistant strains and 6.77 ± 0.41 days with other strains. The duration of hospital stay was significantly different ($p<0.001$) among patients infected with different strains (Table 4). Complication was found in 6.7% of the cases. Hepatitis (3.3%), encephalopathy (1.7%) and life threatening ($<20x10^3$/cu-mm) thrombocytopenia (1.7%) were the observed complications. But there was no mortality (Table 5).

Table 1: Clinical features and laboratory findings

Symptoms	Frequency	Percentage
Fever	60	100.0
Cough	21	35.0
Abdominal pain	34	56.7
Anorexia	46	76.7
Nausea/ vomiting	36	60.0
Diarrhea	22	36. 7
Constipation	19	31.7
Rash	15	25
signs		
Raised temperature	60	100.0
Relative bradycardia	21	35.0
Hepatomegaly	23	38.3
Splenomegaly	28	46.7
Rose spot	13	21.7
Laboratory findings		
Anaemia (Hb<12.0gm)	33	55.0
Leucopenia(<3000/cu-mm)	26	44.3
Thrombocytopenia(150x 10^3/cu-mm)	16	26.7
ESR (mean ± SD) (mm in the first hour)	41.5 (SD± 10.1)	

Table 2: Antibiotic susceptibility pattern of S. typhi

Drugs	Sensitive	Resistant
Ampicillin	31 (51.7)	29 (48.3)
Chloramphenicol	39 (65.0)	21 (35.0)
Cotrimoxazole	22 (36.7)	26 (46.7)
Azithromycin	42 (70.0)	18 (30.0)
Ciprofloxacin	33 (55.0)	27 (45.0)
Cefixime	47 (78.4)	13 (21.6)
Ceftriaxone	51 (85.0)	9 (15.0)
Imipenem	60 (100.0)	0 (0.0)

Table 3: Distribution of patients by epidemiologic strain of *S. typhi*

Epidemiologic strain	Frequency	Percentage
Resistant strain	35	58.3
Epidemic strain	7	11.7
Multi-drug resistant strain	12	20.0
Other	6	10.0

Table 4: Duration of hospital stay among patients*

Different strain	Hospital stay (days)		p-value
	Mean	SD	
Resistant strain (n=35)	6.14	± 0.88	
Epidemic strain (n=7)	7.71	± 0.95	
			*p<0.001
Multidrug resistant strain (n=12)	8.83	± 0.58	
Other (n=4)	6.77	± 0.41	

*One way ANOVA was applied to analyze the data

Table 5: Complications of S. typhi infection

Complications	Frequency	Percentage
Hepatitis	2	3.3
Encephalopathy	1	1.7
Thrombocytopenia	1	1.7
Death	0	0.0
No complications	56	93.3

Discussion

Enteric fever (typhoid and paratyphoid) is a systemic infection caused by several *Salmonella enterica* serotypes including *S. Typhi* and *S. paratyphi* A. The Indian subcontinent including Bangladesh has the highest incidence of the disease in the world.[14]Enteric fever continues to be a public health problem in Bangladesh. *S. typhi* has become resistant to antibiotics like ampicillin, chloramphenicol and cotrimoxazole, and also to previously efficacious agents like ciprofloxacin.[9] The emergence of strains of *S. typhi* resistant to multiple antibiotics has posed a serious health problem. The first major epidemic of multidrug resistant *S. typhi* was reported in 1972 in Mexico. Since then, an increasing frequency of antibiotic resistance has been reported from all parts of the world, but more so from the developing countries.[15]A recent study revealed that typhoid fever was endemic in urban areas with a high incidence of multidrug resistant strains.[16]Optimum empiric therapy for enteric fever greatly depends on the local antimicrobial resistance patterns because wide variation in the sensitivity pattern of various strains of typhoid bacilli to antibiotics in different geographic regions has been observed. This also calls for compilation of data from specific zones for epidemiological analysis.[1,17] This cross-sectional observational study was conducted in the medicine inpatient of Sylhet Women's Medical College Hospital, Sylhet, Bangladesh, during the period from January 2014 to December 2014 with a view to find out the drug resistance pattern of Salmonella in this locality.

Among the *S. typhi* isolates in this study, susceptibility was found to be 51.7% for ampicillin, which was lower than the results (55% and 67.5%) found by Kalu*et al.*[18]and Muthu*et al.*[10] respectively, but higher (40.1% and 48%) than that found by Madhulika*et al.*[19] (40.1%) and Bhatia *et al.*[20] respectively. However, 98.4% susceptibility was reported by Yashavanth-Vidyalakshmi.[21] This study and the one done by Madhulika*et al.*[19], showed similar susceptibility to cotrimoxazole (36.7% and 35.5% respectively) but Nagashety*et al*[22]and Yashavanth-Vidyalakshmi found higher results (82.1% and 97.4% respectively).

With the poor susceptibility profile with ampicillin, cortrimoxazole and chloramphenicol (65% susceptibility in this study), and emergence of MDR *S. typhi* worldwide, fluoroquinolones, which are available since the 1980s and having both good in-vitro susceptibility and in-vivo efficacy, have been good alternative choices to the first-line drugs until early 1990s.[22] Nalidixic acid is the prototype and the first member of the quinolone group. But it is now seldom used due to the emergence of resistant serotypes of Salmonella. It has also been observed that nalidixic acid resistant *S. typhi* has decreased susceptibility to other fluoroquinolones as well. Since 1993, *S. typhi* with decreased susceptibility to ciprofloxacin has been isolated with an increased frequency in Vietnam.[23] Subsequently, an extensive outbreak of nalidixic acid resistant *S. typhi* with decreased susceptibility to ciprofloxacin occurred in Tajikistan.[24]Threlfall *et al*[25] observed that the isolation of *S. typhi* with reduced susceptibility to ciprofloxacin increased from 0.9% in 1991 to 23% in 1999 in the Central Public Health Laboratory, UK. Most of those isolates were from patients who returned recently from the Indian subcontinent, particularly India and Pakistan.[24] The present study showed that 55% of *S. typhi* isolates were sensitive to ciprofloxacin and similar reports were observed inmany other studies, suggesting that these strains are endemic in the Indian subcontinent.[26]

Cefixime, a third generation oral cephalosporin, showed sensitivity in 78.4% and 76.7% of *S. typhi* isolates in this study and the study by Islam *et al* [7] respectively. Ceftriaxoneresistant *S. typhi* was found in 15% of the isolates in this study and Saha*et al*[27]found similar results. All strains were sensitive to imipenem in this study and several other studies.[7]So, this is of paramount importance that this drug should be used judiciously only in selected cases where first-line and second-line drugs fail to produce satisfactory response. High cost and need for parenteral administration also limit the use of this drug in enteric fever in spite of its excellent sensitivity profile. Over the recent years, along with cefixime and ceftriaxone, azithromycin has also been a good option for the treatment of quinolone resistant typhoid fever.[28] Unfortunately, in this study, resistance rate was quite high (30%) with azithromycin.

Begum *et al.*[13] found 2.8% of the isolates to be multidrug resistant during October-November 2010 but 12.2% multidrug resistant strain during January-December 2012, suggesting a rising trend of multidrug resistant strains of S. typhi over time. In our study, 58.3% resistant, 11.7% epidemic and 20% multidrug resistant strains of *S. typhi* were found among the blood culture isolates. This also implies a persistent rise of MDR strains over time. The clinico-financial impact of this is on the longer duration of hospital stay (6.77 ± 0.41 days) with MDR strain infected patients. According to Kato *et al.*[14] the median fever clearance time was 6 days (range 3–12 days).Parande*et al.*[29] reported that the mean duration of hospital stay for enteric fever patient was 7.91 ± 5.45 days and the range was 2-35 days. In this study complications were found in 6.7% cases but there was no mortality. Hepatitis (3.3%), encephalopathy (1.7%) and thrombocytopenia (1.7%) were the common complications.

Activity of antimicrobials on *S. typhi* appears to be changing over the past decades.[30]From nalidixic acid to ciprofloxacin and development of resistance has steadily progressed. Although this is not a major problem at present, but there are sporadic reports of ceftriaxone resistant *S. typhi*.[27] Moreover, detection of integrons in isolates of *S. typhi* from Asia suggests that this organism has the potential for acquisition of new resistant genes.[31,32]So, there is a rising concern about the emergence of effectively untreatable typhoid in the developing countries.[23] Rational use of antibiotics and effective hospital infection control practice may play a crucial role in preventing the emergence and spread of resistant organisms. At this point of time, it is also necessary to develop and evaluate newer antimicrobials with different target sites and mechanisms of action as alternatives for treating enteric fever effectively.[33]

Conclusion

Considering the progressive rise in multidrug resistant enteric fever cases, it is clear that larger scale epidemiological studies and surveillance strategies are required in this issue. Patterns of antibiotic sensitivity and resistance should be known and understood to treat enteric fever cost effectively.

Limitation of the study

This study was conducted in a in a tertiary hospital only with a small sample size. Moreover, this was a cross sectional study conducted over a short time frame. So, valid epidemiological inferences cannot be drawn based on such small scale study.

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