Antimicrobial Susceptibility Pattern of Salmonella enterica Serovar typhi and Para typhi Isolates from Suspected Enteric Fever Patients in Chattogram

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ABSTRACT

Background: *Enteric fever, a systemic infection caused by Salmonella enterica serovar typhi and Para typhi is one of the most common infections in developing countries. Regardless of high endemicity of enteric fever in Bangladesh, there is paucity of studies on prevalence and drug-resistance of the pathogen. This study was done to evaluate the status in antimicrobial susceptibility patterns of Salmonella enterica serovar typhi and Salmonella enterica Para typhi obtained from blood culture in suspected patients of Chattogram.*

Materials and methods: *A cross-sectional descriptive type of study was done at the Ibne Sina diagnostic center for a period of two-year from February 2021 to January 2023. A total of 1230 clinically suspected septicemic patients were enrolled.The samples were collected and processed following standard microbiological techniques and an antibiotic susceptibility test was done on pure culture isolates using disc-diffusion method for the commonly used antibiotics. The data were analyzed by using SPSS version 27 and the results were summarized by using tables and graphs.*

Results: *Among 1230 suspected cases, blood culture positive were 139 (11.3%). Among them male was more prevalent 65.22% to develop enteric fever and female 34.78%.Out of 139 isolated organisms Salmonella enterica serovar typhi was the most common 46.04%, followed by CoNS 16.55%, E. coli 12.95%, Klebsiella spp. 10.07%, Pseudomonas spp. 6.47%, Staphylococcus aureus 4.32% and Salmonella enterica Para typhi 3.60%. Young Adult and Children are more prone to develop enteric fever by Salmonella enterica typhi 43.48% and 42.03%. Among the 64 Salmonella enterica typhi isolates, least resistance was shown to Amikacin 04 (6.25%) Ceftazidime 06 (9.37%) and Amoxiclav 12 (18.75%).High resistance to Azithromycin 56 (87.5%). Salmonella enterica para typhi isolates were excellently sensitive to most of the antibiotics and showed 100% sensitivity to Amikacin, Gentamycin, imipenem and Tazobactam-Piperacillin, followed by 80% sensitive to Amoxiclav, Ceftriaxone, Chloramphenicol, Ciprofloxacin and Levofloxacin.*

Conclusions: *Current study demonstrated a scenario of enteric fever occurrence and trend of antibiotic use in this disease in Bangladesh. Antibiotics should be used judiciously and according to culture and sensitivity report; over-the-counter sales of antibiotics must be stopped to prevent risk of developing newer strains of resistant organisms called superbug.*

Introduction

Enteric fever (Typhoid and paratyphoid fever) is a serious bloodstream infection caused by *Salmonella*

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enterica serovar typhi and *Para typhi* A, B and C.[1] Enteric fever is transmitted predominantly by the fecal–oral route and manifests with several clinical outcomes including malaise, fever, chills, nausea, abdominal discomfort, transient rash and hepatosplenomegaly.[2] Enteric fever is still a major health issue in developing nations even with improvements in personal hygiene, sanitation and the availability of efficient treatments. Each year, developing nations report between 11.9 and 20.6 million cases of typhoid and paratyphoid fever, with observed fatality rates ranging from 129,000 to 223,000.[3] Moreover, a major proportion of these cases and mortalities is concentrated in South Asia, where it exhibits the seasonal variation, peak in the rainy reason, from June to August.[4] In case of Nepal, the burden of enteric fever is concentrated in the densely populated regions: major city areas and low-lying southern Terai.[5] Reduced access to clean drinking water and improper

sanitation facilities in these populous regions further deteriorates the quality of life, which leads to the frequent onset of infectious diseases including typhoid and paratyphoid fever. Salmonella has been estimated as the most common single pathogen isolated from blood cultures in, which is indicative of higher prevalence of enteric fever among Nepali population.[6]

When treating enteric fever, the most effective medications are antimicrobials such as chloramphenicol, ampicillin, co-trimoxazole and fluoroquinolones.[7] Without antibiotic medication, the case fatality rate is estimated to be between 10% and 30%; with the right course of treatment, the fatality rate drops to 1% to 4%.[2] However, the widespread and illogical prescribing and use of these antibiotic medications has developed Multidrug-Resistance (MDR), against Salmonella virulent strains.[6]

Treatment failures, a reduced range of medication regimens, elevated severities and fatalities are all caused by MDR strains.[8] Early drug resistance in Salmonella isolates began in the late 1980s when antibiotic resistance rendered the conventional first-line medications (Trimethoprim-sulfamethoxazole, Ampicillin and Chloramphenicol) ineffective, forcing physicians to use fluoroquinolones, particularly ciprofloxacin.[9] However, a catastrophic rise in infectious illnesses might result from the current global spike in fluoroquinolone resistance.[10]

Nepal has endured several epidemics of enteric fever with varied patterns of drug resistance in the last few decades.[11] Since the very first report of MDR strain of Salmonella in 1991, there has been continuous change in epidemiology and drug-susceptibilities. To cope with increasing resistance to first-line antibiotics, fluoroquinolones were introduced.[12,13] However, several findings suggest the increasing burden of nalidixic acid- resistant strains with reduced susceptibilities to quinolones, which subsequently has led to the introduction of third generation cephalosporin and azithromycin.[14] Despite several previous attempts, there is paucity of reliable data and ample scientific studies on the prevalence and antibiogram of *Salmonella enterica Serovar typhi* and *Para typhi*. This study was conducted to determine the prevalence and antibiotic susceptibility profile of *Salmonella spp.* isolated from clinical specimens obtained at Ibne Sina diagnostic center, Chattogram. This study further aimed to investigate if there was re-emergence of the susceptibility of the bacterial strains to the conventional drugs.

Materials and methods

A cross-sectional descriptive type of study was done for a period of two years from February 2021 to January 2023. A total of 1230 blood samples from clinically suspected septicemia patients were enrolled at the Ibne Sina diagnostic center, Chattogram. Under strict aseptic precautions, I0 ml of venous blood was collected and inoculated into adult aerobic blood culture bottles of BD BACTEC FX 40 automated blood culture system. Inoculated blood culture bottles were loaded into the system and incubated at 37°C, monitored for up to 5 days when there was no signal. In case of growth, the automated system gave an alert signal. Positive bottles were then sub-cultured on Blood agar and MacConkey's agar medium. Isolates was identified by their characteristic appearance on their respective media, Gram staining and confirmed by the pattern of biochemical reactions using the standard method.[7]

Salmonella enteritidis serovar *S. typhi* and *S. para typhi* were identified by conventional phenotypic methods using TSI, MIU medium and Citrate utilization test. Antimicrobial susceptibility testing was performed for all blood culture isolates in Mueller-Hinton agar medium by Kirby–Bauer disc diffusion method as recommended in the National Committee for Clinical Laboratory Standards (NCCLS) guidelines.[8] The media and antibiotic discs were obtained from HI media (India) Laboratories.

Results were summarized by using tables and graphs and analyzed by using SPSS version 22. Percentages were used to analyze epidemiological variables and significance among percentages was calculated with Chi square test and p value of < 0.05 was considered statistically significant.

Results
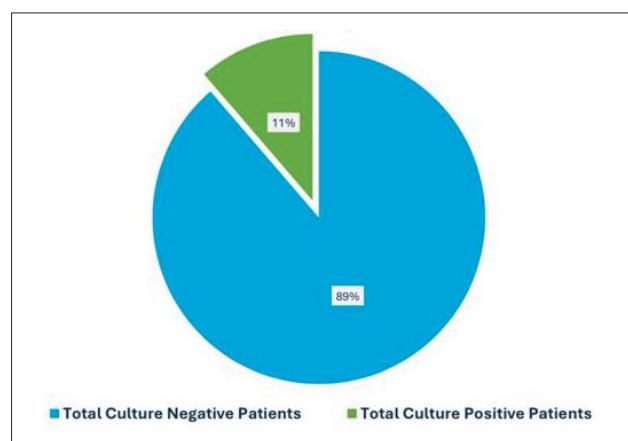
Figure 1 Results of Blood culture among suspected septicemia patients (n=1230)

Among 1230 suspected cases blood culture positive was 139 (11.3%) and negative was 1091 (88.7%)

Table I Distribution of organisms Isolated from Blood culture (n=139)

Name of the organisms	Number	Percentage (%)
Salmonella enterica Serovar typhi	64	46.04
Coagulase Negative Staphylococcus (CoNS)	23	16.55
Escherichia coli	18	12.95
Klebsiella spp.	14	10.07
Pseudomonas spp.	09	06.47
Staphylococcus aureus	06	4.32
Salmonella para typhi	05	3.60
Total	139	100.00

Among the 139 isolated organisms *Salmonella enterica Serovar typhi* was the most common 46.04%, followed by CoNS 16.55%, *E. coli* 12.95%, *Klebsiella spp.* 10.07%, *Pseudomonas spp.* 6.47%, Staphylococcus aureus 4.32% and *Salmonella Para typhi* 3.60%.

Table II Age distribution of culture positive enteric fever patients (n=69)

Age category	*Salmonella enterica Serovar typhi*		*Salmonella Para typhi*		Total *Salmonella Serovar*
Children < 18 years	29	42.03%	04	5.80%	33
Young Adult 18-35 years	30	43.48%	01	1.45%	31
Middle-aged adult 36-55 years	03	4.35%	00	00	03
Older adult 56 and above	02	2.90%	00	00	02
Total	64	92.75%	05	7.25%	69 (100%)

Here it was observed Young Adult and Children were more prone to develop enteric fever by *Salmonella enterica Serovar typhi* 43.48% and 42.03%, but less culture positivity seen by Salmonella Para typhi 5.80% for Children and 1.45% in Young Adult.

Table III Distribution of *Salmonella* isolates according to gender of patients (n=69)

Sex	*Salmonella spp.* Isolated n (%)	p-value
Male	45 (65.22%)	>0.05
Female	24 (34.78%)	
TOTAL	69 (100%)	

Male Female Ratio: 1.88 : 1
Chi square test done. p- value = 0.9530, p > 0.05, statistically no significant difference at 95% Confidence Interval.

Above table showed male were more prevalent to develop enteric fever 65.22% whereas female was 34.78%.
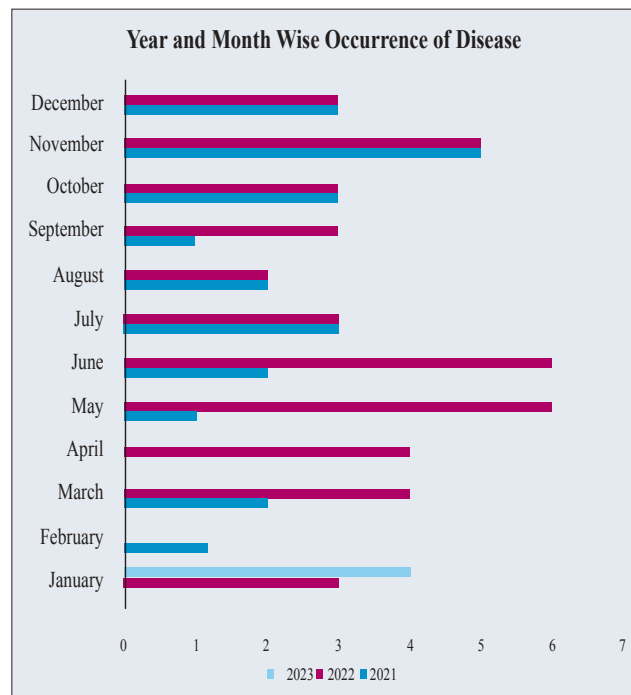
Figure 2 Year and month wise occurrence of disease

Enteric fever occurs round the year in Chattogram with slightly increased incidences in monsoon (May-July) and in winter season (November-January).

Antibacterial resistance patterns of the Salmonella enterica Serovar typhi and Salmonella Para typhi blood stream isolates are shown in Table IV.

Table IV Drug resistance pattern of *Salmonella typhi* and *Para typhi* (n=69)

Antibiotics	*Salmonella enterica Serovar typhi* (64)		Salmonella Para typhi (05)	
	Sensitive (S)	Resistant (R)	Sensitive (S)	Resistant (R)
Amikacin	60 (93.75)	04 (6.25)	05 (100)	00
Amoxiclav	52 (81.25)	12 (18.75)	04 (80)	01 (20)
Ampicillin	46 (71.88)	18 (28.12)	00	05 (100)
Azithromycin	08 (12.5)	56 (87.5)	00	05 (100)
Cefixime	16 (25)	48 (75)	01 (20)	04 (80)
Ceftazidime	58 (90.63)	06 (9.37)	01 (20)	04 (80)
Ceftriaxone	58 (90.63)	06 (9.37)	04 (80)	01 (20)
Chloramphenicol	60 (93.75)	04 (6.25)	04 (80)	01 (20)
Ciprofloxacin	58 (90.63)	06 (9.37)	04 (80)	01 (20)
Cotrimoxazole	34 (53.13)	30 (46.87)	03 (60)	02 (40)
Gentamycin	58 (90.63)	06 (9.37)	05 (100)	00
Imipenem	00	00	05 (100)	00
Levofloxacin	58 (90.63)	06 (9.37)	04 (80)	01 (20)
Tazobactam-Piperacillin	00	00	05 (100)	00

● Number within parentheses indicates percentage.

Among the 64 *Salmonella enterica Serovar typhi* isolates, there was least resistance to Amikacin 04 (6.25%), Ceftazidime 06 (9.37%) and Amoxiclav 12 (18.75%) and high resistance to Azithromycin 56 (87.5%). Conventional first-line medications to Salmonella also showed very good sensitivity to Chloramphenicol 93%, Ampicillin 71% and Cotrimoxazole 53%. *Salmonella para typhi* isolates were excellently sensitive to most of the antibiotics and showed 100% sensitivity to Amikacin, Gentamycin, Imipenem and Tazobactam-Piperacillin, followed by 80% sensitive to Amoxiclav, Ceftriaxone, Chloramphenicol, Ciprofloxacin and Levofloxacin.

Discussion

Enteric fever stands as a primary endemic disease in Bangladesh and other Low-to-Middle-Income (LMIC) countries. The high prevalence of this illness in these nations can be attributed to several factors, including crowded urban areas with poor access to hygienic facilities and clean water, lower socioeconomic conditions, inefficient surveillance, and control measures.[15]

In this study, a total of 139 culture-positive isolates were examined. Among these 139 isolates, 64 (46.04%) were *S. typhi* and 5 (3.60%) were *S. paratyphi,* which demonstrated similarity with the study of Choudhary et al. where the prevalence of *S. typhi* was 57.8% and *S. paratyphi* was 41.6%.[16] According to Maharjan A et al. the growth for *S. enterica* (Serovar paratyphi A) was 3.1%.[17] The low growth rate in blood culture can be attributed to the practice of self-medication, which is more prevalent in developing countries. In this study, the prevalence of *S. enterica serovar typhi* was significantly higher (46.04%) compared to *S. enterica* serovar *Para typhi* A (3.60%). This finding aligns with earlier studies conducted in Nepal, which indicated that 65.1% of cases were attributed to *Salmonella enterica* serovar *typhi,* while 34.9% were linked to *Salmonella enterica* serovar *Para typhi* A. Such consistency across research underscores the importance of ongoing investigation in this field, encouraging further exploration to enhance understanding and improve public health strategies.[18,19]

While no definitive explanation exists for the variation in enteric fever serotypes, the greater prevalence of *S. enterica* serovar *typhi* may be attributed to its waterborne transmission, which typically involves smaller inoculum compared to *S. enterica* serovar *Para typhi*. The latter is generally spread through food-borne transmission, necessitating larger inocula.[16]

The study findings indicate a higher incidence of typhoid and paratyphoid fever during the months of May to July, which coincide with the summer and rainy season. This seasonal pattern aligns with the observations reported by Maharjan A et al. who had also noted an increased occurrence of enteric fever during the monsoon period. The seasonal variation in enteric fever cases can be attributed to environmental factors associated with the rainy season. Specifically, the increased risk may be due to flooding: heavy rains can lead to floods, which may compromise water quality, water treatment plant issues.[17,20]

Although, no significant association was found between gender and disease incidence, thisstudy revealed a higher proportion of cases in males (65.22%) compared to females (34.78%). Table III finding aligns closely with the results reported by Maharjan A et al. who observed a similar gender distribution (65% males vs. 35% females). The higher incidence of enteric fever among males may be attributed to their increased participation in outdoor activities, potentially exposing them to sources of infection more frequently.[17,21,22]

In this study, Young Adult and Children are more prone to develop enteric fever by Salmonella typhi 43.48% and 42.03% respectively, Similar studies found iby Maharjan A et al. patients of the age group 21–30 years (47.5%) were the most affected by enteric fever, followed by the age group 11–20 years (32.5%).[17]

In recent study, *Salmonella typhi*isolates showed least resistance to Amikacin 04 (6.25%) Ceftazidime 06 (9.37%) and Amoxiclav 12 (18.75%) and high resistance to Azithromycin 56 (87.5%). According to Uddin et al., 2022 from Bangladesh reported, the highest antimicrobial susceptibility to *S. typhi* was observed for meropenem (77.5%), gentamicin and imipenem (72.5%) and cefotaxime (70%) respectively.[23] These findings highlight differences in antibiotic susceptibility patterns between the two studies. The first study shows high resistance to Azithromycin, while another study reports high susceptibility to several antibiotics, particularly Meropenem. This variation could be due to differences in geographical location, antibiotic usage patterns or local resistance trends. It's important to note that antibiotic resistance patterns can vary significantly across regions and over time, emphasizing the need for ongoing surveillance and appropriate antibiotic stewardship.[23]

In present study, isolates of *Salmonella para typhi* demonstrated excellent antibiotic sensitivity profiles, showcasing a remarkable 100% sensitivity to Amikacin, Gentamicin, Imipenem and Tazobactam-Piperacillin.

Additionally, these isolates revealed an encouraging 80% sensitivity to Amoxicillin-Clavulanate, Ceftriaxone, Chloramphenicol 93%, Ampicillin 71%, Cotrimoxazole 53%. These findings are particularly significant, as they align with the research conducted by Veeraraghavan et al. in India, which reported a comparable trend of 100% sensitivity to Ceftriaxone, Chloramphenicol and Cefixime.[23]

This consistent pattern of sensitivity is promising and indicates a positive outlook for the treatment of infections caused by *Salmonella typhi* and *Salmonella para typhi*. It highlights the importance of ongoing monitoring and responsible antibiotic use to maintain the efficacy of these treatments. As science is advancing day by day, attempt should continue to advocate for further research and collaborative efforts to ensure the effective management of Salmonella infections and potentially contribute to the global knowledge pool in this area. Combined efforts can make significant strides in combating antibiotic resistance and fostering better health outcomes, for better treatment and to overcome drug resistance against the microorganisms.

Conclusion

Enteric fever is still a significant cause of morbidity and mortality in developing countries. The diagnostic dilemma in most cases is mainly due to the diverse clinical presentation and lack of accurate diagnostic methods.Global death occurrence yearly 128000-161000 due to enteric fever, the incidence rate in Bangladesh is 252/100000 (WHO). Lots of complications like intestinal perforation, gastrointestinal hemorrhage, hepatitis, cholecystitis, myocarditis, shock, encephalopathy, pneumonia, anemia etc can occur due to this disease, some of which are very fatal condition. This study revealed a high rate of resistance among Salmonella isolates to Azithromycin, whereas the re-emergence of susceptibility was observed to the conventional drugs including third-generation Cephalosporins, Chloramphenicol, Ciprofloxacin which is suggestive of possible re-introduction of such drugs in case management.

Acknowledgements

Disclosure

The authors declared no conflicts of interest.

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