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In Vitro investigation on antimicrobial sensitivity pattern of enteric fever causing bacteria isolated from different clinical sources in Dhaka City, Bangladesh

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Abstract
Background: Enteric fever continues to remain a major public health problem, especially in Dhaka city, Bangladesh due to poor sanitation and water supply as well as self medication. In this study we evaluate the prevalence and antibiotic sensitivity pattern of *Salmonella* typhi and *Salmonella* paratyphi isolates.
Methods: A total of 106 clinical isolates were collected and processed and identified by standard microbiological method and biochemical tests and antimicrobial susceptibility testing was carried out by a standard disc diffusion method.
Result: Out of all clinical isolates 83.49% of these isolates were *Salmonella* typhi and 16.50% were *Salmonella* paratyphi *A*. *Salmonella* typhi was found to be most sensitive to levofloxacin, aztreonam, imipenam and less sensitive to ciprofloxacin (25.58%), azithromycin (27.9%), amoxyclav (37.2%). *Salmonella* paratyphi *A* was found to be most sensitive to ceftriaxone, levofloxacin, aztreonam, cefixime, gatifloxacin, imipenam and showed least sensitive to azithromycin (23.53%), ciprofloxacin (29.41%). Overall 44.7% (n= 46) of the isolates were multidrug-resistant.
Conclusion: This study suggests the further monitoring of efficacy of older and newer antimicrobial agents for the proper treatment and management of enteric fever.

Keywords: *Salmonella* typhi, *Salmonella* paratyphi *A*, Clinical isolates, Antimicrobial sensitivity, Multi-drug resistance.

Introduction

Typhoid and paratyphoid fevers are commonly grouped together under the collective term enteric fever. Enteric fever is a systemic infection caused by *Salmonella* typhi and *Salmonella* paratyphi *A, B* or *C* that can only survive in human beings. Despite the use of newly developed antibacterial drugs, enteric fevers such as typhoid and paratyphoid caused by multidrug-resistant bacterial strains are one of major health problems in Bangladesh, especially for the children [1]. Typhoid fever is the most serious form of enteric fever that causes bacteremia and inflammatory destruction of the intestine and other organs [2]. which is most in South Central Asia and Southest Asia with more than 100 cases per 100000 person per year with 22 million new cases of enteric fever annually which kills an estimated 200,000 people every year [3]. where there is rapid population growth, substandard water supply and lack of sanitation. In Bangladesh, enteric fever is not only a problem for patients, but also for physicians, because patients often present to medical care after taking one or more antibiotics by themselves or according to the advice of physicians, resulting in no growth of *Salmonella* typhi or paratyphi in blood cultures. Though antimicrobial therapy markedly reduces the morbidity and mortality, emergence of resistance to first line antibiotics poses challenge in its management [4].

Several treatment failure of enteric fever patients with chloramphenicol, azithromycin ciprofloxacin and some other fluoroquinolones have been reported in Bangladesh, where both ciprofloxacin and chloramphenicol considered as the first line choice of drug for the treatment of this fatal infection [5, 2, 6]. Further, resistance to third generation cephalosporins have also been reported earlier in Bangladesh and other countries [7-9]. However, as first line drugs are not used in enteric fever because of the high resistance rate, so they are regaining their sensitivity such as chloramphenicol [10, 11].

Recently, continued dependence on Ciprofloxacin and azithromycin for the empirical treatment of enteric fever in many developing countries, including Bangladesh has led to the emergence of resistance of *Salmonella* typhi and paratyphi to those drugs.

These observation will provide suitable guidelines for physicians to prescribe specific antibiotics for enteric fevers in specific cases for the patients of Bangladesh on the basis of sensitivity and resistance patterns of enteric fever causing bacteria against particular antibiotic.

Materials and Methods

A total of 106 samples (Blood, Stool) of enteric fever patients were collected from different diagnostic centers of Dhaka city, Bangladesh, from the period of August 2015 to March 2016. The samples were carefully and aseptically transferred to the laboratory for further examinations. The collected samples were cultured in nutrient agar media and stored at 2-8 ^{0}C for further examinations.

Identification of Organisms

For identification of different microorganisms selective media were used in this study. For example, Salmonella Shigella agar and Xylose-Lysine Deoxycholate agar were used for the identification of *Salmonella* typhi and *Salmonella* paratyphi.

Biochemical tests were also performed for specific identification of isolated microorganisms. Triple sugar ion agar test, citrate utilization test, motility test, indole test and urease tests were performed to identify bacteria.

Antibiotic Susceptibility Test

Antibiotic susceptibility test of isolates on commonly used antibiotics was performed on Muller-Hinton agar medium by disk diffusion technique according to the Clinical Laboratory Standard Institute (CLSI) guidelines [12]. Paper disks were impregnated with antibiotics namely: ceftriaxone (30 µg), levofloxacin (5 µg), azithromycin (15 µg), aztreonam (30 µg), cefixime (5 µg), gatifloxacin (5 µg), imipenam (10 µg), cephotaxime (30 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), doxycycline (30 µg), tetracycline (30 µg), amoxyclav (30 µg), chloramphenicol (30 µg), streptomycin (10 µg) (Hi Media Laboratory Ltd, Mumbai, India) respectively and incubated at 37°C for 24 hours. The medium containing antibiotic disc was quality controlled daily by standard culture. After defined incubation period, the diameter of the zone of inhibition was measured and interpretation of results based on CLSI guidelines [12].

Results

All the laboratory test results were assessed carefully and These results present the recent situation of antimicrobial sensitivity pattern of the isolates those were collected from the hospitalized patients of the different diagnostic center of Dhaka city, Bangladesh.

The age of study patients ranged from 1 to 60 years, Out of the 106 clinical isolates, 61 (57.55%) were from male and 46 (42.45%) were from female, in which 56.31% (n= 58) were found to be infected in case of male patients and in terms of female patients (n= 45, 43.69%) were found to be infected. The overall positive growth rate was relatively higher in male (56.31%) compared to female (43.69%) patients (Table 1).

Table 1: Isolates with enteric fever causing bacteria in two sex groups

Sex	No. of samples (%)	No. of positive cases (%)
Male	61 (57.55)	58 (56.31)
Female	45 (42.45)	45 (43.69)
Total	106 (100)	103 (97.17)

The distribution of growth, positive cases with enteric fever causing bacteria by age and sex, among them the highest number of patients with positive growth rates (n= 33, 32.04%) were belonged to the age of group 1-10 years. Similar trends in growth positivity were found between the age group of 40-50 (n= 1, 0.97%) and 51-60 (n= 1, 0.97%) years (Figure 1).

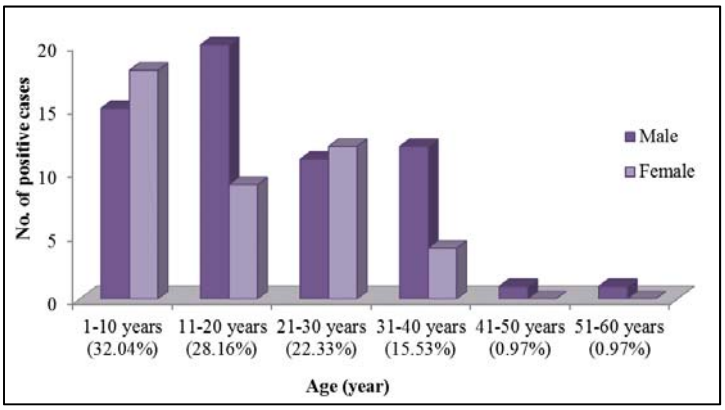

Fig 1: Distribution of positive cases with enteric fever causing bacteria by age and sex

By the whole of 101 blood samples and 5 stool samples cultured, the overall growth positivity was found in case of 97.17% (n= 103) isolates. The most common infecting organisms isolated in this study were presumptively identified as *Salmonella* typhi (n= 86, 83.49%) and *Salmonella* paratyphi *A* (n=17, 16.50%). *Salmonella* paratyphi *B* and *Salmonella* paratyphi *C* were not isolated in this study (Figure 2).

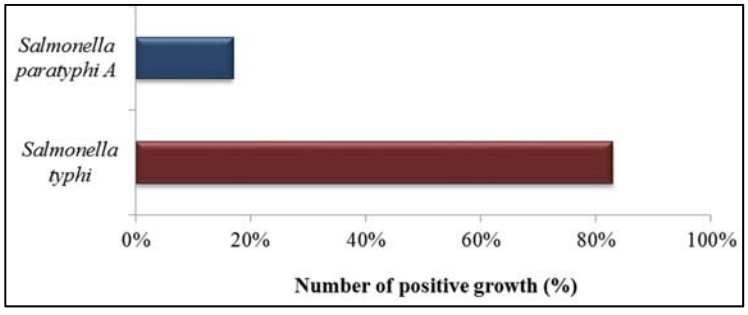

Fig 2: Distribution of isolated organisms

Among the tested fifteen antibiotics *Salmonella* typhi (Figure 3) was found to be most sensitive to levofloxacin (100%), aztreonam (100%), imipenam (100%) followed by ceftriaxone (96.51%), gatifloxacin (96.5%), cephotaxime

(93.02%), cefixime (93.02%), chloramphenicol (80.23%). It was found to be least sensitive to ciprofloxacin (25.58%) followed by azithromycin (27.90%), amoxyclav (37.20%), co-trimoxazole (54.65%), tetracycline (63.95%), doxycycline (69.76%), streptomycin (67.44%).

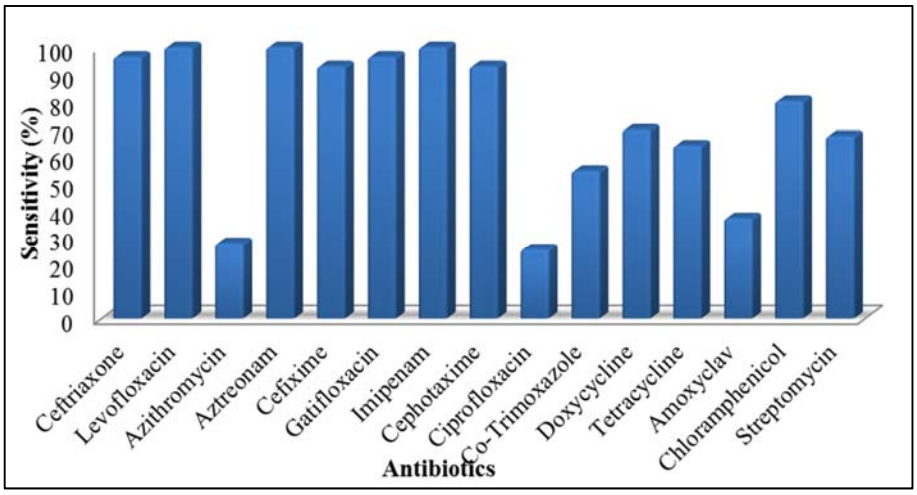

Fig 3: Antimicrobial sensitivity pattern against clinical isolates of *Salmonella* typhi

Among the tested fifteen antibiotics *Salmonella* paratyphi *A* (Figure 4) was found to be most sensitive to ceftriaxone (100%), levofloxacin (100%), aztreonam (100%), cefixime (100%), gatifloxacin (100%), imipenam (100%) followed by cephotaxime (94.12%), tetracycline (82.35%). It was found to be least sensitive to azithromycin (23.53%) followed by ciprofloxacin (29.41%), amoxyclav (52.94%), doxycycline (64.71%), chloramphenicol (64.71%), streptomycin (64.71%), co-trimoxazole (76.47%).

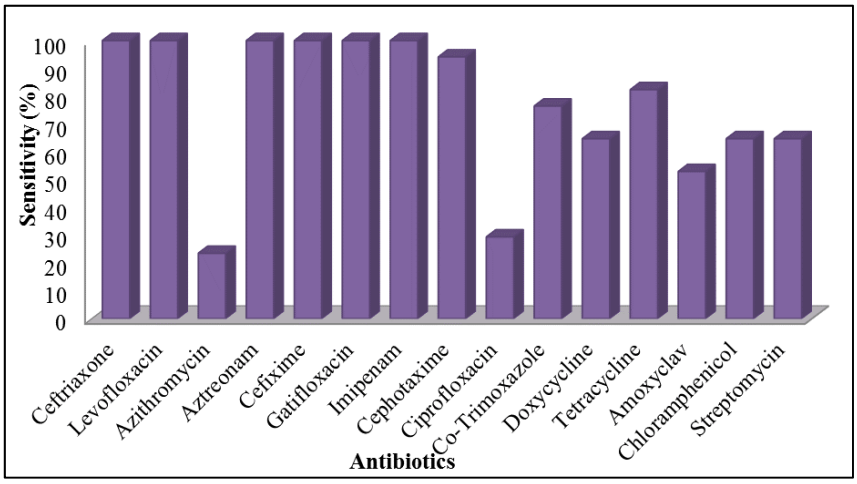

Fig 4: Antimicrobial sensitivity pattern against clinical isolates of *Salmonella* paratyphi *A*

Out of 103 isolates, (Table 2) 45 (43.68%) isolates of *Salmonella* typhi were sensitive to all antibiotics and 12 (11.7%) isolates of *Salmonella* Paratyphi *A* were sensitive to all antibiotics. Total 46 (44.7%) isolates were resistant to two or more drugs.

Further, 13% of Multi-drug resistance (MDR) *Salmonella* typhi were observed against cefixime, azithromycin and ciprofloxacin; same rates were observed in terms of cotrimoxazole and amoxyclav. On the other hand 10.8% of *Salmonella* isolates were combinely resistant to tetracyclines (tetracyclines and doxycycline) whereas in terms of *Salmonella* paratyphi *A*, the resist pattern was decreased up to 2.17%. In case of *Salmonella* typhi 8.7% resistance to tetracycline and amixoclav was also observed.

Table 2: Susceptibilities of multidrug resistance against clinical isolates of *Salmonella* typhi and paratyphi *A*

Multi-drug Resistant	*Salmonella* typhi	*Salmonella* paratyphi *A*	Total
None	45	12	57
MDR	41	5	46
Total	86	17	103

Discussion

Enteric fever is an endemic disease in the tropic and sub-tropic regions of the world and has become a major public health problem in developing countries including Bangladesh. Isolation of *Salmonella* species occurs throughout the year. The occurrence of enteric fever was higher in summer month

[13] mainly rainy season, but now a day it spreads highly among the community in all seasons of the year. Poverty and lack of proper health care facilities hamper national growth and incite disease burden in Bangladesh. Several studies has been conducted by many researchers in different parts of the world, establishing the significance of *Salmonella* typhi and *Salmonella* paratyphi *A* in the causation of salmonellosis. The above two species of *Salmonella* were encountered in the present study. Among the *Salmonella* species isolated *Salmonella* typhi (83.49%) was predominant, compared to *Salmonella* paratyphi *A* (16.50%).

The highest prevalence rate was observed in adult whose age was between 1 to 20 years whwreas only 2 positive cases were found in between 41 to 60 years age patients. Similar trends were observed earlier [13-15, 10].

In this study, males (56.31%) were marginally higher incidence compared to female (43.69%). These were similar to those reported earlier [6, 15, 11].

The result of this study showed that, 44.7% of isolates were resistant to two or more drugs where 43.68% were *Salmonella* typhi and 11.7% were *Salmonella* paratyphi *A*. Most of these multi-drug resistant isolates showed reduced susceptibility to ciprofloxacin, azithromycin and co-trimoxazole.

In this study Ceftriaxone was 100%, susceptible to *Salmonella* paratyphi *A,* which wasn't supported by Poudel *et al* (2014) [4]. who showed approximately 5.4% resistance to this drug. In case of *Salmonella* typhi 3.49% (n= 3) showed resistance to Ceftriaxone, this finding was closer to the finding reported earlier in Pakistan, Bangladesh and Nepal [9, 7, 10, 4, 8]. In the present study, 6.98% (n= 6) resistance to cefixime was observed against s*almonella* typhi, which was observed earlier in Bangladesh [16, 17, 5].

Ciprofloxacin and azithromycin has been considered a better choice as an antibiotic to treat enteric fever. In this study, 25.58% (*Salmonella* typhi) and 29.41% (*Salmonella* paratyphi *A*) of isolates displayed susceptibility to ciprofloxacin. In India Choudhary *et al* (2013) [18] showed that ciprofloxacin was 55% (n= 177) sensitive to *Salmonella* typhi and paratyphi. Ciprofloxacin resistant strains of *Salmonella* typhi in typhoid patients with decreased ciprofloxacin susceptibility are likely to complicate typhoid fever treatment procedures in several countries [19-21, 5].

The present study showed that 27.90% *Salmonella* typhi *and* 23.53% *Salmonella* paratyphi *A* were susceptible to azithromycin. Significant numbers of isolates were resistant to azithromycin as seen in other studies [22, 2, 18, 5].

As first line drugs are not used in enteric fever because of the high resistance rate, so they are regaining their sensitivity. From this study it can be concluded that Chloramphenicol is still a little bit sensitive to *Salmonella* typhi (80.23%) and *Salmonella* paratyphi *A* (64.71%) even though these antibiotic is no longer used for the treatment of typhoid fever in the study area on account of adverse reactions. In Nigeria, Ishaleku *et al* (2015) [11] have reported of 85.9% and 86.4% sensitivity against *Salmonella* typhi and *Salmonella* paratyphi respectively. In Bangladesh, low susceptibility to chloramphenicol was observed in case of *Salmonella* typhi [6, 2].

Co-trimoxazole is the second cost effective drug used to treat typhi and paratyphi infection. In this study, it was worth noting of lower sensitivity to cotrimoxazole 54.65% (*Salmonella* typhi), 76.47% (*Salmonella* paratyphi *A*). This was similar to those reported earlier 58% by Shahriar and Kabir (2010) [2], 64.3% by Prajapati *et al* (2008) [14], 74.4% by Mubeena *et al* (2006) [23] and 82.73% by Akter *et al* (2012) [24]. However Hasasn *et al* (2011) [6] showed that only 37.5% (n= 6) were sensitive to co-trimoxazole in case of *Salmonella* typhi in Bangladesh, which was much lower than this present study.

Conclusion

High physicians consultation cost, easy access to the antibiotics and lack of awareness about the health consequences of self-medication, high morbidity and economic loss have lead to the emergence of multidrug-resistance *Salmonella* strains in Bangladesh. Antibiotics are unnecessarily prescribed for common cold, cough, normal fever conditions and diarrhea in Bangladesh. Physicians, therefore, should always be aware of the antibiotic sensitivity/resistance profile of the organism for the rational use of antibiotics. Therefore, it might be recommended that blood/stool/urine cultures should be sent to diagnostic centre for characteristic of a particular pathogen before prescribing antibiotics in suspected enteric fever patients to prevent further resistance. Improvement in sanitation and water supply systems has been suggested as a method to control epidemics of enteric fever in Bangladesh as these factors are the tip of the iceberg.

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