Antibiotic resistance in salmonella typhi and salmonella paratyphi a among enteric fever patients of dhaka, bangladesh

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Abstract

Enteric fever is a severe public health threat because of rising antibiotic resistance of Salmonella spp. in developing countries, especially in endemic areas like Bangladesh. This retrospective study was aimed to assess the effectiveness of a range of 17 commonly used antimicrobials against Salmonella typhi (S. typhi) and Salmonella paratyphi A (S. paratyphi A) isolated from 601 enteric fever cases in Dhaka, Bangladesh. Conventional biochemical tests were used to identify Salmonella strains, and the Kirby-Bauer disc diffusion method to perform the antibiotic sensitivity in SAIC Digital Diagnostic Lab, Dhaka. We followed the National Committee for Clinical Laboratory (NCCL) guidelines to interpret the antibiogram results and applied statistical software SPSS (version 22.0) to analyze the obtained data. The male patients (54.74%) predominated over their female counterparts (45.26%). The patients' ages ranged from 1 month to 75 years, with a mean of 19.74±12.79 years. Of 601 Salmonella spp. isolates, S. typhi infections (56.57%) prevailed over those of S. paratyphi A (43.42%). Both strains showed >85% antimicrobial insusceptibility to three major antibiotics: ciprofloxacin, gentamicin, and ampicillin. S. typhi showed significantly greater resistance, 65.29%, to azithromycin than S. paratyphi A, 14.9% (p<0.001). Both pathogens reported over 95% sensitivity to ceftriaxone, cefixime, ceftazidime, amoxiclav, cephalaxin, aztreonam, imipenem, and cefuroxime. We observed an increased rate of antibiotic resistance of Salmonella spp. to several critical antimicrobials which were earlier effective against that pathogens. This outcome of current antibiotic susceptibility patterns of S. typhi and S. paratyphi A would contribute the medical practitioners to making informed decisions and providing better treatment to the patients in concern.

Keywords: Biochemical tests, Salmonella, Antimicrobials, Drug sensitivity

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1. Introduction

Enteric fever is a life-threatening systemic illness caused by Gram-negative S. typhi and S. paratyphi A (Crump and Mintz, 2010). Each year, it attacks almost 16 million people and over 153,000 deaths worldwide, most of them belong to South Asia and sub-Saharan Africa. In 2017, nearly 17 million people worldwide got infected and badly 117,000 victims lost their precious lives with a mortality rate of 4 to 5% (Global Burden of Disease Study, 2017). Its widespread prevalence in Asia and Africa is primarily for inadequate food and water safety. This fatal contagious disease has become endemic in developing and tropical countries like Bangladesh (Crump and Mintz, 2010; Kirk et al., 2015). Between 2003 and 2004, Bangladesh reported enteric fever incidence as 200 episodes per 100,000 individuals per year compared to 394.2 episodes per 100,000 individuals in South Asia (Saha et al., 2018). One recent study by Ahmed D et al., explored the bacterial etiology of bloodstream infections and found S. typhi and S. paratyphi A as the most frequently isolated organism (36.9% of samples) with a high percentage of those strains were multidrug-resistant (MDR) (Ahmed et al., 2017). Much to the reason of our apprehension, younger children have experienced the highest incidence of enteric fever compared to similar cases of Vietnam and other comparable regions (Brooks et al., 2005). We know this deadly infection as typhoid when caused by S. typhi and paratyphoid fever when by S. paratyphi. That pathogens transmit through the oral/fecal route and manifest the morbidity by the signs of fever, abdominal pain, and nonspecific symptoms, including nausea, vomiting, headache, and anorexia (Connor and Schwartz, 2005; Sur et al., 2007). When ingested, these Salmonella spp. bacteria colonize the small and large intestines, invade the gastrointestinal barrier, and then spread to the vital organs such as the liver, spleen and bone marrow (Raffatellu et al., 2008). However, timely and suitable antibiotic treatment cures enteric fever. But, available antibiotics as the effective treatment options are reducing day by day because of their growing antimicrobial resistance against S. typhi (Das et al., 2017; S. K. Saha et al., 1997). This situation has been deteriorating in low and middle-income countries abruptly because of higher rate of antimicrobial resistance of S. typhi and S. paratyphi A strains caused by multiple factors like incomplete treatment, overuse, and over-the-counter availability of antibiotics. Several reports confirmed the MDR of S. typhi against ampicillin, chloramphenicol, and cotrimoxazole in the early 1970s and ciprofloxacin resistance by these pathogens first began in the 1990s (Olarte and Galindo, 1973). Nowadays, roughly 90% of clinical isolates from the urban settings of endemic regions showed decreased sensitivity to ciprofloxacin (Das et al., 2017; Iyer et al., 2017). Later, this trend also shifted to other classes of antibiotics such as azithromycin and ceftriaxone (Das et al., 2017). A recent study from Pakistan also revealed the S. typhi has induced extensive drug-resistance to ciprofloxacin and ceftriaxone (Klemm et al., 2018). Therefore, this study was carried out to observe the current antibiotic susceptibility patterns of S. typhi and S. paratyphi A isolated from the blood samples of enteric fever cases. We hope this study’s outcomes would benefit healthcare professionals in making informed decisions and providing better treatment for enteric fever patients in the coming days.

2. Methods

A retrospective study spanning approximately one year (January 2019 to November 2019) was conducted based on the laboratory records of the SAIC Digital Diagnostic Lab database, Dhaka. 601 blood culture-positive samples collected from the enteric fever patients were assigned for the study. The Institutional Review Board and chairperson of the SAIC Digital Diagnostic Lab, Dhaka, acknowledged the required ethical approval for the study. We ensured the patients did not receive any antibiotics before 8 hours of their sample collection. Gram-staining and conventional biochemical methods were used to identify the Salmonella isolates. A culture media enriched with brain-heart infusion (BHI) broth was used to support the likely growth of pathogens. Following the inoculation, the media was incubated and sub-cultured into Salmonella-Shigella agar, blood agar, and Mac-Conkey agar. Triple sugar iron (TSI) agar was used initially to differentiate the isolated Salmonella strains, resulting in alkaline slant, acidic butt and H2S production. S. typhi produced H2S but not gas, whereas S. paratyphi A generated gas but not the H2S. Both strains were motile but showed negative reactions in indole, citrate and urease tests. Finally, to determine the antibiotic susceptibility of Salmonella isolates, the Kirby-Bauer disc-diffusion method was performed on Muller-Hinton agar plates. Subsequently, the antimicrobial sensitivity patterns were interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards, 1997). The list of 17 antibiotics tested was given in the Table 1. Finally, Microsoft Excel-2019 was used to tabulate and illustrate the data graphically, whereas SPSS-22 to perform descriptive statistics, including Chi-square and Student-t tests at 0.05 level of significance.
Table 1: Antibiotics used in the study

| Antibiotics     | Types                                      |
|-----------------|--------------------------------------------|
| Cefepime (30 μg)| Cephalosporins of 4th generation          |
| Ceftriaxon (30 μg)| Cephalosporins of 3rd generation         |
| Imipenem (10 μg)| Cephalosporins of 3rd generation          |
| Tetracycline (30 μg)| Glycylcyclines of 3rd generation     |
| Cefixime (5 μg)  | Cephalosporins of 3rd generation          |
| Ceftazidime (30 μg)| Cephalosporins of 3rd generation  |
| Cephalexin (30 μg)| Cephalosporins of 1st generation         |
| Cotrimoxazole (25 μg)| Trimethoprim/sulfamethoxazole   |
| Piperacillin (75 μg)| Cephalosporins of 3rd generation  |
| Aztreonam (30 μg)| Beta-lactam antibiotics                |
| Ampicillin (10 μg)| Penicillin                              |
| Cefuroxime (30 μg)| Cephalosporins of 2nd generation         |
| Ciprofloxacin (5 μg)| Fluoroquinolone of 2nd generation   |
| Gentamicin (10 μg)| Aminoglycosides                          |
| Amikacin (30 μg)| Aminoglycosides                          |
| Amoxiclav (30 μg)| Combination of amoxicillin, a β-lactam antibiotic and potassium clavulanate, a β-lactamase inhibitor |
| Azithromycin (15 μg)| Azalide, a type of macrolide antibiotic |

3. Results

Of 601 Salmonella isolates, 340 (56.57%) and 261 (43.42%) were confirmed as S. typhi and S. paratyphi A, respectively. Among the patients, the number of males (54.74%) predominated their female counterparts (45.26%). The proportion of both genders based on the infections by S. typhi and S. paratyphi A was insignificantly similar (p>0.05). Males and females both suffered more by S. typhi than S. paratyphi A about 60% males and 57% females were tested positive for by S. typhi. The patients’ ages ranged from 1 month to 75 years, with a mean of 19.74±12.79 years. The average age of the patients infected by S. typhi and S. paratyphi A was nearly the same, 19.64±13.39, and 19.87±11.97 years, respectively. The majority of the cases, about 83%, aged between 5 to 40 years old. Patients aged 5 to 20 years contributed to the maximum enteric fever cases (47.42%) followed by 21 to 40 years (35.77%). The least number of patients (1.5%) belonged to the age group >60 years. When S. typhi and S. paratyphi cases were distributed within different age groups, we observed that the number of typhoid patients was more than the paratyphoid patients in each age group. Within the groups of 41-60 and >60 years, the typhoid patients nearly doubled the paratyphoid. The infection by the both pathogens was most common among the age groups of 5 to 20 years, followed by 21 to 40 years (Table 1). S. typhi and S. paratyphi A showed >85% antimicrobial insusceptibility against three major antibiotics ciprofloxacin, gentamycin, and ampicillin. However, they were nearly 20% resistant against cotrimoxazole, piperacillin, ampicillin, and azithromycin. Interestingly, five out of 17 antimicrobials tested: cefixime, ceftazidime, cephalexin, aztreonam,
and amoxyline presented almost invariable effectiveness against nearly all typhoid and paratyphoid cases. Ten drugs were the highest sensitive to \textit{S. typhi}, showing over 95% susceptibility such as ceftriaxone (99.71%), ceftazidime (99.71%), cefixime (99.41%), cephalexin (98.51%), cefuroxime (98.23%), imipenem (97.35%), amoxiclav (97.31%), aztreonam (97.30%), and tetracycline (97.31%). In striking resemblance with \textit{S. typhi}, eight out of that ten antimicrobials had over 95% efficacy against \textit{S. paratyphi A} as following ceftriaxone (99.2%), cefixime (98.9%), ceftazidime (98.9%), amoxiclav (98.1%), cephalexin (97.7%), aztreonam (96.5%), imipenem (96.2%), and cefuroxime (96.2%). On the other hand, \textit{S. typhi} demonstrated as high as over 85% resistance to the antibiotics like gentamycin (99.12%), amikacin (99.41%), and ciprofloxacin (85.50%), whereas other antimicrobials showed lower resistance to this pathogen; azithromycin (65.29%), cotrimoxazole (77.65%), piperacillin (66.19%), and ampicillin (19.53%) (Table 2). Likewise, \textit{S. paratyphi A} too showed over 85% insensitivity to the gentamycin (99.1%), amikacin (98.5%), and ciprofloxacin (88.5%) followed by cotrimoxazole (12.7%), piperacillin (13.6%), and azithromycin (14.9%). (Table 2). When the sensitivity of each antibiotic was allocated against the type of \textit{Salmonella} spp. several significant variations (p<0.05) was observed in their susceptibility. Cefepime showed significantly unparallel resistance to \textit{S. typhi} (.35%) and \textit{S. paratyphi A} (16.9%) (p<0.001). Cotrimoxazole was two times ineffective against \textit{S. typhi} (22.65%) than \textit{S. paratyphi A} (12.7%) (p=0.002). \textit{S. typhi} (19.53%) was about double insensitive to ampicillin than \textit{S. paratyphi A} (8.1%) (p<0.001). Overwhelmingly, \textit{S. typhi} (65.29%) was about five times more resistant to azithromycin than \textit{S. paratyphi A} (14.9%) (p<0.001).

### Table 2: Distributions of positive cases based on sex and age of the patients

| Patients' sex and age | Salmonella spp. | Total n (%) | Statistical Tests |
|-----------------------|-----------------|--------------|-------------------|
|                       | Salmonella paratyphi A | Salmonella typhi |                |
|                       | n (%)            | n (%)        |                  |
| Sex                   |                  |              |                  |
| Male                  | 145 (44.1)       | 184 (55.9)   | 329 (54.74)      | \( \chi^2 = 0.123 \) p = 0.726 |
| Female                | 116 (42.6)       | 156 (57.4)   | 272 (45.26)      |                  |
| Total n (%)           | 261 (43.42)      | 340 (56.57)  | 601(100)         |                  |
| Age group             |                  |              |                  |
| <5 years              | 31 (47.7)        | 34 (52.3)    | 65 (10.82)       | \( \chi^2 = 6.184 \) p = 0.186 |
| 5-20 years            | 115 (40.4)       | 170 (59.6)   | 285 (47.42)      |                  |
| 21-40 years           | 104 (48.4)       | 111(51.6)    | 215 (35.77)      |                  |
| 41-60 years           | 8 (29.6)         | 19 (70.4)    | 27 (4.49)        |                  |
| >60 years             | 3 (33.3)         | 6 (66.7)     | 9 (1.50)         |                  |
| Mean±SD of age (years)| 19.87±11.97      | 19.64±13.39  | 19.74±12.79      | t = 0.218, p = 0.827 |
| Median age (years)    | 19.00            | 17.00        | 18.00            |                  |
| Range of age          | –                | –            | 1 month to 75 years |                  |

**Note:** \( \chi^2 = \) Chi-square Value, p=significance value at (\( \alpha = 0.05 \)).
Table 3: Patterns of antibiotic sensitivity of both Salmonella

| Antibiotics | Sensitivity | Salmonella spp. | Chi-square | p     |
|-------------|-------------|-----------------|------------|-------|
|             |             | Salmonella typhi (%) | Salmonella paratyphi An (%) |          |       |
|             |             | 285(99.65) | 217(83.1) | 49.20 | <.001 |
| Cefepime    |Sensitive     |             |            |       |
|             |Resistant     | 1(0.35)    | 44(16.9)   |       |
| Ceftriaxone |Sensitive     | 339(99.71) | 259(99.2) | .663  | .416  |
|             |Resistant     | 1(0.29)    | 2(0.8)     |       |
| Imipenem    |Sensitive     | 331(97.35) | 251(96.2) | .677  | .411  |
|             |Resistant     | 9(2.65)    | 10(3.8)    |       |
| Tetracycline|Sensitive     | 332(96.51) | 244(93.5) | 2.380 | .123  |
|             |Resistant     | 13(3.78)   | 17(6.5)    |       |
| Cefixime    |Sensitive     | 338(99.41) | 258(98.9) | .564  | .453  |
|             |Resistant     | 2(0.59)    | 3(1.1)     |       |
| Ceftazidine |Sensitive     | 338(99.71) | 258(98.9) | .664  | .413  |
|             |Resistant     | 1(0.29)    | 2(1.1)     |       |
| Cephalexin  |Sensitive     | 331(98.51) | 250(97.7) | .583  | .445  |
|             |Resistant     | 5(1.29)    | 6(2.3)     |       |
| Cotrimoxazole|Sensitive    | 263(77.35) | 227(87.3) | 9.752 | .002  |
|              |Resistant     | 77(22.65)  | 33(12.7)   |       |
| Piperacillin |Sensitive     | 262(78.68) | 216(86.4) | 5.76  | .016  |
|             |Resistant     | 71(21.32)  | 34(13.6)   |       |
| Aztreonam   |Sensitive     | 324(97.30) | 251(96.5) | .286  | .593  |
|             |Resistant     | 9(2.70)    | 9(3.5)     |       |
| Ampicillin  |Sensitive     | 272(80.47) | 239(91.9) | 15.49 | <.001 |
|             |Resistant     | 66(19.53)  | 21(8.1)    |       |
| Ceftazoxime |Sensitive     | 333(96.23) | 251(96.2) | 2.415 | .120  |
|             |Resistant     | 6(1.77)    | 10(3.8)    |       |
| Ciprofloxacin|Sensitive    | 49(14.50)  | 30(11.5)   | 1.160 | .280  |
|             |Resistant     | 289(85.50) | 231(88.5)  |       |
| Gentamycin  |Sensitive     | 3(0.88)    | 1(0.9)     | .557  | .456  |
|             |Resistant     | 337(99.12) | 260(99.1)  |       |
| Amikacin    |Sensitive     | 2(0.59)    | 4(1.5)     | 1.315 | .252  |
|             |Resistant     | 336(99.41) | 257(98.5)  |       |
Table 3 (Cont.)

| Antibiotics     | Sensitivity | Salmonella typhi (%) | Salmonella paratyphi An (%) | Chi-square | p     |
|-----------------|-------------|----------------------|-----------------------------|------------|-------|
|                 |             |                      |                             |            |       |
| Amoxyclav       | Sensitive   | 325(97.31)           | 255(98.1)                   | .378       | .539  |
|                 | Resistant   | 9(2.69)              | 5(1.9)                      |            |       |
| Azithromycin    | Sensitive   | 118(34.71)           | 222(85.5)                   | 152.370    | <.001 |
|                 | Resistant   | 222(65.29)           | 39(14.9)                    |            |       |

4. Discussion

Enteric fever is a growing public health concern in developing and tropical countries, including Bangladesh. Indiscriminate use of antibiotics intensifies the problem by making previously effective drugs resistant to the Salmonella spp. In the present study, we tried to investigate the existing antibiotic susceptibility of S. typhi and S. paratyphi A in Dhaka city for last 11 months of 2019. We found S. typhi (56.57%) affected more individuals than S. paratyphi A (43.42%), which is consistent with the previous studies. One study presented that S. typhi were 66.6% and S. paratyphi A were 33.3% responsible for the enteric fever (Guha et al., 2005). Strikingly similar to our finding, Raza et al. (2012) found that 55.8% of the cases were diseased by S. typhi and 44.2% with S. paratyphi A. For both type of Salmonella infections, male patients were dominant over the females, with a proportion 1.20:1. In several related studies, it was also observed that the males were more susceptible to Salmonella spp. compared to female individuals (Chowta and Chowta, 2005; Kumar et al., 2008). We found patients aged 5 to 20 years accounted for the maximum enteric fever cases (47.42%) and children under-5 years were less vulnerable to Salmonella spp. infections than their older peers. Likewise, another study observed highest number of patients (63.8%) were within the 6-15 years of age group, followed by 13(22.41%) in 16-25 years age group (Sattar et al., 2017). But some studies found under-5 year children are more frequently affected by typhoid fever than paratyphoid fever (Naheed et al., 2010; Sinha et al., 1999). Whereas, Brooks et al., (2005) found that children above-5 years were more susceptible to enteric fever than under-5 years, which is comparable to our findings. Although it has been suggested that young children are less prone to typhoid fever (Ferreccio et al., 1984; Khanam et al., 2015).

In this study, S. typhi was mostly sensitive to cefepime (99.65%), ceftriaxone (99.71%), tetracycline (96.51%), cefixime (99.41%), cefazidime (99.71%), cephalaxin (98.51%), cotrimoxazole (77.35%), piperacillin (78.68%), aztreonam (97.30%), amoxiclav (97.31%) and cefuroxime (98.23%). Not align with our findings, a similar study in Pakistan reported that the resistance S. typhi was 91.43% for cefixime, 85.71% for tetracycline, and 68.57% for ceftriaxone, respectively (Barman, et al., 2019). Rahman et al. (2015) presented that S. typhi was resistant to some antibiotics like gentamycin (99.1%), ampicillin (98.5%), cotrimoxazole (22.65%), piperacillin (21.32%). In correspondence with us, a likewise study in Pakistan reported that the resistance S. typhi was 66.1% for ampicillin, 68.5% for ceftriaxone (Qamar et al., 2014). In sharp contrast to us, a community-based 2001 to 2003 in Indonesia showed a low resistance of S. typhi (only 2.5%) against ampicillin, with no resistance against ceftriaxone, or ciprofloxacin (Punjabi et al., 2013). The antibiotic resistance
pattern may vary among the countries. We found both Salmonella spp. were resistant to azithromycin. Contrarily, two studies found azithromycin highly sensitive (Chandey & Multani, 2012). Again, the current study revealed S. paratyphi A was sensitive to cefepime (83.1%), ceftriaxone (99.2%), imipenem (96.2%), tetracycline (93.5%), cefixime (98.9%), ceftazidime (98.9%), cephalaxin (97.7%), cotrimoxazole (87.3%), piperacillin (86.4%), aztreonam (96.5%), amikacin (91.9%), amoxiclav (98.1%) and cefuroxime (96.2%). Accordingly, S. paratyphi A was sensitive to ceftriaxone (100%) (Bhatia et al., 2007). We observed S. paratyphi A showed resistance to some antibiotics like gentamycin (99.1%), cotrimoxazole (12.7%), piperacillin (13.6%), amikacin (98.5%), azithromycin (14.9%) and ciprofloxacin (88.5%). In contrast, Naheed et al., (2010) found all S. paratyphi A isolates were susceptible to all antimicrobial agents that they tested. In Bangladesh, alarmingly, both S. typhi and S. paratyphi A lost the susceptibility to azithromycin and ciprofloxacin. Azithromycin’s insusceptibility to Salmonella spp. poses an emerging public health concern as treatment failures have been reported (Molloy et al., 2010). Over-use of ciprofloxacin and azithromycin because of their over-the-counter accessibility and oral route administration along with incomplete treatment contributed to the antibiotic resistance of these drugs in Bangladesh. We observed, not any single antibiotic had complete susceptibility to the total S. typhi isolates tested. Unless this increasing antibiotic resistance rate for Salmonella is checked, options for treating enteric fever cases would be lost shortly. Bangladesh Government should cryingly implement a national guideline on the proper usage of antibiotics.

5. Conclusion
The study explored much-needed information about current antibiotic susceptibility patterns of S. typhi and S. paratyphi A to help the medical practitioners in making informed decisions and providing better treatment for enteric fever patients. Male and young aged individuals were more susceptible to enteric fevers compared to their counterparts. Both S. typhi and S. paratyphi A were equally highly resistant to some commonly used critical antibiotics. Several antimicrobials presented significant variation in resistance against S. typhi and S. paratyphi A. We expect researchers and policymakers to find this study helpful in prioritizing their research scopes to tackle the upcoming challenges of antibiotic resistance to prevent infectious diseases.

6. Declarations

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Ethical Approval
Ethical approval was obtained from the institutional review board of the university and the chairman of the Diagnostic Center.

Conflict of Interest
None.

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