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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 that of by *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. paratyhi A,* 14.9% (p<0.001). Both pathogens reported over 95% sensitivity to ceftriaxone, cefixime, ceftazidime, amoxiclav, cephalexin, 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 non-specific 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 H_2S production. *S. typhi* produced H_2S but not gas, whereas *S. paratyphi A* generated gas but not the H_2S. Both strains were motile but showed negative reactions in indole, citrate and urea 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 â-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, azetreonam,

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 n (%)	*Salmonella typhin* (%)		
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: χ^2 = Chi-square Value, p=significance value at ($\alpha = 0.05$).

and amoxyline presented almost invariable effectiveness against nearly all typhoid and paratyphoid cases. Ten drugs were the highest sensitive to *S. typhi*, showing over 95% susceptibility such as ceftriaxone 339(99.71%), ceftazidime 338(99.71%), cefepime 285(99.65%), cefixime 338(99.41%), cephalexin 331(98.51%), cefuroxime 333(98.23%), imipenem 331(97.35%), amoxiclav 325(97.31%) aztreonam 324(97.30%) and tetracycline 332(96.51%). In striking resemblance with *S. typhi*, eight out of that ten antimicrobials had over 95% efficacy against *S. paratyphi A* as following ceftriaxone 259(99.2%), cefixime 258(98.9%), ceftazidime 258(98.9%), amoxiclav 255(98.1%), cephalexin 250(97.7%), aztreonam 251(96.5%), imipenem 251(96.2%), and cefuroxime 251(96.2%). On the other hand, *S. typhi* demonstrated as high as over 85% resistance to the antibiotics like gentamycin 337(99.12%), amikacin 336(99.41%), and ciprofloxacin 289(85.50%), whereas other antimicrobials showed lower resistance to this pathogen; azithromycin 222(65.29%), cotrimoxazole 77(22.65%), piperacillin 71(21.32% and ampicillin 66(19.53%) (Table 2). In similar with the mentioned resistance rate by the *S. typhi*, *S. paratyphi A* was also sensitive to cefepime 217(83.1%), tetracycline 244(93.5%), cotrimoxazole 227(87.3%), piperacillin 216(86.4%), amikacin 239(91.9%). Likewise, *S. paratyphi A* too showed over 85% insensitivity to the gentamycin 260(99.1%), amikacin 257(98.5%), and ciprofloxacin 231(88.5%) followed by cotrimoxazole 33(12.7%), piperacillin 34(13.6%), azithromycin 39(14.9%). (Table 2). When the sensitivity of each antibiotic was allocated against the type of *Salmonella* spp. several significant variations ($p<0.05$) was observed in their susceptibility. Cefepime showed significantly unparallel resistance to *S. typhi* (.35%) and *S. paratyphi A* (16.9%) ($p<0.001$). Cotrimoxazole was two times ineffective against *S. typhi* (22.65%) than *S. paratyphi A* (12.7%) ($p=0.002$). *S. typhi* (19.53%) was about double insensitive to ampicilin than *S. paratyphi A* (8.1%) ($p<0.001$). Overwhelmingly, *S. typhi* (65.29%) was about five times more resistant to azithromycin than *S. paratyphi A* (14.9%) ($p<0.001$).

Table 3: Patterns of antibiotic sensitivity of both *Salmonella*

Antibiotics	**Sensitivity**	***Salmonella* spp.**		***Chi-square***	***p***
		***Salmonella typhi*n (%)**	***Salmonella paratyphi A*n (%)**		
Cefepime	Sensitive	285(99.65)	217(83.1)	49.20	<.001
	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)		
Ampicilin	Sensitive	272(80.47)	239(91.9)	15.49	<.001
	Resistant	66(19.53)	21(8.1)		
Cefuroxime	Sensitive	333(98.23)	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* spp.		*Chi-square*	*p*
		Salmonella typhi n (%)	*Salmonella paratyphi A* n (%)		
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%), ceftazidime (99.71%), cephalexin (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 Bangladeshi study in 2015 represented that the resistance rates of *S. typhi* were 97.14% for cotrimoxazole, 91.43% for cefixime, 85.71% for tetracycline, and 68.57% for ceftriaxone, respectively (Rahman, 2015). Our low resistance of ceftriaxone to *S. typhi* was also earlier found by another study (Britto *et al*., 2018). Similarly, Ahmed *et al*., (2019) showed *Salmonella* spp. was highly sensitive to cefixime and ceftriaxone (Ahmed *et al*., 2019). In this study, *S. typhi* was highly sensitive to imipenem (97.35%). Drug imipenem (carbapenem) maintained high sensitivities to *S. typhi* in many past studies. Rahman *et al*., (2015) reported increased sensitivity of *S. typhi* to imipenem (88.57%). Two studies in Indonesia and China also noticed low resistance of *S. typhi* to imipenem (Hardjo Lugito and Cucunawangsih, 2017; Yaxian *et al*., 2015). We found alarmingly high resistance of *S. typhi* against ciprofloxacin (85.50%) and azithromycin (65.29%). A similar trend was found in a relevant study revealing excessive resistance of azithromycin and ciprofloxacin as 95.29% and 77.14% respectively (Barman, 2018). Another study in Bangladesh also experienced reduced ciprofloxacin sensitivity for 74% S. typhi strains, compared to 50% in the United Kingdom (Threlfall and Ward, 2001). Similarly, decreased ciprofloxacin susceptibility for *S. typhi* has been witnessed by studies in India recently (Chandel and Chaudhry, 2001). We found *S. typhi* was resistant to some antibiotics likes 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 88.2% for ciprofloxacin, 66.1% for ampicillin (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%), cephalexin (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. typh*i 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 of 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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