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Antibiotic resistance pattern of *Salmonella typhi* in patients attending a teaching hospital in Bangladesh

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Abstract

Enteric fever or typhoid is caused by the human-specific gram-negative pathogen *Salmonella enterica* serovar *typhi* (*S. typhi*). The incidence of typhoid fever remains high in rural areas and the emergence of multidrug resistance has exacerbated the situation. The present study examined the antimicrobial susceptibility patterns of *Salmonella typhi* to the antibiotics commonly used in Bangladesh. This study also examined the status of prescription antibiotic use. From July 2016 to June 2017, a total of 40 bacterial pathogens were isolated from blood samples from 100 clinically suspected patients at a teaching hospital in Savar, Dhaka. The blood samples were subjected to analysis byWidal test, microscopy, culture and susceptibility testing using conventional bacteriological methods. A total of 40% of cases had an established bacterial etiology with *S. typhi*. The highest antimicrobial susceptibility to *S. typhi* was observed for meropenem (77.5%), gentamicin and imipenem (72.5%), and cefotaxime (70%), respectively. The maximum resistance was observed in the penicillin group, such as (80%) followed by cotrimoxazole (75%). Carbapenem-type beta-lactam antibiotics (meropenem, imipenem) and third-generation cephalosporins (cefotaxime, ceftriaxone, and ceftazidime) were the most effective drugs for typhoid fever. Prescription survey results in four cities in Bangladesh showed that typhoid patients were mainly prescribed third-generation cephalosporins such as cefixime (36.73%) and ceftriaxone (18.37%). The findings are important for an adequate identification of the pathogens and their antibiotic resistance for the correct selection of antibiotics in typhoid fever. The study has implications for the development of guidelines for prescribing antibiotics for typhoid fever in Bangladesh.

Key words: Enteric fever, antimicrobial resistance, *Salmonella typhi*, typhoid fever, blood culture, Widal test.

INTRODUCTION

Typhoid or enteric fever is a bacterial disease spread worldwide, transmitted by ingestion of food or water contaminated with feces from an infected person, which contain the bacterium *Salmonella enterica*, serovar *typhi* (WHO, 2018). According to a recently revised global estimate; it causes 21.6 million illnesses each year resulting in 216,500 deaths (Marathe *et al*., 2012). Typhoid accounts for approximately 16-30 million cases each year, occurring mainly in the developing world with a 10% mortality rate. Recent developments in mapping the Salmonella genome have provided insight into its pathogenicity and the development of antibiotic resistance and human immunity (Jamil *et al*., 2004). Enteric fever is very contagious. An infected person can shed the bacteria from

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their body with their stool when they have a bowel movement or, less commonly, when they urinate. If someone else eats food or drinks water contaminated with a small amount of infected feces or urine, they can get typhoid. The course of untreated enteric fever is usually divided into four individual stages, each lasting about a week. During these phases, the patient becomes exhausted and emaciated (Jamil *et al*., 2004; Farzana *et al*., 2015). The causative pathogens of typhoid can be spread through poor hygiene habits and public sanitation, and sometimes through flying insects that feed on faeces. *Salmonella typhi* only lives in humans. The pathophysiology of typhoid is not a simple process that goes through several stages. It can be diagnosed by taking blood, bone marrow, or stool cultures and using the Widal test. Typhoid fever can be prevented by maintaining hygiene and using vaccines. The aim of this study was to investigate the rate of resistance among typhus pathogens and to determine the current antibiotic resistance pattern of these bacterial isolates. Knowing the likely predominant strains along with their antimicrobial resistance pattern will aid in better patient management and antibiotic policy setting.

MATERIALS AND METHODS

Study design and area: This was a retrospective study of typhoid bacterial isolation pattern for all ages and both sexes to determine their antibiotic susceptibility pattern in Enam Medical College Hospital in Savar, Dhaka. The study period was from July 2016 to June 2017.

Study population and specimen collection: A total of 100 blood samples were collected from patients visiting different wards of the hospital. Blood samples were taken for culture sensitivity, Widal's test and ICT from 100 clinically suspected cases of typhoid to isolate the causative agents, both *Salmonella typhi* and *paratyphi*, to the microbiology laboratory for immediate analysis. Culture medium such as trypticase soy broth has been used to culture all common causative agents of bacteremia. Blood collection, incubation and subculture on MacConkey agar were performed according to standard methods (Collee *et al*., 1996). All *Salmonella typhi* isolates were tested for their antimicrobial susceptibility pattern on Mueller-Hinton agar media by the disc diffusion method against 14 antibiotics such as ampicillin (10 μg), ciprofloxacin (5 μg), cotrimoxazole (25 μg), ceftriaxone (30 μg), azithromycin (15 μg) and ceftazidime (30 μg). The disk strength and zone-size interpretation were in accordance with the National Committee for Clinical Laboratory Standards (NCCLS).

Isolation and identification of *Salmonella*: *Salmonella* isolation was performed using different types of plating media. The most commonly used media selective for *Salmonella* are Xylose Lisin Deoxycholate (XLD) Agar, Bismuth Sulphite Agar, SS Agar, Hektoen Enteric (HE) Medium, Brilliant Green Agar. All of these media contain both selective and diverse ingredients and are commercially available (Bauer *et al*., 1966; Akkerman *et al*., 2005).

Interpretation of zone: The antimicrobial susceptibility test can be performed based on the interpretation of the zones of inhibition created by each drug. Drugs have been classified into two susceptibility categories, Susceptible (S) and Resistant (R), based on

the National Committee for Clinical Laboratory Standards (NCCLS). However, intermediate strains were not considered in this study.

Study design: This was a retrospective study of the bacterial isolation pattern of respiratory specimens for all ages and genders and to determine their antibiotic susceptibility pattern in a teaching hospital in Savar, Dhaka. Samples taken for processing were sputum. The duration of the study was from July 2016 to June 2017.

Specimen collection: In the present study, a total of 100 sputum samples were collected from patients visiting different wards of the hospital. All patients had clinical signs of respiratory tract infection and a single positive culture per patient was included in the analysis. Specimens were collected aseptically from 100 patients. Each patient was instructed to collect the sputum samples and was immediately taken to the microbiology laboratory for analysis. Sputum samples were collected in well-labelled, sterile, screw-top glass bottles.

Specimen culture: The sputum was decontaminated using the standard alkaline decontamination method (WHO, 2012). The digested sputum samples were cultured on chocolate agar, sheep blood agar (5%) and MacConkey agar (Oxoid, UK) plates. On top of the chocolate agar, bacitracin (10units) and optochin discs (5g) (Oxoid, UK) were placed at the secondary inoculation to screen *S. pnemoniae*. The chocolate agar plates were incubated in an incubator (5-10% CO_2) at 37°C for 24-48 hours, while blood agar and MacConkey agar were incubated in an aerobic atmosphere at 37°C for 24 hours. Suspect colonies were then subcultured onto appropriate solid culture media for purification and thereafter preserved on appropriate agar slants and stored in a refrigerator (4°C)for subsequent analysis.

Identification of isolates was performed using standard microbiological techniques, which included colony morphological studies, Gramstain reactions, and a battery of biochemical tests as appropriate. A colonycount of 104 CFU/mL was considered significant for sputum, while 105 CFU/mL for other samples indicated infection.

Antibiotic susceptibility testing: The antimicrobial susceptibility of the test strains of fourteen antibiotics was determined using the Kirby-Bauer disk diffusion method (Bauer, 1966) according to the guidelines of the Clinical Laboratory Standards Institute (CLSI). Antibiotic discs were obtained from commercial suppliers of Becton Dickinson and Company, USA; Oxoid Ltd. Great Britain; Mast diagnosis, UK. The susceptibility patterns of the bacterial pathogens were determined to the following antimicrobial agents: azithromycin (AZT), amoxicillin(AMX), imipenem (IPM), cefotaxime (CFT), ceftriaxone (CTX), cefuroxime (CEF), ciprofloxacin (CPX), cloxacilin (CLX), gentamicin (GTM), meropenem (MPM), vancomycin (VCM), ceftazidime (CFZ), cotrimoxazole (CMX) and cefixime (CFM). A lawn of the test pathogen was prepared by spreading 100 L of inoculum smoothly over the entire surface of the agar plate using a sterilized spreader. The plates were dried prior to application of the antibiotic disc. Then, some commercially available antibiotic discs were carefully and firmly placed on the agar plates, which were then left at room temperature for 1 hour to allow the antibiotics to diffuse into the agar

medium. The plates were incubated at 37°C for 24 h and the diameters of the zones of inhibition were compared to those of the standard isolates (E. coli, ATCC 25922; P. aeruginosa, ATCC27853; S. aureus, ATCC 25923; S. pneumoniae, ATCC) 49619; K. pneumoniae, ATCC 700603) to determine sensitivity or resistance based on the guidelines of the Clinical and Laboratory Standards Institute, Wayne, Pennsylvania.

Quality control: Each batch of culture media used has been tested for sterility. Standard control strains of *S. typhi* strains CT18 (Parkhill *et al*., 2001) and Ty2 (Deng *et al*., 2003) and for *S. paratyphi* A strain ATCC9150 (McClelland *et al*., 2004) were used during cultivation and Antibiotic susceptibility testing will be used as a control throughout the study.

Survey of prescriptions: The study was conducted in four district cities in Bangladesh from July 2016 to June 2017to show the current trend of antibiotic use in relation to medical prescriptions. The prescribers came from different groups: general practitioners, general practitioners, specialists and consultants (Biswas *et al*., 2014). Prescribing data was randomly collected from the patients who came to buy the drugs at the drug stores. In this article, only the prescriptions of the qualified doctors were analyzed and presented. It should also be ascertained whether or not the patients have used antibiotics to treat the present situations of enteric fever.

Ethical consideration: During the study, the general principles (section 12) of the WMA Declaration of Helsinki were followed. Data were collected after obtaining written informed consent from each study participant. This survey-based research did not require further institutional ethics committee approval.

Statistical analysis: After collecting appropriate information, the data was entered into Microsoft Excel-2010 for statistical analysis. The data has been presented as actual numbers, percentages and proportions. The results were presented both in graphs and in tables. The data were subjected to a simple descriptive statistical analysis including frequency distribution and percentage.

Interpretations of prescriptions: The study was carried out in four districts in Bangladesh to show the current trend of antibiotic use in relation to medical prescriptions. It was also determined whether or not antibiotics are used to treat the present cases of enteric fever.

RESULTS AND DISCUSSION

Out of 100 suspected cases of typhoid fever, blood culture positive for *S. typhi* were 40 (40%) and remaining 60 (60%) were negative.

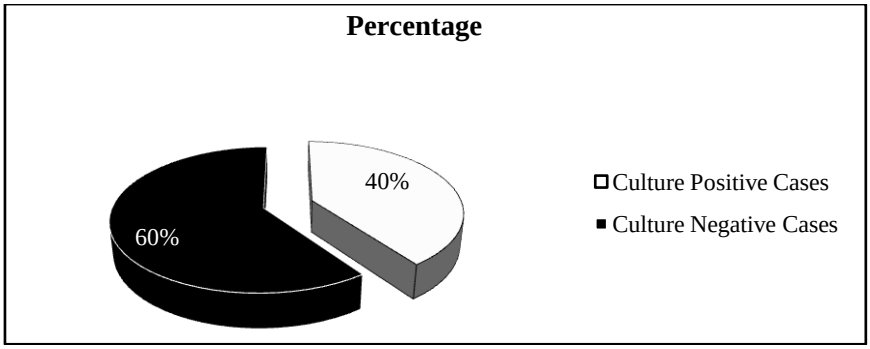

Fig. 1. Rate of isolation of *S. typhi* in blood culture at EMCH in Savar, Dhaka, Bangladesh from July 2016-June 2017

Table 1. Symptoms of typhoid fever at EMCH in Savar, Dhaka, Bangladesh from July 2016-June 2017

Symptoms of Typhoid fever	Percentage
Fever	70%
Vomiting	8.50%
Abdominal pain	6.50%
Myalgia	4.50%
Cough	4%
Diarrhoea	2.50%
Headache	3%
Others	1%

EMCH = Enam Medical College & Hospital

Table 2. Presence of bacteria (*S. typhi*) in both male and female at EMCH in Savar, Dhaka, Bangladesh from July 2016-June 2017

Age Groups	No. of Isolates	Sex		Percent (%)
		Male	Female	
0-5	5	4	1	12.5%
6-10	5	3	2	12.5%
11-20	8	5	3	20%
21-30	7	5	2	17.5%
31-40	5	3	2	12.5%
41-50	3	2	1	7.5%
51-60	4	1	3	10%
>60	3	1	2	7.5%
	Total=40	=24	=16	

EMCH = Enam Medical College Hospital, Savar, Dhaka

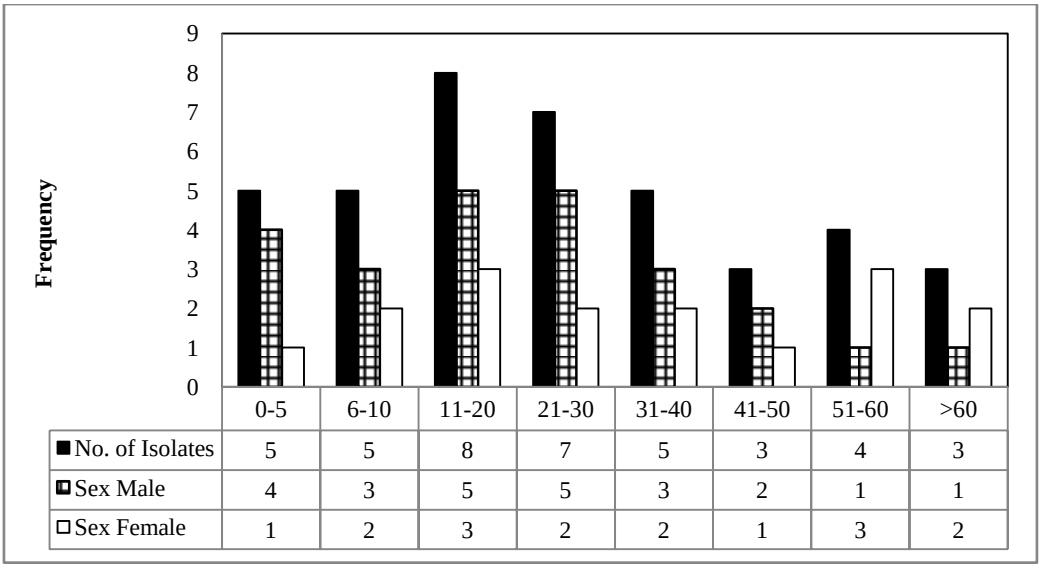

Fig. 2. Presence of *S. typhi* in relation to sex at EMCH in Savar, Dhaka, Bangladesh from July 2016-June 2017

EMCH = Enam Medical College & Hospital

The bar diagram was plotted to describe the presence of the total number of isolates in both males and females (Figure 2). The maximum number of bacteria was present in the age groups 11-20. On the other hand, the lowest numbers of bacteria were present in both the 41-50 years old and the over 60 years old.

Table 3. Percentage of susceptibility pattern of antibiotics against *S. typhi* at EMCH in Savar, Dhaka, Bangladesh from July 2016-June 2017

Antimicrobial Agents	Sensitivity to *S. typhi*	Resistance to *S. typhi*
Azithromycin (AZT)	52.5%	47.5%
Ciprofloxacin (CPF)	62.5%	37.5%
Ceftriaxone (CFT)	40%	60%
Ceftazidime (CZD)	65%	35%
Cefixime (CFX)	42.5%	57.5%
Imipenem (IMP)	72.5%	27.5%
Cefuroxime (CFU)	65%	35%
Amoxycilin (AMO)	20%	80%
Gentamicin (GEN)	72.5%	27.5%
Co-trimoxazole (COT)	25%	75%
Clindamycin (CLD)	65%	35%
Cloxacillin (CLX)	17.5%	82.5%
Meropenem (MPM)	77.5%	22.5%
Cefotaxime (CFM)	70%	30%
Vancomycin (VAN)	40%	60%

EMCH = Enam Medical College & Hospital

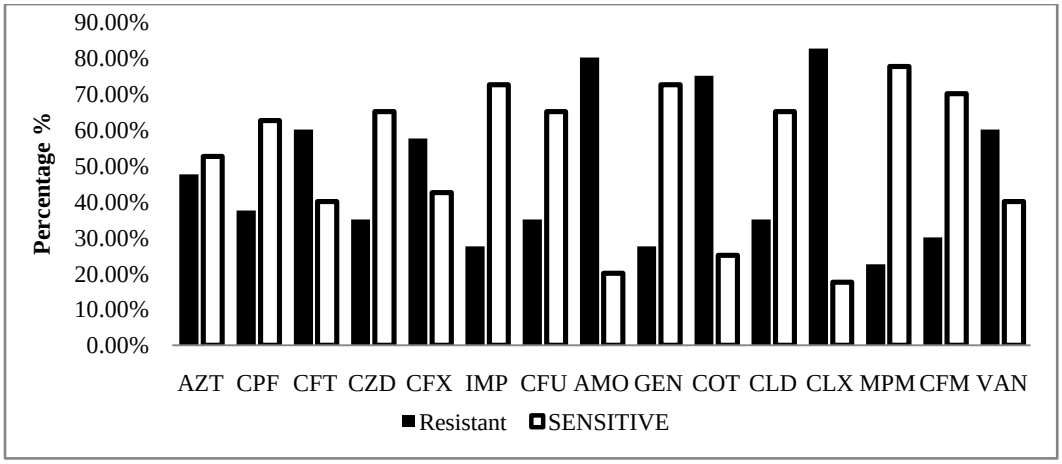

Fig. 3. The relationship between the susceptibility pattern and antibioticat EMCH in Savar, Dhaka, Bangladesh from July 2016-June 2017

EMCH = Enam Medical College & Hospital

Table 4. Age and sex distribution of collected prescriptions for cases of typhoid fever in four cities in Bangladesh

Age Group (in Year)	Frequency of Prescription (TNP=98)		Individual Percentage (%)		Total Frequency	AVP (%)
	Male	Female	Male	Female		
5-15	36	18	56.25	52.94	54	54.08
15-30	16	6	25.00	17.65	22	22.45
31-45	4	6	6.25	17.65	10	13.27
46-60	6	3	9.38	8.82	9	7.14
>60	2	1	3.13	2.94	3	3.06

TNP=Total Number of Prescriptions, AVP=Average Percentage

The survey was conducted among 98 patients to identify susceptibility to typhoid in both men (64) and women (34). We found that most prescriptions for typhoid were between the ages of 5 and 15 for both males (56.25%) and females (52.94%). However, it was surprising that the fewest typhoid patients were found in the age range > 60 years in both men (3.13%) and women (2.94%).

Table 5. Frequency and pattern of prescribed antibiotics in typhoid fever in the collected prescriptions

Group of Antibiotics	Frequency (TNP = 98)	Percentage
Cefixime	36	36.73
Ceftriaxone	18	18.37
Azithromycin	17	17.35
Ciprofloxacin	8	8.16
Cefuroxime axetil	11	11.22
Cotrimoxazole	8	8.16
Others	36	36.73

TNP=Total Number of Prescription

The third-generation cephalosporins such as cefixime (36.73%) and ceftriaxone (18.37%) have been used primarily to treat typhoid fever. In contrast, azithromycin (17.35%), cefuroxime axetil (11.22%), ciprofloxacin, and cotrimoxazole were only prescribed in a few cases.It was also mentioned that 36.73% used other antibiotics to treat the diseases.

Table 6. Pattern of antibiotic prescription and usages in enteric fever in four districts in Bangladesh

Parameters	Prescription Pattern	Frequency (TNP=98)	Percentage
Pattern of antibiotics	Single antibiotic	36	36.73
Prescription	Multiple antibiotics	62	63.27
Information on direction for	Complete	53	54.08
antibiotic use	Not mentioned	35	35.75
Clinical test for prescribing	With test	15	15.31
antibiotics	Without test	83	84.69
Completion of full antibiotic	Yes	63	64.29
course	No	35	35.71
Patient's compliance	Disease recovery	64	65.31
	Noncompliance	34	34.69

TNP=Total Number of Prescriptions

The table above shows the pattern of antibiotic prescribing in the treatment of typhoid based on patient compliance, information on the direction of antibiotic use, completion of full antibiotic treatment and clinical testing in susceptible cases. The study was conducted in four district cities in Bangladesh to determine patient response to prescribed medication. Here, 63.27% of patients administered several antibiotics compared to 36.73% of single antibiotics. Information was only directed for the 54.08% who should take antibiotics and the rest of the patients (35.75%) was not mentioned. On the other hand, 64.29% of the patients completed the course of antibiotics completely. Most patients were prescribed without clinical testing, which must be essential for identifying the pathogens responsible for antibiotic resistance. Out of 98 patients, 65.31% agreed to the use of antibiotics, which can be increased with an appropriate diagnostic test and the use of antibiotics as recommended by doctors and registered pharmacists.

Enteric fever is a common disease caused by *S. typhi* or other enteric fever *Salmonella* (*S. paratyphi* A, B and C), which only infect humans and are transmitted by the oral-faecal route (Bhutta *et al*., 1994). In the case of enteric fever, men have been found to be more prone to bacteremia than women.In our study, we found that 40 (40%) of the blood cultures were positive for *S. typhi* and the remaining 60 (60%) of 100 cases were negative, whereas previous study showed that 16% were positive and 84% were negative (Hasan *et al*., 2011).

Our study also found that male (24) patients were more susceptible to salmonellosis than females (16). Most (08) bacteria were found in the age range of (11-20), followed by 05 (age range 0-5, 6-10, 31-40), 04 (age range 51-60). On the other hand, the lowest number of isolates (03) were present over the age range of >60years. Our study reported that fever (70.0%),vomiting (8.5%), abdominal pain (6.5%), myalgia (4.5%),cough(4%), diarrhea

(2.5%), headache (3.0%) occurred. Although other studies explained slightly different features such as fever (97.0%) and vomiting (50.0%) (Jamil *et al*., 2004). We found that meropenem (77.5%) showed the maximum effect since beta-lactam antibiotics are considered safe (Leung *et al*., 2000) and are also used in pregnant women with typhoid (Seoud *et al*., 1988).

Although fluoroquinolones have shown positive reports of successful use, they have not generally been used in pregnancy due to safety concerns (Koul *et al*., 1995; Leung *et al*., 2000). Third generation cephalosporins such as cefotaxime (70%), cefuroxime (65%), ceftazidime (65%) and cefixime (42.5%) have been shown to be more sensitive than other oral antibiotics. Of the third-generation cephalosporins, oral cefixime (15-20 mg per kg per day for adults, 100-200 mg twice daily) has been widely used in children in a variety of geographic settings and has been found satisfactory (Bhutta *et al*., 1994; Girgis *et al*., 1995; Pandit *et al*., 2007). In this case, third-generation cephalosporins such as ceftriaxone can be used. However, the cost and route of administration make ceftriaxone less suitable for treating patients in some low- and middle-income countries, and the third-generation oral cephalosporin, cefixime, appears to outperform other oral agents in terms of both fever reduction duration and duration treatment inferior to failure (Frenck *et al*., 2000). Antibiotic resistance varied from patient to patient. Our study also examined the current situation of antibiotics for the treatment of enteric fever against *Salmonella typhi*. Amoxicillin and cloxacillin have shown high resistance to enteric fever or salmonellosis including co-trimoxazole (75%), vancomycin (60%), azithromycin (47.5%), meropenem (22.5%), gentamicin (27.5%) and ciprofloxacin (37.5) (Butler, 1999).

Recent data on the use of azithromycin in children indicated that it might be safely administered as an alternative agent to treat uncomplicated typhoid fever (Frenck *et al*., 2000) and that azithromycin, 1 gram once daily for 5 days, is also a useful treatment of typhoid, although the disease takes longer to resolve (Butler 1999; Mirza *et al*., 1996). We also found that most prescriptions for typhoid were in the 5 to 15 years age group for both males (56.25%) and females (52.94%). However, it was surprising that the fewest typhoid patients were found in the age range > 60 years in both men (3.13%) and women (2.94%). Third-generation cephalosporins such as cefixime (36.73%) and ceftriaxone (18.37%) were mainly prescribed to treat typhoid fever. It was also mentioned that 36.73% used other antibiotics to treat the diseases. Here, 63.27% of patients administered several antibiotics compared to 36.73% of single antibiotics. Information was only directed for the 54.08% who should take antibiotics and the rest of the patients (35.75%) was not mentioned. On the other hand, 64.29% of the patients completed the entire course of antibiotics.

Conclusion: Current study demonstrated a scenario of enteric fever prevalence and trend of antibiotic use in this disease along with antibiotic resistant pattern in Bangladesh. Inappropriate antibiotic prescription and use lead to emergence of resistance, resulting socio-economic burden in our country. Decreased ciprofloxacin susceptibility and, more recently, fluroquinolone resistance led to greater use of third generation cephalosporins in

enteric fever. Although azithromycin showed promise for the management of feverin various parts of the world,study revealed relatively poor sensitivity in Bangladesh. Sensitivity to less and infrequent use of first line antibiotics such as chloramphenicol and co-trimoxazole make us rethink about reinstitution of their uses. 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.

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