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Biochemical Characterization and Antibiotic Susceptibility Analysis of *Escherichia coli* and *Salmonella typhi* Isolated from Clinical Sample

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Abstract

Poor sensitivity of pathogenic bacteria to bacteria is a serious health issue worldwide specially in the developing and underdeveloped countries making greater concern to the scientists. This study followed the CLSI (Clinical and Laboratory Standards Institute) guidelines 30, Kirby-Bauer disc diffusion method to test sensitivity. We analyzed the antibiotic sensitivity pattern of *Escherichia coli* and *Salmonella typhi* isolated from clinical samples, collected from two hospitals of Dhaka, Bangladesh. The sensitivity of twelve different single antibiotics (cefepime, cefotaxime, chloramphenicol, ciprofloxacin, nitrofurantoin, meropenem, imipenem, amikacin, gentamicin, azithromycin, tetracycline, fosfomycin), and two combinations (piperacillin-tazobactam and amoxicillin-clavulanate) were evaluated against *E. coli* and *S. typhi* isolates. *E. coli* isolates showed significantly less susceptibility than *S. typhi* isolates to the antibiotics tested. Three antibiotics showed significant effectiveness against *E. coli* isolates, (chloramphenicol, nitrofurantoin, and fosfomycin). Ciprofloxacin was the least effective antibiotic to *S. typhi* isolates (only 20% isolates showed sensitivity). 85% *E. coli* isolates were MDR (Multi Drug Resistant), whereas only 5% *S. typhi* isolates were MDR. The highest MAR (Multiple Antibiotic Resistance) index value among *E. coli* isolates was 0.86, whereas highest value among *S. typhi* isolates was 0.21. The lowest MAR value among *E. coli* isolates was 0.21, whereas two *S. typhi* isolates had MAR value of zero (0). Almost all the single and combination antibiotics demonstrated poor sensitivity to the *E. coli* and *S. typhi* isolates. Thus, it makes infections much harder to treat and makes other medical procedures and treatment, such as surgery, caesarean sections and cancer therapy, much riskier.

Key words: *Escherichia coli*, *Salmonella typhi*, susceptibility, resistance, biochemical characterization.

Introduction

Human intestines are typically home to a particular kind of bacteria called *Escherichia coli* (Katouli, 2010). Certain animal stomach contains it as well. In fact, the majority of *E. coli* strains are beneficial to the health of human digestive system (Nakkarach *et al.*, 2020). Yet, if we consume tainted food or contaminated water, certain strains can make us sick with diarrhea (Ishii and Sadowsky, 2008). One of the most prevalent causes of many common bacterial diseases in both humans and animals is still *E. coli* (Allocati *et al.*, 2013). For many years, impoverished nations have been plagued by the gram-negative bacterium *Salmonella typhi*, which causes typhoid disease (Allocati *et al.*, 2013). Infections caused by *E. coli* and *S. typhi* varies depending on the geographic location of a country (Erb *et al.*, 2007). *E. coli* bacteria is very much

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available in contaminated foods of Bangladesh, one study showing 61% stored foods of rural Bangladesh contains it (Doza *et al.*, 2018). *S. typhi* is also very much a cause of concern for healthcare professionals in Bangladesh, one study showing 26% blood samples collected from hospital enrolled patients had *S. typhi* infections (Garrett *et al*., 2022).

Antimicrobial resistance (AMR) is a global health concern. As a result of AMR, antibiotics become ineffective and infections become difficult or impossible to treat, increasing the risk of disease spread, severe illness, disability and death (Lushniak, 2014, Komolafe, 2003). Because of rising antibiotic resistance, treating *E. coli* and *S. typhi* infections are becoming progressively difficult. Certain antibiotic agents may not work on certain germs, they are employed. Antibiotic susceptibility testing, also known as antibiotic sensitivity testing, quantifies how susceptible bacteria are to antibiotics. The results of a sensitivity test enable a physician to switch from empiric therapy, in which an antibiotic is chosen based on clinical suspicion regarding the infection site and common causative bacteria, to a directed therapy, which bases the antibiotic choice on knowledge of the organism and its sensitivities (Leekha *et al*., 2011).

GLASS (Global Antimicrobial Resistance Surveillance System) is a case-finding surveillance system that combines clinical information from patients with the infection with AMR data of key pathogens (World Health Organization, 2016). GLASS priority pathogens currently include the pathogens: *Escherichia coli, Acinetobacter spp., Salmonella spp., Shigella spp., Staphylococcus aureus, Neisseria gonorrhoeae, and Streptococcus pneumoniae* (Veeraraghavan and Walia, 2019). All of these bacteria are multi-drug resistant (MDR), meaning they can withstand the effects of at least three different classes of antibiotics (Magiorakos *et al*., 2012).

We conducted this study to analyze the susceptibility pattern of *E. coli* and *S. typhi* isolates against twelve single antibiotics and two antibiotic combinations in Dhaka city, Bangladesh. This study will help healthcare professionals in Bangladesh to more accurately prescribe antibiotics to treat *E. coli* and *S. typhi* infections.

Materials and Methods

Sample collection: A total of 40 bacterial cultures (20 *E. coli* and 20 *S. typhi*) were analyzed in the study. All the samples were collected randomly from two selected hospitals of Dhaka city, namely Bangabandhu Sheikh Mujib Medical University Hospital (BSMMU) and Popular Diagnostic Centre Ltd. Thirty-seven samples were collected from BSMMU on the basis of a Memorandum of Understanding (MoU) between the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University and the Department of Clinical Pharmacy and Pharmacology, University of Dhaka. Three samples were collected from the Popular Diagnostic Centre Ltd. through the approval of the chairman of microbiology department.

Biochemical characterization: For the identification of *E. coli* and *S. typhi* isolates, we used three biochemical tests, namely Citrate test, KIA (Kligler's Iron Agar) test and MIU (Motility Indole Urease) test. Both *E. coli* and *S. typhi* isolates were citrate negative. Simmons' citrate agar remained green (no colour change) in the presence of citrate-negative bacterial species *E. coli* and *S. typhi*, which were not capable of metabolizing citrate (MacWilliams, 2009). Carbohydrate fermentation was detected using Kligler's Iron Agar (KIA). *E. coli* isolates were able to ferment both lactose and glucose, which was indicated by the characteristic yellow colour in both slant and butt of the test tubes, because of acid production. The *E. coli* isolates were producing gas (CO_2) as well, which was indicated by the space at the tube's bottom and the break in the agar at the center of the tube. *S. typhi* isolates produced hydrogen sulfide (H_2S), which was indicated by the characteristic black colour at the site of inoculation (De Ryck, 1994).

E. coli isolates were motile and indole positive. The extracellular spiral thread-like structures known

as flagella are present in *E. coli* cells. A clockwise or counterclockwise rotating motor is located at the base of each flagellum, which causes the motility (Mittal *et al*., 2003). Tryptophan, an amino acid, can be changed into indole by bacteria that possess the tryptophanase enzyme. When Kovac's reagent is introduced to indole, it interacts to produce red dye known as rosindole dye (which suggests the presence of indole). *E. coli* isolates exhibited indole positivity. *S. typhi* isolates also moved from the line of inoculation, but they were indole negative.

Antibiotic susceptibility test: Antibiotic susceptibility tests were performed by Kirbey-Bauer disc diffusion method following the CLSI (The Clinical & Laboratory Standards Institute) guidelines 30. Muller Hinton Agar (MHA) was used as the media to check the zone of inhibition caused by antibiotic discs. Following the measurement of zone of inhibition via a meter scale in millimeter (mm), susceptibility and resistance results were interpreted according the guidelines specified by the CLSI guidelines 30 (Table 1).

Table 1. Critical values for zone of inhibition of antibiotics used.

Antibiotics used	Coded	Family	Disc load	Critical values of inhibition diameters (mm)	
				Sensitive	Resistant
Cefepime	CPM	Beta lactams	30 µg	≥25	≤18
Cefotaxime	CTX	Beta lactams	30 µg	≥26	≤22
Piperacillin-tazobactam	PIT	Beta lactams	100/10 µg	≥21	≤17
Amoxicillin-clavulanate	AMC	Beta lactams	20/10 µg	≥18	≤13
Chloramphenicol	C	Phenicols	30 µg	≥18	≤12
Ciprofloxacin	CIP	Fluoroquinolones	5 µg	≥31	≤20
Nitrofurantoin	NIT	Nitrofurans	300 µg	≥17	≤14
Meropenem	MEM	Carbapenems	10 µg	≥23	≤19
Imipenem	IPM	Carbapenems	10 µg	≥23	≤19
Amikacin	AK	Aminoglycosides	30 µg	≥17	≤14
Gentamicin	GEN	Aminoglycosides	10 µg	≥15	≤12
Azithromycin	AZM	Macrolides	15 µg	≥13	≤12
Tetracycline	TE	Tetracyclines	30 µg	≥15	≤11
Fosfomycin	FO	Phosphonic acids	200 µg	≥16	≤12

*Critical values are appearing in the CLSI guidelines 30.

Data analysis: Susceptibility data, interpreted according to the CLSI guidelines 30, were used to conclude *E. coli* and *S. typhi* isolates as MDR or Non-MDR. The tables were produced by simple statistical calculation in Microsoft word. The figures of MDR and MAR Index were made using Microsoft PowerPoint.

Results and Discussion

Susceptibility and resistance profile of Escherichia coli isolates: Chloramphenicol was the only antibiotic to which 100% *E. coli* isolates were susceptible. Fosfomycin and nitrofurantoin also showed similar effectiveness, which inhibited the growth of most of the *E. coli* isolates (95%). Tetracycline and meropenem showed little effectiveness in inhibiting the growth of *E. coli* isolates, 35% samples showed susceptibility to both

of them. Imipenem and piperacillin-tazobactam combination also showed similar effectiveness against *E. coli*, both having susceptibility to 30% of the *E. coli* isolates.

Cefotaxime, a third-generation cephalosporin, an FDA approved antibacterial for the treatment of anaerobic, gram-positive and gram-negative bacteria, (Carmine *et al*., 1983), showed highest (95%) resistivity against, and hence the lowest effectivity to inhibit the growth of *E. coli* isolates Cefepime and ciprofloxacin also showed greater (90%) resistivity against *E. coli* isolates. Among other antibiotics, amoxicillin-clavulanate combination was effective against 15% *E. coli* isolates, azithromycin to 20%, amikacin to 45%, gentamicin to 40% and azithromycin to 20% isolates (Table 2).

Table 2. Susceptibility and resistance profile of *Escherichia coli* isolates (N=20).

Antibiotics	Susceptible		Resistant	
	Number	Percentage	Number	Percentage
Cefepime (30 µg)	2	10	18	90
Cefotaxime (30 µg)	1	5	19	95
Piperacillin-tazobactam (100/10 µg)	6	30	14	70
Amoxicillin-clavulanate (20/10 µg)	3	15	17	85
Chloramphenicol (30 µg)	20	100	0	0
Ciprofloxacin (5 µg)	2	10	18	90
Nitrofurantoin (300 µg)	19	95	1	5
Meropenem (10 µg)	7	35	13	65
Imipenem (10 µg)	6	30	14	70
Amikacin (30 µg)	9	45	11	55
Gentamicin (10 µg)	8	40	12	60
Azithromycin (15 µg)	4	20	16	80
Tetracycline (30 µg)	7	35	13	65
Fosfomycin (200 µg)	19	95	1	5

Susceptibility and resistance profile of salmonella typhi isolates: S. typhi isolates showed significant susceptibility to most of the antibiotics analyzed, except ciprofloxacin. A total of 16 out of 20 isolates (80%) were resistant to ciprofloxacin. A total of 100% *S. typhi* isolates were sensitive to 7 antibiotic samples, namely nitrofurantoin, meropenem, imipenem, amikacin, gentamicin, azithromycin and piperacillin-tazobactam combination, which typically exhibits increased activity against the majority of pathogens that produce beta-lactamases (Schoonover *et al.*, 1995), which hydrolyze the peptide link of the distinctive four-membered beta-lactam ring, inactivating beta-lactam antibiotics and making them useless (Majiduddin *et al*., 2002).

Interestingly, three antimicrobial agents such as cefepime, tetracycline and fosfomycin also showed significant effectiveness against *S. typhi* isolates. All of the them were capable to inhibit the growth of 95% *Salmonella typhi* isolates. A total of 90% *S. typhi* isolates were susceptible to cefotaxime, 85% were susceptible to two antimicrobial agents, one being amoxicillin-clavulanate combination and another one was chloramphenicol, which inhibits the bacterial protein synthesis (Coutsogeorgopoulos, 1966). Interestingly, both antibiotic combinations showed significant ability to inhibit the growth of *S. typhi* isolates; piperacillin-tazobactam being effective against 100% isolates and amoxicillin-clavulanate being able to inhibit the growth of 85% *S. typhi* isolates. Ciprofloxacin, a fluoroquinolone antibiotic,

is FDA-approved for treating bacterial infections like pneumonia and urinary tract infections (Thai *et al*., 2023), has shown the least efficacy in inhibiting the growth of *E. coli* isolates. All antibacterial agents except ciprofloxacin were effective against more than or equal to 85% *S. typhi* isolates. *S. typhi* isolates were surprisingly more susceptible to 14 antibiotic agents we studied, than *E. coli* isolates (Table 3).

Table 3. Susceptibility and resistance profile of *Salmonella typhi* isolates (N=20).

Antibiotics	Susceptible		Resistant	
	Number	Percentage	Number	Percentage
Cefepime (30 µg)	19	95	1	5
Cefotaxime (30 µg)	18	90	2	10
Piperacillin-tazobactam (100/10 µg)	20	100	0	0
Amoxicillin-clavulanate (20/10 µg)	17	85	3	15
Chloramphenicol (30 µg)	17	85	3	15
Ciprofloxacin (5 µg)	4	20	16	80
Nitrofurantoin (300 µg)	20	100	0	0
Meropenem (10 µg)	20	100	0	0
Imipenem (10 µg)	20	100	0	0
Amikacin (30 µg)	20	100	0	0
Gentamicin (10 µg)	20	100	0	0
Azithromycin (15 µg)	20	100	0	0
Tetracycline (30 µg)	19	95	1	5
Fosfomycin (200 µg)	19	95	1	5

MDR profile of E. coli and S. typhi: Multidrug-resistant (MDR) organism means when a microbe is resistant to three or more classes of antibiotics. Out of 20 *E. coli* isolates, 17 (85%) were MDR. Two important factors that contribute to the intrinsic resistance of Gram-negative bacteria, such as *E. coli*, are their outer membrane is inaccessible to many molecules, and the expression of multiple efflux pumps which efficiently lower the intracellular level of some antibiotics (Poirel *et al*., 2018). Among the *E. coli* isolates, 15% were shown to have MDR trait (Figure 1).

One out of twenty *S. typhi* isolates (5%) were MDR, *i.e.*, resistant to three or more classes of antibiotics. A total of 95% *S. typhi* isolates were Non-MDR, which was significantly higher than *E coli* isolates (15%). *E. coli* isolates were highly resistant to the different types of antibiotics (having 85% MDR) compared to the *S. typhi* isolates (5% MDR) (Figure 1).

Resistance phenotype of E. coli: Three *E. coli* isolates were resistant to 3 different antibiotics (which was the lowest amount of antibiotics to which any *E. coli* isolate was resistant). Out of 20 *E. coli* isolates, 19 (95%) were resistant to cefotaxime, a cephalosporin antibiotic of beta lactam group. Eighteen isolates (90%) were not effective against cefepime (a cephalosporin antibiotic) and ciprofloxacin (a quinolone antibiotic). It is apparent from the findings that cefepime, cefotaxime and ciprofloxacin might be almost ineffective to treat *E. coli* infections. Other antibiotics, like piperacillin-tazobactam, amoxicillin-clavulanate, meropenem, imipenem, amikacin, gentamicin, azithromycin and tetracycline were also significantly ineffective to inhibit the growth of *E. coli* isolates.

Only three antibiotics which showed significant effectiveness against *E. coli* isolates were nitrofurantoin, (a nitrofuran antibiotic), chloramphenicol, (a phenicol antibiotic) and fosfomycin (a phosphonic acid bactericidal agent)

which works by deactivating the enzyme UDP-N-acetylglucosamine-3-enolpyruvyltransferase (sometimes referred as MurA). Deactivation of MurA enzyme causes the prevention of the synthesis of bacterial cell wall (McOsker and fitzpatrick, 1994). Fosfomycin was also very much effective in inhibiting the growth of *E. coli* isolates. No *E. coli* isolate, except one, was resistant to fosfomycin. The broad spectrum, bactericidal, low toxicity and time-dependent inhibitory action of fosfomycin include its ability to block the MurA enzyme, which catalyzes the initial steps in the formation of peptidoglycans (Silver, 2017). Only one *E. coli* isolate was resistant to both nitrofurantoin and fosfomycin. All of the *E. coli* isolates were sensitive against chloramphenicol. Meropenem and imipenem, the two broad-spectrum carbapenem antibiotics showed same effectiveness against *E. coli* isolates except one, which was resistant to only imipenem. Almost all antibiotics were ineffective to resist the growth of *E. coli* isolates, which is really a disquieting, as the ineffectiveness of antibiotics to inhibit the growth of *E. coli* isolates indicate that we may be out of chemical tools to treat the vast number of infections caused by *E. coli* bacteria (Table 3).

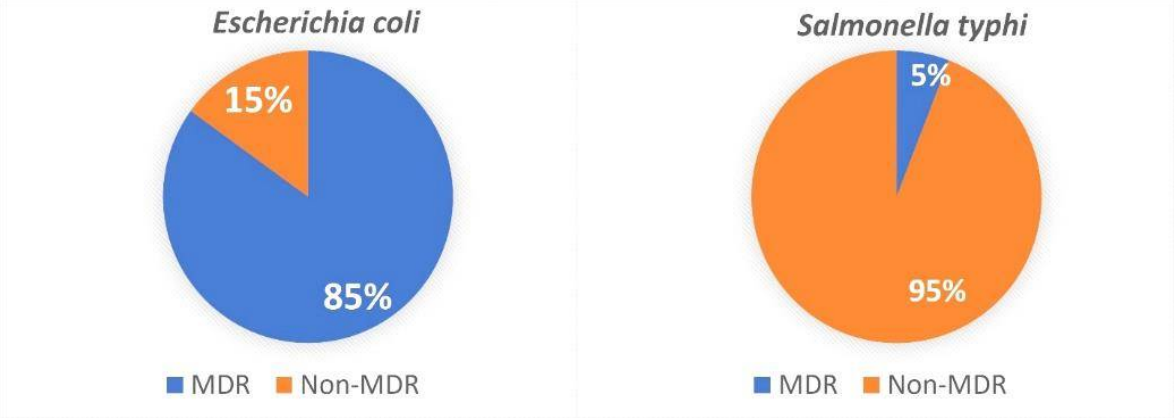

Figure 1. MDR data of *Escherichia coli* and *Salmonella typhi* isolates.

Resistance phenotype of S. typhi: Two *S. typhi* isolates did not show any resistance to any of the fourteen antibiotics we tested. Eleven isolates were resistant to only one antibiotic (ciprofloxacin). Three isolates were resistant to chloramphenicol and ciprofloxacin. Two other isolates were resistant to two different antibiotics, of them one was against cefotaxime and amoxicillin-clavulanate combination, another one was against ciprofloxacin and amoxicillin-clavulanate combination. None of the *S. typhi* isolates were resistant to 5 or more antibiotics. Ciprofloxacin has shown the least efficacy in inhibiting the growth of *S. typhi*, which was similar for *E. coli* isolates as well (Thai *et al*., 2023). Three other antimicrobial agents which were resistant to some *S typhi* isolates, were chloramphenicol (a protein synthesis inhibitor and broad-spectrum antibiotic), Cefotaxime (a third-generation intravenous cephalosporin antibiotic) and amoxicillin-clavulanate, (a combination of penicillin and beta lactamase inhibitor). In case of combination antibiotics, no *S. typhi* isolate was resistant to piperacillin-tazobactam combination, whereas three isolates were resistant to amoxicillin-clavulanate combination.

S. typhi isolates could be inhibited by almost all of the investigated antibiotics. Except ciprofloxacin, there was no other antibiotic to which *S. typhi* isolates showed resistance. However, in case of *E. coli*, the scenario was completely opposite. These data suggest that *S. typhi* isolates are comparatively more susceptible to antimicrobial agents than *E. coli* isolates. The findings indicate that treating *S. typhi*

infections still remains within the comfortable range for most of the antimicrobial agents, which is opposite in case of *E. coli* isolates and really a matter of global health concern (Table 4).

Table 4. Resistance phenotype of *E. coli* and *S. typhi* isolates.

Samples	CPM	CTX	PIT	AMC	C	CIP	NIT	MEM	IPM	AK	GEN	AZM	TE	FO
E. coli 1	√	√	√	√	-	√	-	√	√	√	√	√	-	-
E. coli 2	√	√	√	√	-	√	-	√	√	-	-	√	-	-
E. coli 3	√	√	√	√	-	√	-	√	√	√	√	√	√	
E. coli 4	√	√	√	√	-	√	√	√	√	√	√	√	√	-
E. coli 5	√	√	√	√	-	√	-	√	√	√	√	√	√	-
E. coli 6	√	√	√	√	-	√	-	√	√	√	√	√	√	-
E. coli 7	√	√	√	√	-	√	-	√	√	√	√	√	√	√
E. coli 8	√	√	√	√	-	√	-	-	-	√	-	√	√	-
E. coli 9	√	√	√	√	-	√	-	√	√	√	√	√	-	-
E. coli 10	√	√	√	√	-	√	-	√	√	√	√	√	-	-
E. coli 11	√	√	√	√	-	√	-	√	√	√	√	√	√	-
E. coli 12	-	-	-	√	-	√	-	-	-	-	√	√	√	-
E. coli 13	√	√	√	√	-	√	-	√	√	-	-	-	√	-
E. coli 14	√	√	-	√	-	√	-	-	√	-	-	√	√	-
E. coli 15	√	√	-	√	-	√	-	-	-	-	√	√	√	-
E. coli 16	√	√	-	-	-	-	-	-	-	-	-	√	-	-
E. coli 17	√	√	-	-	-	√	-	-	-	-	-	-	-	-
E. coli 18	-	√	√	√	-	√	-	√	√	-	-	-	√	-
E. coli 19	√	√	-	-	-	-	-	-	-	-	-	-	√	
E. coli 20	√	√	√	√	-	√	-	√	√	√	√	√	-	-
S. typhi 1	-	-	-	-	-	√	-	-	-	-	-	-	-	-
S. typhi 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. typhi 3	-	-	-	-	-	√	-	-	-	-	-	-	-	-
S. typhi 4	-	-	-	-	-	√	-	-	-	-	-	-	-	-
S. typhi 5	-	-	-	-	-	√	-	-	-	-	-	-	-	-
S. typhi 6	-	-	-	-	-	√	-	-	-	-	-	-	-	-
S. typhi 7	-	-	-	-	-	√	-	-	-	-	-	-	-	-
S. typhi 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. typhi 9	-	-	-	-	-	√	-	-	-	-	-	-	-	-
S. typhi 10	-	-	-	-	√	√	-	-	-	-	-	-	-	-
S. typhi 11	-	-	-	-	√	√	-	-	-	-	-	-	-	-
S. typhi 12	-	-	-	-	-	√	-	-	-	-	-	-	-	-
S. typhi 13	-	√	-	√	-	-	-	-	-	-	-	-	-	-
S. typhi 14	-	-	-	-	-	√	-	-	-	-	-	-	-	-
S. typhi 15	-	-	-	-	√	√	-	-	-	-	-	-	-	-
S. typhi 16	-	-	-	√	-	√	-	-	-	-	-	-	-	-
S. typhi 17	-	-	-	-	-	√	-	-	-	-	-	-	-	-
S. typhi 18	-	-	-	-	-	√	-	-	-	-	-	-	-	-
S. typhi 19	-	-	-	√	-	√	-	-	-	-	-	-	√	-
S. typhi 20	√	√	-	-	-	-	-	-	-	-	-	-	-	√

*Resistant (√), Sensitive (-), Cefepime (CPM), Cefotaxime (CTX), Amoxicillin-clavulanate (AMC), Piperacillin-tazobactam (PIT), Chloramphenicol (C), Ciprofloxacin (CIP), Nitrofurantoin (NIT), Meropenem (MEM), Imipenem (IPM), Amikacin (AK), Gentamicin (GEN), Azithromycin (AZM), Tetracycline (TE), Fosfomycin (FO).

MAR Index: The MAR (Multiple Antibiotic Resistance) index is computed as the ratio of the number of antibiotics to which an isolate exhibits resistance to the total number of antibiotics to which the organism is exposed. An -MAR value larger than 0.2 indicates that the regular use of antibiotics is the high-risk cause of contamination (Silver, 2017). The lowest MAR value among *E. coli* isolates was 0.21 and the highest was 0.86, which is really alarming for fighting *E. coli* infections. Two isolates had MAR index value of 0.86 (resistant to 12 out of 14 antibiotics), whereas three isolates had MAR index value of 0.21 (resistant to 3 out of 14 antibiotics).

In contrast, the lowest MAR value among the *S. typhi* isolates was 0 (resistant to 0 out of 14 antibiotics). as observed in two *S. typhi* isolates. This suggests that those samples were sensitive all of the tested antimicrobial agents. The highest MAR value among *S. typhi* isolates was 0.21 (resistant to 3 out of 14 antibiotics). Two *S. typhi* had such value (0.21) (Figure 2).

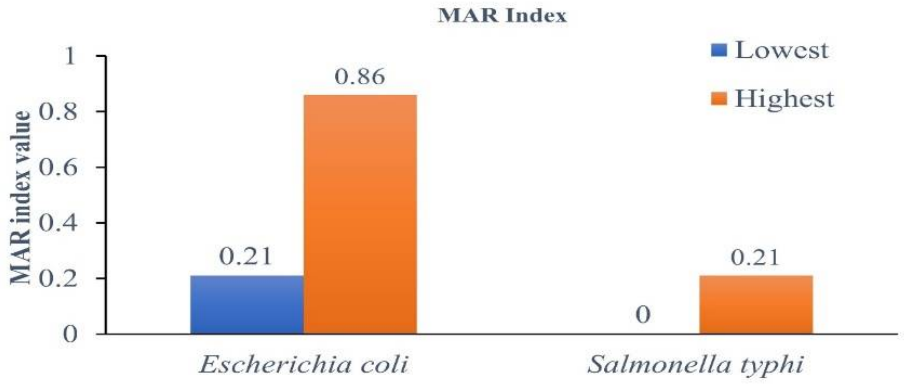

Figure 2. MAR index for *E. coli* and *S. typhi*. isolates

Most of our study data were consistent with prior research works by others in different regions of Bangladesh, though few were inconsistent as well. A study conducted in square hospital Ltd. showed that 17.65% isolates were susceptible to cefepime (Biswas *et al*., 2014). This finding was nearly in agreement with the finding of our study where 10% isolates were susceptible to cefepime. Another study conducted in Bangladesh showed 95.3% *E. coli* isolates were susceptible to nitrofurantoin (Acherjya *et al*., 2018), which is in agreement with our study where 95% of isolates were susceptible to nitrofurantoin. In our study, 35% *E. coli* isolates were susceptible to tetracycline, which is very close to the data (33.33%) of a study conducted in a private diagnostic center in Bangladesh (Mollick *et al*., 2016).

A survey on antibiotic sensitivity pattern of different antibiotics conducted by Shahriar *et al*. in Dhaka city showed 7.5% *E. coli* isolates were susceptible to ciprofloxacin (Shahriar *et al*., 2010). One prior mentioned study by Rama Biswas showed 11.77% *E. coli* samples were susceptible to ciprofloxacin (Biswas *et al*., 2014). Both of these studies were in agreement with our study data where 10% isolates were susceptible. In our study, 22.5%, 41%, 31% and 52.2% isolates were susceptible to chloramphenicol, imipenem, amikacin and gentamicin respectively, which are comparatively close to other published data in Bangladesh (Acherjya *et al*., 2018, Shahriar *et al*., 2010, Akhtar *et al*., 2016). Susceptibility data in other published studies on *E. coli* isolates to cefotaxime, amoxicillin-clavulanate, meropenem and azithromycin weren't

consistent with our study data (Acherjya *et al*., 2018; Akhtar *et al.*, 2016).

Our study findings on *S. typhi* isolates were almost entirely similar with already other research conducted in Bangladesh except in case of azithromycin. In our study 99.65%, 88.88%, 97.31%, 88%, 19.80%, 100%, 100%, 93.40%, 100% and 96.51% isolates were susceptible to cefepime, cefotaxime, amoxicillin-clavulanate, chloramphenicol, ciprofloxacin, meropenem, imipenem, amikacin, gentamicin and tetracycline respectively. These data are in accordance with other published research work conducted in various regions of Bangladesh (Chowdhury *et al*., 2022; Rahman *et al*., 2024; Ali and Sultana, 2016; Haque *et al*., 2020; Faisal *et al.*, 2017). A study conducted in Bangladesh by Ali and Sultana (2016) to find out the antibiotic sensitivity pattern of *S. typhi* in children showed 88.4% isolates were susceptible to azithromycin, which isn't fully in agreement with our study data (100%).

S. typhi is responsible for life threatening conditions including typhoid fever. From this study it's evident that almost all *S. typhi* isolates were susceptible to most of the antibiotics, except ciprofloxacin. So, all these thirteen antibiotics can be used to treat *S. typhi* caused infections including typhoid fever. *E. coli* can cause diarrhea and food poisoning, or more serious conditions like pneumonia (Marrie *et al*., 1998). Only three antibiotics showed significant effectiveness against *E. coli* isolates, namely chloramphenicol, nitrofurantoin and fosfomycin. So, our study suggests only few options left for us to treat *E. coli* infections. It makes infections much harder to teat and makes other medical procedures and treatment, such as surgery, caesarean sections and cancer therapy, much riskier. However, to get more precise information about the susceptibility pattern of *E. coli* and *S. typhi*, further research should be conducted on them. Regular monitoring of antibiotic resistance pattern of *E. coli* and *S. typhi* is vital to the treatment of infections caused by these microbes.

Author contribution

The experiment design, data interpretation, and manuscript draft were done by Md Tarekur Rahaman. All of the tests and data collection were carried out by Md Tarekur Rahaman. Sarder Arifuzzaman, Md. Tarekur Rahaman, and Zubair Khalid Labu prepared the figures and formatted the manuscript. Muhammad Asaduzzaman, Sreedam Chandra Das and Md. Saiful Islam supervised and helped in drafting the manuscript. Each of the authors proofread the manuscript. Each author has reviewed and approved the final draft.

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Disclosure Statement

There is no conflict of interest, the authors claim.

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