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Original Research

Isolation and identification of enteric bacteria from different water sources at Mymensingh city, Bangladesh and their antibiogram study

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Abstract

The goal of the current study was to separate and characterize the enteric bacteria found in water samples taken from various Mymensingh municipal sources and places. A total of 40 water samples were examined. Through the use of polymerase chain reaction (PCR), biochemical testing, staining, and culturing enteric bacteria were isolated and identified. The antibiotic resistance phenotype was examined using the disk diffusion assay. Out of 40 isolates that tested positive for bacterial growth, 3 (7.5%) were identified as *Salmonella* spp., 6 (15%) as *Shigella* spp., 14 (35%) as *E. coli*, and 3 (7.5%) as *Vibrio* spp. Molecular detection of Salmonella spp. were confirmed by PCR-based detection of the *bcfC* gene, *Shigella* species by the *invC* gene, *E. coli* by the *16S rRNA* gene, and Vibrio spp. by the *groEL* gene. Furthermore, PCR-confirmed isolates were tested for antibiotic resistance to 12 routinely used antibiotics. All Salmonella isolates were 100% resistant to Amoxicillin but 100% sensitive to Azithromycin. However, varied sensitivity was seen against Cefepime (80%), Gentamycin (80%), Levofloxacin (75%), Cotrimoxazole (74%), and Amikacin (70%). The isolates also showed resistance to Ceftazidime (70%), as well as Ceftriaxone (33%). *Shigella* spp. isolates showed increased resistance to Cefepime (83%) and Ceftazidime (67%). Additionally, resistance to Amoxicillin (17%) and Colistin (10%) was shown to be lower. In addition, Azithromycin, Levofloxacin, Cotrimoxazole, Ceftriaxone, Amikacin, and Gentamicin showed nearly no resistance or significant susceptibility *E. coli* isolates showed stronger resistance patterns to amoxicillin (100%), and colistin (93%). Cefepime (72%), Cotrimoxazole (72%), and Azithromycin (72%). The lower levels of resistance to ceftriaxone, amikacin, ceftazidime, levofloxacin, and gentamicin were also seen. The higher resistance patterns were shown by the isolated *Vibrio* spp. against Ampicillin (100%), Cefepime (81%), Cefixime (72%), Amoxicillin (70%), Erythromycin (67%), and Ceftazidime (67%). On the other hand, isolates with high and moderate sensitivity were found to be gentamicin, levofloxacin, doxycycline, cotrimoxazole, and amikacin. The study's conclusions point to recent and ongoing contamination of the water sources, putting residents who depend on the water for drinking at risk for gastrointestinal illnesses.

1. Introduction

Life depends on water. Water supply has been essential to society for many uses, including drinking, agriculture, industry, domestic use, and more, from the beginning of human civilization. The lack of access to clean drinking water is a major cause of health issues in poor nations (Hasan *et al.,* 2019; Bylund *et al.*, 2017; Thayer *et al.*, 2012). Health and disease are significantly impacted by water, sanitation, and hygiene. Without a question, access to clean water is essential for survival and cannot be achieved without it (Hutton and Chase, 2016).

Several water sources in developing nations are dangerous due to the presence of dangerous chemical, biological, and physical contaminants (Cheesbrough, 1985). Infectious disorders that are spread by water are known as waterborne diseases because the pathogen or causal organism is present in the water and is consumed (Hurst, 2018).

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Water-borne infections, including diarrhea and gastrointestinal disorders, have been the source of many outbreaks. These illnesses are caused by various bacteria, viruses, and protozoa (Kristanti *et al.*, 2022).

The traditional waterborne enteric pathogens are *Shigella* spp. (four species causing dysentery), *Salmonella enterica* (subsp. *enterica* ser. Typhi, causing typhoid), and *Vibrio cholerae* (sero-groups O1 and O139, causing cholera). These pathogens have been largely controlled by water treatment/disinfection, and as a result, they are rarely a problem via drinking water in developed regions. However, *Shigella* sonnei, along with closely related shiga toxin and verotoxin-producing *E. coli* persist in the sewage of developed nations due to person-to-person and foodborne transmission (Ashbolt *et al.*, 2015).

A genus of rod-shaped, gram-negative, non-spore-forming enterobacteria, mostly motile, is called salmonella. They are facultative anaerobes and chemo organotrophs, deriving their energy from organic substances through oxidation and reduction reactions. Growing most species on media containing ferrous sulfate allows one to easily detect hydrogen sulfide that they create. There are two phases that most isolates go through: a motile phase (I) and a non-motile phase (II). It is possible to transition non-motile cultures into the motile phase after primary culture. Closely linked to the

Escherichia genus, salmonella can be found in both warm- and cold-blooded animals, including humans, as well as in food-borne illnesses including typhoid fever and paratyphoid fever (Oludairo *et al.,* 2022; Stella *et al.*, 2018).

Shigella are non-motile, facultative anaerobic, gram-negative rods that do not generate spores. Shigella's pathogenicity, physiology (inability to digest lactose or decarboxylate lysine), and serology set it apart from the closely related *Escherichia coli* bacterium. The most frequent cause of bacillary dysentery is Shigella. This illness is not the same as enterotoxigenic diarrhea caused by *Escherichia coli*, which results in excessive amounts of watery diarrhea. The genus contains four sero-groups: A. with twelve serotypes of *S. dysenteriae*, B. with six serotypes of *S. flexneri*, C. with eighteen serotypes of *S. boydii*, and D with one serotype of *S. sonnei* (Strockbine *et al.*, 2015).

Although *E. coli* is considered to be a more accurate indication of faecal pollution, it still has a number of drawbacks that should be taken into account before relying solely on the findings of *E. coli* testing for faecal contamination (Rana *et al.,* 2024; Hossain *et al.,* 2024; Li *et al.*, 2021). It has even been demonstrated that *E. coli* can flourish in certain natural aquatic settings. The emergence of dangerous strains of *Escherichia coli* and their pervasiveness in sources of drinking water are concerning (Saima *et al.*, 2021). There is little evidence to correlate serotype to pathogenicity, and there is no biochemical marker to distinguish pathogenic from non-pathogenic strains of *E. coli* (Geurtsen *et al.*, 2022). *E. coli* strains that cause disease have been isolated from mountain streams, drinking water sources, and tap water. According to reports, one of the ways that pathogenic *E. coli* strains spread is by water contamination; this is the case for many outbreaks of the newly developing O157:H7 strain of enterohemorrhagic *E. coli* (Gebregziabher *et al.*, 2024; Ashbolt, 2015).

The water-borne pathogens EPEC, ETEC, EIEC, and EHEC are well-known. It has a relatively limited lifespan outside of the environment, and it might not be able to thrive if there are other faecal bacteria present. In water, *E. Coli* O157:H7 can enter a viable but non-culturable (VBNC) condition but cannot live for extended periods of time (Gambushe *et al.*, 2022)

Vibrio are tiny, curved, Gram-negative rods that have a single polar flagellum. Facultative anaerobes with both respiratory and fermentative metabolisms are called vibrios (Rana *et al.,* 2024). Sodium is an absolute need for most species and encourages growth in all. The structures known as fimbriae, or pili, found on *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* cells are composed of the protein TcpA. One of the main factors influencing in vivo colonization is TcpA production, which is co-regulated with the expression of the cholera toxin (Cabral, 2010).

The World Health Organization has identified antibiotic resistance in bacteria as one of the biggest risks to human health (WHO, 2014). Antibiotic-resistant bacteria are able to multiply due to the selection pressure that is created when antibiotics are overused or misused (Islam *et al.*, 2024; Woappi *et al.*, 2016; Levy *et al.*, 2005). The environmental contamination is thought to be the most effective method for both the selection of resistant populations and the transfer of resistance genes via mobile genetic elements (Larsson and Flach, 2022; Ahmad *et al.*, 2021). This species may serve as an indicator of bacteria that spread antibiotic resistance in aquatic environments because of their great prevalence there and a variety of antibiotic-resistant mechanisms (Siri *et al.*, 2023). It may serve as a storehouse for ARGs that can spread horizontally through genes to other dangerous bacteria (Tao *et al.*, 2022).

The current study was conducted to look into the occurrence of enteric pathogens (such as *E. coli*, *Salmonella* spp., *Shigella* spp., and *Vibrio* spp.) in various sources of water. Molecular characterization of isolated enteric pathogens, including the discovery of drug-resistant and virulence factor genes. Finally, an antibiogram research was conducted to choose appropriate antibiotics for therapeutic purposes, hence reducing economic loss.

2. Materials and Methods

2.1 Ethical approval

No ethical approval is required for this study.

2.2 Study areas

The entire study was carried out in the Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, from March 2021 to October 2022. The places pond water samples were collected from the different ponds inside of Mymensingh city where as river water samples were collected from the Brahmaputra which flow beside Mymensingh city (Figure 1).

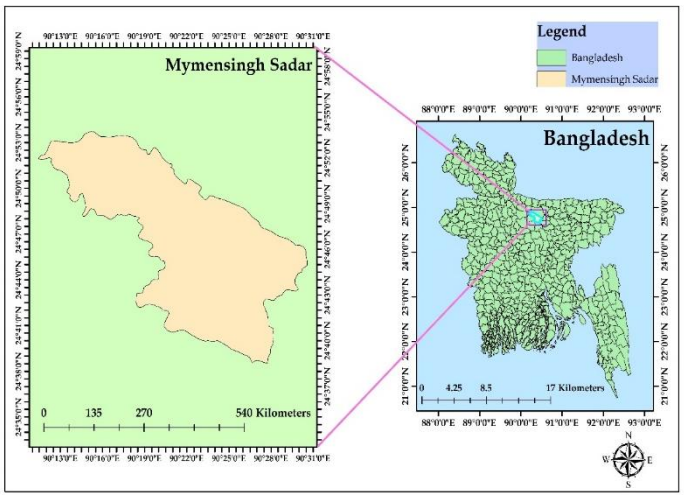

Figure 1. Map of the study area, where the whole study was conducted.

2.3 Sample collection and processing

A total of 40 water samples (pond water, tap water, tube-well water, and river water) were collected, among which were 10, 10, 10, and 10 samples from rivers, ponds, water taps, and tube-wells, respectively. Each type of sample was collected from five different places of Mymensingh city where 2 samples were collected from each of the five different locations (Table 1). All water samples of this study were carefully handled and kept in transport box and maintained the temperature at 4 °C and immediately after collection all samples transported to the department of microbiology and hygiene for bacterial analysis. Using sterile falcon tubes, the samples (100 ml each) were collected, and then they were carefully transferred to the proper sterile containers and brought to the laboratory for bacteriological investigation. The water that had been collected was centrifuged for 3 minutes at 10,000 rpm. Sediments were re-suspended in sterile PBS for culture, while supernatants were disposed of.

Table 1. Sample types, number of samples, and sampling location.

River water (n=10)	Pond water (n=10)	Tube-well water (n=10)	Tap water (n=10)
Shesmor ghat	Shesmor pond	Shesmor	Kewatkhali
Ladies' hall ghat	Baitul Aman mosque pond	Railway	Ganginar par
Baisakhi chattor ghat	Railway colony pond	Durgabari road	Shesmor
Thanar ghat	Teacher's Training College pond	BAU 1st gate	SR Hall, BAU
Kalibari ghat	Tinkona pond	Jabbar's moor	SNA Hall, BAU

2.4 Isolation and identification of bacteria

After being re-suspended, the primary culture of the samples was inoculated with NB and incubated for an entire night at 37 ºC. The enriched culture from nutrient broth was streaked on to selective agar media and incubated at 37 ºC for 24 hours. To obtain pure cultures, a single colony that had surfaced on the selective media was streaked onto selective media. The standard procedure was followed when

examining various samples culturally for bacteriological analysis (ICMSF, 1988). Based on colony morphology, Gram's staining reaction, and biochemical test, bacteria were identified. The pure culture was stained by Gram using the technique outlined by Cheesbrough (2006).

2.5 Molecular identification of Salmonella spp., Shigella spp., E. coli, and Vibrio spp. by PCR

2.5.1 Extraction of genomic DNA from the bacterial isolates

Each isolate's pure bacterial colony was inoculated into NB and kept at 37 °C for the overnight. Next, 1 ml of cultured broth was centrifuged for 3 min at 12,000 rpm. The supernatant was disposed of and replaced with 200 µl of purified water. After that, the tube was placed in boiling water and left there for 20 min. It was then immediately placed on ice for a brief period of time roughly for 10 min and centrifuged for 10 min at 12,000 rpm. During PCR, the supernatant was collected and used as a DNA template. Master Mix for PCR 10 µl, 1 µl each of the forward and reverse primers, 4 µl of the DNA template, and 4 µl of nuclease-free water. The final final volume of PCR product was 20 µl. The primers were performed to amplify the DNA of *bcf*C, *inv*C, *16S rRNA*, and *groEL* genes of *Salmonella* spp., *Shigella* spp., *E. coli*, and *Vibrio* spp., respectively (Table 2, 3, 4, 5, and 6).

Table 2. The sequence of primers for bacteria.

Target genes	Primer sequence (5'–3')	Amplicon size (bp)	References
bcfC	F: GGG TGG GCG GAA AAC TAT TTC R: CGG CAC GGC GGA ATA GAG CAC	993 bp	Zhu *et al.* (2015)
invC	F: TGCCCAGTTTCTTCATACGC R: GAAAGTAGCTCCCGAAATGC	875 bp	Ojha *et al.* (2013)
16S rRNA	F: AATTGAAGAGTTTGATCATG R: CTCTACGCATTTCACCGCTAC	704 bp	Sarker *et al.* (2018)
groEL	F: TCCARAACATGGGCGCACAA R: ACGTTTTGYTCTTCGTTGTCRC	1117 bp	Hossain *et al.* (2014)

'F'= forward; 'R'= reverse

2.5.2 Thermal profile for the amplification of PCR

Table 3. The thermal profile of *bcfC* gene specific primer of *Salmonella* spp.

Conditions	Temperature (°C)	Time	Cycle (s)	References
Initial denaturation	94	2 min		
Denaturation	94	20 sec		Zhu *et al.* (2015)
Annealing	57	45 sec	30	
Extension	72	1 min		
Final extension	72	10 min		
Holding	4	Until use		

Table 4. The thermal profile for *inv*C gene specific primer *Shigella* spp.

Conditions	Temperature (°C)	Time	Cycle (s)	References
Initial denaturation	95	5 min		
Denaturation	94	30 sec		
Annealing	56	45 sec	30	Ojha *et al.* (2013)
Extension	72	60 sec		
Final extension	72	10 min		
Holding	4	Until use		

Table 5. Thermal profile for *16S rRNA* gene specific primer of *E. coli.*

Conditions	Temperature (°C)	Time	Cycle (s)	References
Initial denaturation	95	5 min		
Denaturation	94	40 sec		
Annealing	56	30 sec	30	Sarker *et al.* (2018)
Extension	72	30 sec		
Final extension	72	10 min		
Holding	4	Until use		

Table 6. Thermal profile for *groEL* gene specific primer of *Vibrio* spp.

Conditions	Temperature (°C)	Time	Cycle (s)	References
Initial denaturation	94	5 min		
Denaturation	94	30 sec		
Annealing	69	30 sec	30	Hossain *et al.* (2014)
Extension	72	30 sec		
Final extension	72	7 min		
Holding	4	Until use		

2.6 Antibiotic sensitivity testing

The antimicrobial susceptibility assay was detected using the disc diffusion method in accordance with the Clinical and Laboratory Standards Institute's (CLSI, 2016) guidance. The disc diffusion method was used to test antimicrobial drug susceptibility against 12 widely used antibiotics (Bauer *et al.*, 1966). The following antibiotic discs were employed for *Salmonella* spp., *Shigella* spp., *E. coli*, and *Vibrio* spp.: Amoxycillin (AMX, 20 µg), Gentamicin (GEN, 10 µg), Amikacin (AK, 30 µg), Ceftriaxone (CTR, 30 µg), Ceftazidime (CAZ, 30 µg), Azithromycin (AZM, 15 µg), Levofloxacin (LE, 5 µg), Colistin (CL, 10 µg), Cefepime (CPM, 30 µg), Aztreonam (AT, 30 µg), Ampicillin (AMP, 10 µg), Erythromycin (E, 15 µg) Doxycycline (DO, 30 µg), Cefixime (CPM, 30 µg).

3. Results

3.1 Cultural, staining, and biochemical characteristics

3.1.1 Bacterial growth on nutrient agar

The appearance of homogenous turbidity in the nutrient broth indicated the growth of bacteria. The appearance of various types of colony characteristics (large, circular, convex, greyish-white, smooth, and translucent) on nutrient agar media indicated the presence of mixed bacterial population. Then each type of colony was sub-cultured on different selective media for specific bacterial colony as well as to obtain pure culture of *Salmonella* spp., *Shigella* spp., *E. coli*, and *Vibrio* spp. (Figure 2).

Figure 2. Growth of mixed bacterial population on nutrient agar plate.

3.1.2 Subculture of bacterial isolates on different media for obtaining pure isolates of *Salmonella* spp., *Shigella* spp., *E. coli,* and *Vibrio spp.*

Among the 40 samples, 3 isolates showed colony characteristics consistent with *Salmonella* spp. on various media such as SS, XLD, BGA, and MAC Agar. On *Salmonella Shigella* (SS) agar media, all six suspected *Shigella* isolates formed characteristic big, round, convex, and colorless colonies. All 14 probable *E. coli* isolates formed smooth, round, black or green colonies with a metallic sheen on EMB agar. Following primary culture, all probable *Vibrio* spp. isolates (n=3) formed characteristic smooth, round, yellow or greenish colonies on TCBS media.

Under the microscope, all of the putative *Salmonella*, *Shigella*, *E. coli*, and Vibrio isolates appeared to be gram-negative, pink-colored, tiny rod-shaped bacteria organized singly or in pairs. *Salmonella*

isolates that are culture positive and urease positive change color from yellow to purple-red extremely quickly after organisms are inoculated into test tubes. However, unfavorable reactions that occur slowly imply positive results for *Salmonella* spp. All of the suspected Salmonella isolates tested negative for urease. The positive *Shigella* isolates did not ferment dextrose, maltose, lactose, or sucrose in comparison to gas generation. The isolates were only mannitol-positive, indicating acid generation.

The suspected *Shigella* isolates tested positive for Methyl Red and the Indole test. The suspected *Shigella* isolates produced a pink ring and tested negative for VP.

The positive isolates of *E. coli* and *Vibrio* spp. fermented dextrose, maltose, lactose, and sucrose, as well as produced acid and gas. All probable *E. coli* isolates were positive for the Methyl Red test. However, *Vibrio* spp. isolates were negative for the Methyl Red test. Both putative isolates were VP-positive (Figure 3).

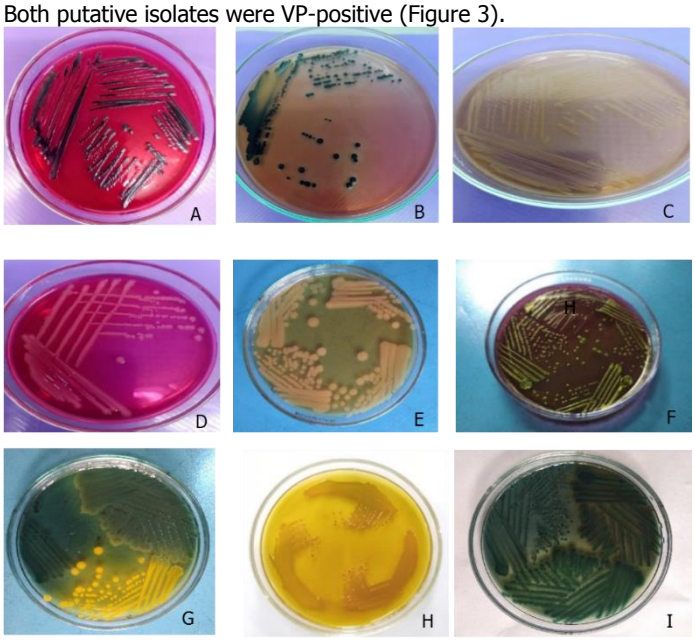

Figure 3. The *Salmonella* spp. produced a red-colored colony with a black center, smooth, and small round colonies on XLD agar (Figure 3A) and the production of translucent black, smooth, and black-centered colonies on SS agar (Figure 3B). Along with the *Salmonella* spp. produced a round, smooth, small, opaque colorless colonies on MacConkey agar (Figure 3C) and development of translucent pink, smooth, and small round colonies on Brilliant Green Agar (Figure 3D). The *Shigella* spp. are seen as large, circular, convex, and transparent colonies on the SS (Salmonella-Shigella) agar plate (Figure 3E). Smooth, spherical colonies on EMB agar that are colored green or black with a metallic sheen for *E. coli* (Figure 3F). Smooth, circular, yellow or greenish colonies on TCBS media after primary culture of Vibrio (Figure 3G). Particularly smooth, circular, and yellow (Figure 3H) or greenish (Figure 3I) colonies on TCBS media for *Vibrio* spp.

3.2 Molecular confirmation of suspected Salmonella spp., Shigella spp., E. coli, and Vibrio spp. isolates

DNA from all probable Salmonella isolates was used in the PCR assay. DNA from pure culture was extracted using the standard boiling procedure. *Salmonella* spp. were validated by amplification of the *bcfC* gene (993 bp). Finally, 3 isolates were identified as *salmonella* spp. (Figure 4A). Among the 40 samples, 6 were positive for *Shigella* spp. using a genus-specific primer of the *invC* gene (Table 7). The amplicon size of 875 bp was observed using the UV trans-illuminator (Figure 4B). Of the 40 samples analyzed, 14 tested positive for *E. coli* using a primer specific to the *16S rRNA* gene. The resulting amplicon, measuring 704 bp, was detected with a UV trans-illuminator (Figure 4C).

Out of the 10 isolates initially suspected to be Vibrio, 3 were confirmed as Vibrio species using genus-specific *groEL* primers, which yielded a positive band at 1117 bp (Figure 4D). Further analysis with multiplex PCR and species-specific *groEL* primers identified all three confirmed isolates as *Vibrio cholerae*, producing a positive band at 418 bp (Figure 4E). No isolates were identified as *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, or *Vibrio vulnificus* through multiplex PCR.

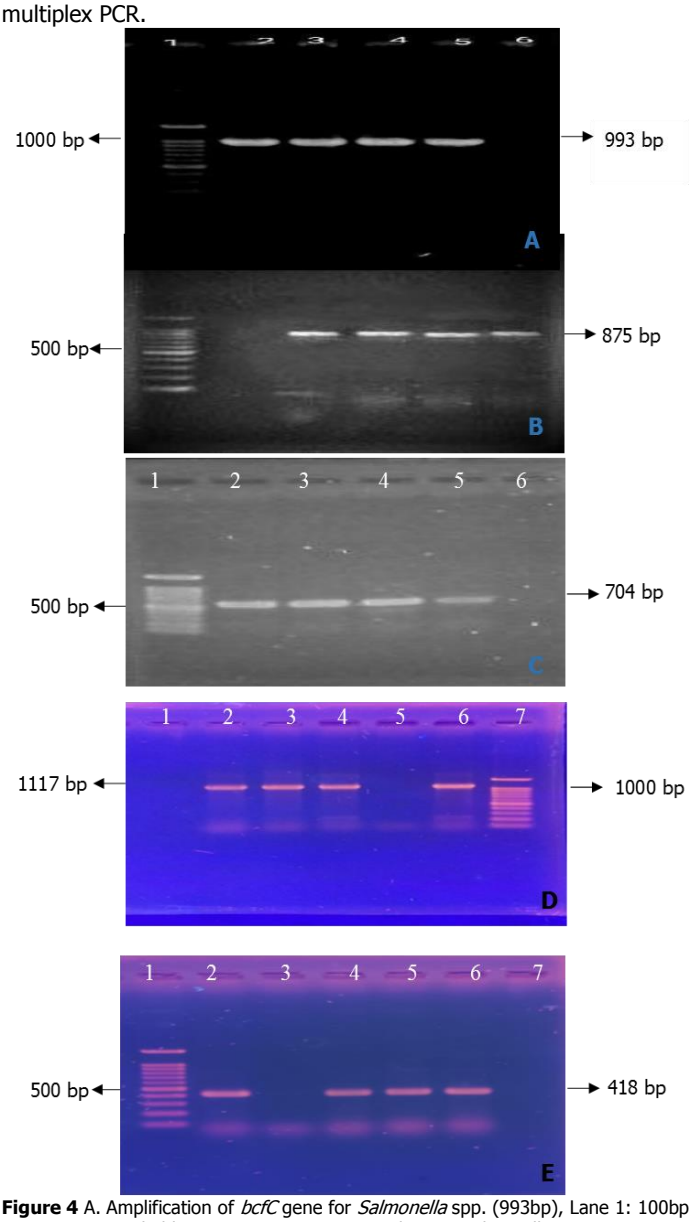

Figure 4 A. Amplification of *bcfC* gene for *Salmonella* spp. (993bp), Lane 1: 100bp DNA ladder, Lane 2: Positive control, 3-5: *Salmonella* spp. Figure 4 B. PCR amplification of Shigella genus specific gene primer (875 bp) of *Shigella* spp. Lane 1: 100bp DNA Marker, Lane 1-4: Representative *Shigella* spp. isolates. Figure 4 C. PCR amplification of *E. coli* genus specific gene primer (*16S rRNA*) of *E. coli.* Lane 1: 100bp DNA Marker, Lane 2-4: Representative *E. coli* isolates. Figure 4 D. PCR amplification of *groEL* gene for specific detection of the genus Vibrio Lane 1: 100bp DNA Marker, Lane-2: Positive control. Lane-3: Negative control, Lane 4-6: Representative Vibrio isolates. Figure 4 E. Amplification of *groEL* gene for the specific detection of *V. alginohyticus* (301), *V. parahaemolyticus* (644), *V. cholerae* (418) and *V. vulnificus* (192).

3.3 Antibiotics sensitivity test of isolated Salmonella spp. Shigella spp., E. coli, and Vibrio spp.

Among the three *Salmonella* spp. isolates, all were resistant to Amoxicillin, 70% to Ceftazidime, 33% to Ceftriaxone, and 25% to Levofloxacin. They were sensitive or intermediately sensitive to Azithromycin, Colistin, Amikacin, and Gentamicin (Table 8).

For the six *Shigella* spp. isolates, 83% were resistant to Cefepime and 67% to Ceftazidime. They showed high or intermediate sensitivity to Azithromycin, Amoxicillin, Ceftriaxone, Colistin, Amikacin, and Gentamicin (Table 9).

Table 7. The overall occurrence of *Salmonella* spp., *Shigella* spp., *E. coli,* and *Vibrio* spp. in river water, pond water, tap water, and tube-well water.

Sources of sample	No. tested of sample	Number of positive *Salmonella* spp. n (%)	Number of positive *E. coli* n (%)	Number of positive *Shigella* spp. n (%)	Number of positive *Vibrio* spp. n (%)
River water	10	2 (20.00)	5 (50.00)	2 (20.00)	2 (20.00)
Pond water	10	1 (10.00)	6 (60.00)	3 (30.00)	1 (10.00)
Tap water	10	0 (0.0)	2 (20.00)	1 (10.00)	0 (0.00)
Tube well water	10	0 (0.0)	1 (10.00)	0 (0.0)	0 (0.0)
Total	40	3	14	6	3

All 14 *E. coli* isolates displayed resistance patterns with 100% resistance to Amoxicillin, 93% to Colistin, 72% to Azithromycin, Cotrimoxazole, and Cefepime, and 57% to Gentamicin. They reacted to ceftriaxone and amikacin with high or intermediate sensitivity (Table 10).

Among the three *Vibrio* spp. isolates, 100% were resistant to Ampicillin, 81% to Cefepime, 72% to Cefixime, 70% to Amoxicillin, and 67% to both Erythromycin and Ceftazidime. They had high or intermediate sensitivity to amikacin, gentamicin, levofloxacin, doxycycline, and cotrimoxazole (Table 11).

Table 8. The percentages of antibiotics sensitivity and resistance patterns of *Salmonella* spp. (n=3) isolates against different antibiotic classes.

Antibiotic classes	Antibiotics	Sensitivity pattern of *Salmonella* spp.		
		Sensitive	Intermediate	Resistant
Beta-lactamase inhibitors	Amoxicillin (AMX)	0%	0%	100%
Macrolides	Azithromycin (AZM)	100%	0%	0%
Polymixins	Colistin (CL)	67%	33%	0%
Quinolones	Levofloxacin (LE)	75%	0%	25%
Sulfonamides	Cotrimoxazole (COT)	74%	26%	0%
Cephalosporin	Ceftriaxone (CTR)	67%	0%	33%
	Ceftazidime (CAZ)	30%	0%	70%
	Cefepime (CPM)	80%	20%	0%
Aminoglycosides	Amikacin (AK)	70%	30%	0%
	Gentamicin (GEN)	80%	20%	0%

The sensitivity and resistance profiles of *Salmonella* spp. isolates from water sources were analyzed against 10 antibiotics from 7 different classes where Amoxicillin (AMX), Azithromycin (AZM), Colistin (CT), Levofloxacin (LE), Cotrimoxazole (COT), Ceftriaxone (CTR), Ceftazidime (CAZ), Cefepime (CPM), Amikacin (AK), and Gentamicin (GEN).

Table 9. The percentages of antibiotics sensitivity and resistance patterns of *Shigella* spp. (n=6) isolates against different antibiotic classes.

Antibiotic classes	Antibiotics	Sensitivity pattern of *Shigella* spp.		
		Sensitive	Intermediate	Resistant
Beta-lactamase inhibitors	Amoxicillin (AMX)	83%	0%	17%
Macrolides	Azithromycin (AZM)	100%	0%	0%
Polymixins	Colistin (CL)	67%	23%	10%
Quinolones	Levofloxacin (LE)	67%	33%	0%
Sulfonamides	Cotrimoxazole (COT)	55%	45%	0%
Cephalosporin	Ceftriaxone (CTR)	83%	17%	0%
	Ceftazidime (CAZ)	16%	17%	67%
	Cefepime (CPM)	0%	17%	83%
Aminoglycosides	Amikacin (AK)	58%	42%	0%
	Gentamicin (GEN)	67%	33%	0%

The sensitivity and resistance profiles of *Shigella* spp. isolates from water sources were assessed against 10 antibiotics across 7 different classes where Amoxicillin (AMX), Azithromycin (AZM), Colistin (CT), Levofloxacin (LE), Cotrimoxazole (COT), Ceftriaxone (CTR), Ceftazidime (CAZ), Cefepime (CPM), Amikacin (AK), and Gentamicin (GEN).

Table 10. The percentages of antibiotics sensitivity and resistance patterns of *E. coli* (n=14) isolates against different antibiotic classes.

Antibiotic classes	Antibiotics	Sensitivity pattern of *E. coli*		
		Sensitive	Intermediate	Resistant
Beta-lactamase inhibitors	Amoxicillin (AMX)	0%	0%	100%
Macrolides	Azithromycin (AZM)	14%	14%	72%
Polymixins	Colistin (CL)	0%	7%	93%
Quinolones	Levofloxacin (LE)	21%	72%	7%
Sulfonamides	Cotrimoxazole (COT)	21%	7%	72%
Cephalosporin	Ceftriaxone (CTR)	64%	0%	36%
	Ceftazidime (CAZ)	22%	21%	57%
	Cefepime (CPM)	21%	7%	72%
Aminoglycosides	Amikacin (AK)	64%	7%	29%
	Gentamicin (GEN)	22%	21%	57%

The sensitivity and resistance profiles of *E. coli* isolates from water sources were tested against 10 drugs from seven distinct classes where Amoxicillin (AMX), Azithromycin (AZM), Colistin (CT), Levofloxacin (LE), Cotrimoxazole (COT), Ceftriaxone (CTR), Ceftazidime (CAZ), Cefepime (CPM), Amikacin (AK), and Gentamicin (GEN).

Table 11. The percentages of antibiotics sensitivity and resistance patterns of *Vibrio cholerae* (n=3) isolates against different antibiotic classes.

Antibiotic classes	Antibiotics	Sensitivity pattern of *Vibrio* spp.		
		Sensitive	Intermediate	Resistant
Beta-lactamase inhibitors	Amoxicillin (AMX)	30%	0%	70%
	Ampicillin (AMP)	0%	0%	100%
Macrolides	Erythromycin (E)	33%	0%	67%
Tetracycline	Doxyciline (DO)	67%	0%	33%
Quinolones	Levofloxacin (LE)	70%	0%	30%
Sulfonamides	Cotrimoxazole (COT)	68%	0%	32%
Cephalosporin	Cefixime (CFM)	28%	0%	72%
	Ceftazidime (CAZ)	30%	3%	67%
	Cefepime (CPM)	6%	13%	81%
Aminoglycosids	Amikacin (AK)	70%	0%	30%
	Gentamicin (GEN)	93%	0%	7%

The display of 11 distinct antibiotics categorized into 7 classes, along with the sensitivity and resistance profiles of *Vibrio cholerae* isolates from water sources where Amoxicillin (AMX), Ampicillin (AMP), Erythromycin (E), Levofloxacin (LE), Doxycycline (DO), Cotrimoxazole (COT), Cefixime (CFM), Ceftazidime (CAZ), Cefepime (CPM), Amikacin (AK), and Gentamicin (GEN) are displayed in a bar diagram.

4. Discussion

In the fields of industry, research, and medicine, the Enterobacteriaceae family of bacteria is one of the most significant (Ramírez-Castillo *et al.*, 2015). Microorganisms play a significant role

in maintaining the quality of water and have the potential to spread pathogenic bacteria, viruses, and parasites into drinking water (Cabral, 2010). Microorganisms like Salmonella, Shigella, *Escherichia coli*, and *Vibrio cholerae* are among those that can make people sick when they consume polluted water (Cabral, 2010).

This study intended to examine the presence, cultural characteristics, biochemical test, molecular characteristics, and antibiotic susceptibility status of *Salmonella* spp*., Shigella* spp*., E. coli*, *and Vibrio* spp*.* strains isolated from different water sources of Mymensingh city in Bangladesh.

The Enterobacteriaceae family is crucial in medicine, industry, and research (Ramírez-Castillo *et al.*, 2015). Microorganisms in water can transfer diseases, including bacteria, viruses, and parasites (Cabral, 2010). Pathogens like Salmonella, Shigella, *E. coli*, and *Vibrio cholerae* can contaminate drinking water (Cabral, 2010).

This study focused on assessing the presence, characteristics, and antibiotic resistance of Salmonella, Shigella, *E. coli*, and Vibrio strains from different water sources in Mymensingh city, Bangladesh.

The occurrence of 7.5% of *Salmonella* spp. in water was comparatively lower than the previously conducted reports of 19.4% and 3.3% of *S. enterica* in pond and well water, respectively, by Gu *et al.* (2021), 0.89% by Momtaz *et al.* (2013), and 4% by Dekker *et al.* (2018). Similarly, the higher findings were reported in southern Georgia and northern Florida at 28.2% by Luo *et al.* (2015) and on the banks of the Zarqa River at 12.8% by Tarazi *et al.* (2021). These fluctuations could be brought on by changes in the seasonal and geographic conditions along the Zarqa River's banks. A total of 3 isolates from the water samples utilized in this investigation were recognized as *Salmonella* spp. based on cultural and biochemical traits. According to Mridha *et al.* (2020), the colony features of *Salmonella* spp. that were isolated in various media resembled those of *Salmonella* spp. Variations in the outcomes could be caused by genetic variables and the type of organisms residing there. The isolates of *Salmonella spp.* exhibited higher resistance patterns against Amoxicillin (100%), Ceftazidime (70%), and Cephalexin (67%). Furthermore, a lower level of resistance was noted against Levofloxacin (25%), Ceftriaxone (33%) and almost no resistance or highly susceptibility to Cefepime, Amikacin, Gentamicin, Azithromycin, Colistin. The previous studies reported the higher resistance patterns of *Salmonella* spp. against amoxicillin by Mian *et al.* (2020). These differences may be due to geographical and seasonal variations.

The occurrence of 15% of *Shigella* spp. in water samples was higher than the previously conducted report of 5.4% by Hsu *et al.* (2010). Similarly, the higher 27% by Saima *et al.* (2018). These discrepancies may arise from differences in sample numbers, methods employed throughout the process, and regional and seasonal distribution. Using drinking water samples from this study, 6 isolates were determined to be *Shigella* spp. based on cultural and biochemical traits. According to Schaumburg *et al.* (2015), the colony features of *Shigella* spp. isolated in various media mirror those of *Shigella* spp. The *Shigella* spp. isolates showed higher resistance patterns to ceftazidime (67%) and cefepime (83%). Furthermore, a lower level of resistance was noted against Amoxicillin (17%), Colistin (10%) and almost no resistance or highly susceptibility was show to Azithromycin, Levofloxacin, Cotrimoxazole, Ceftriaxone, Amikacin, and Gentamicin. According to Matloko *et al.* (2021), who reported that *Shigella* spp. had greater resistance patterns to cefuroxime (36.1%, 13/36), cefepime (30.6%, 11/36), cefuroxime, and aztreonam (27.8%, 10/36) than to the other antibiotics examined (susceptibilities of > 75%), etc.

The occurrence of 35% of *E. coli* in water samples was higher than previously conducted reports of 7.5 8% by Momtaz *et al.* (2013). Similarly, the higher findings found 60.3% by Thani *et al.* (2016) and 77.97% from Larut River were commensal strains, with phylogroup B1 (39.55%) and phylogroup A (38.42%) by Bong *et al.* (2022). Variations in sample numbers, methods types employed throughout the process, and regional and seasonal dispersion could all contribute to these discrepancies. Utilizing drinking water samples for this investigation, a total of 16 isolates were determined to be *E. coli* based on biochemical and cultural traits. According to Julqarnain *et al.* (2022) and Hossain *et al.* (2020), the colony properties of the isolated *E. coli* in various media reflect those of *E. coli*. The higher resistance patterns were shown by the *E. coli* isolates to Amoxicillin (100%), Colistin (93%), Azithromycin (72%), Cotrimoxazole (72%), Cefepime (72%), Ceftazidime (57%), and Gentamicin (57%). Furthermore, a lower level of resistance was noted against Levofloxacin (7%), Ceftriaxone (36%), Amikacin (29%). Prior research has documented the 49.21% of the *Escherichia coli* isolates were susceptible, 12.90% were intermediate, and 37.90% were resistant overall. Vancomycin (94.64%) and erythromycin (85.71%) both exhibited high resistance. There was also a significant level of susceptibility to ceftrioxine (89.29%), gentamicin (91.07%), and ciprofloxacin (94.64%). Adzitey *et al.* (2016) who reported that a comparatively greater proportion of the *Escherichia coli* isolates from the water sample (50%) showed intermediate resistance to amoxycillin/clavulanic acid. Variations may be due to differences in geography, seasonality, sample sizes, and methods.

The occurrence of 7.5% of Vibrio spp. in water samples was higher than previously conducted reports of 1.7% by Alam *et al.* (2014). Similarly, higher findings found 89% by Shanan *et al.* (2011), 35% by Ferdous *et al.* (2018). These differences could be the result of changes in sample numbers, methods utilized throughout the entire process, and regional and seasonal distribution. There were 3 distinct *Vibrio* spp. isolates found according to the cultural and biological traits of the drinking water samples that were utilized for this investigation. Another study conducted by Azwai *et al.* (2016) reported that the colony properties of isolated *Vibrio* spp. in various media are similar to those of *Vibrio* spp., and the higher resistance patterns against 100% Ampicillin, 81% Cefepime, 72% Cefixime, 70% Amoxicillin, 67% Erythromycin, and 67% Ceftazidime were shown by *Vibrio* spp. Conversely, the higher sensitive against Gentamicin, Levofloxacin, Doxyciline, Cotrimoxazole, Amikacin.

5. Conclusions

The present study was carried out to isolate and identify *Salmonella* spp*., Shigella* spp*., E. coli and Vibrio* spp. from water samples of Mymensingh city. In addition, their antibiotic resistance profile was also determined at the phenotypic and genotypic level. The water sources from various locations in Mymensingh municipality are contaminated with enteric bacteria, posing a significant public health risk to residents who rely on the water for drinking. The presence of bacterial species such as Salmonella, Shigella, *E. coli*, and Vibrio highlights the potential for gastrointestinal diseases. The antibiotic resistance profiles of these isolates raise further concern. High levels of resistance to commonly used antibiotics like amoxicillin and ceftazidime suggest that treatment of infections caused by these bacteria may be challenging, and the continued use of antibiotics could exacerbate antimicrobial resistance. However, the isolates showed varied sensitivity to other antibiotics, indicating potential therapeutic options for managing infections. In conclusion, the study underscores the need for urgent measures to improve water quality, implement proper sanitation practices, and develop antimicrobial stewardship programs to address both contamination and the growing issue of antibiotic resistance in the region.

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Data availability

The data generated from this study will be available on the valid request from the corresponding author.

Informed consent statement

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

Authors' contribution

Conceptualization: Mohammad Ferdousur Rahman Khan; **Data collection, study conduct, and manuscript write up**: Dula chakraborty and Limon Biswas; **Methodology preparation:** Mahbubul Pratik Siddique and Najmun Nahar Popy; **Formal analysis, data analysis, first draft develop, and result section interpretation:** Mohammad Ferdousur Rahman Khan, Mahbubul Pratik Siddique, Dula chakraborty, and Shantono Acharjee; **Figure preparation, formal analysis, reviewed, and revised the final version of the manuscript:** Dula chakraborty, Limon Biswas, Shantono Acharjee, and Najmun Nahar Popy, and Mohammad Ferdousur Rahman Khan. All authors critically reviewed the manuscript and agreed to submit final version of the article.

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