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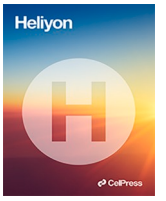

Research article

Screening and identification of multiple antibiotic- resistant genes containing *Salmonella* Typhi from drinking water: A severe public health concern in Bangladesh

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ABSTRACT

Contaminated water poses a significant public health risk due to waterborne microbial diseases, with typhoid caused by *Salmonella* Typhi being the most prevalent bacterial waterborne disease in Bangladesh. This study aimed to assess the presence of antibiotic-resistant genes and their patterns in *Salmonella* Typhi isolated from drinking water sources in Chattogram City, Bangladesh. Out of 150 samples analyzed, 10 isolates were suspected to be *Salmonella* Typhi by selective plating and biochemical test and 8 were confirmed using PCR amplification of the *fliC* gene and subsequent sequencing. Phylogenetic analysis provided insights into the genetic diversity and relationships among the identified isolates. The study revealed the presence of *Salmonella* Typhi in certain drinking water sources, indicating a potential threat to public health. The presence of antibiotic-resistant genes, including *sul2*, *int1*, *tem*, and *gyrA*, in the genomic and plasmid DNA of *Salmonella* Typhi isolates emphasizes the role of contaminated water in spreading antibiotic resistance. All 8 isolates were found to contain at least one antibiotic-resistant gene highlighting the widespread presence of resistance elements. This raises serious concerns about the potential impact on the effectiveness of typhoid treatment. Antibiotic susceptibility testing using the disk diffusion assay confirmed multidrug resistance (MDR) in 87.5 % of the *Salmonella* Typhi isolates, highlighting the urgent need to address the transmission of antibiotic-resistant genes through drinking water sources. The results confirm the widespread environmental transmission of antibiotic resistance in the region and emphasize the potential consequences for the efficacy of typhoid treatment.

1. Introduction

Access to clean and safe water is crucial for the survival and well-being of all living organisms [1]. It is a fundamental requirement for improving public health and preventing the spread of infections and diseases [2,3]. The quality of water plays an important role in

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determining the risk of waterborne illnesses and spread of the infectious diseases [4]. Among waterborne bacterial infections, *Salmonella* spp. is one of the most common causes of morbidity worldwide [5]. Specifically, *Salmonella* Typhi and Paratyphi A, B, and C are the primary causative agents of typhoid fever, particularly prevalent in developing countries [6]. In South Asia, typhoid affects over 7 million people annually and constitutes a major portion of the 80 % of typhoid cases in developing countries [7]. The incidence is estimated to exceed 100 cases per 100,000 population with Bangladesh being a significant contributor to these figures [8].

Salmonella Typhi contamination in water is a major public health issue in developing countries, particularly in the Indian subcontinent and Southeast Asia [9]. Outbreaks in Tajikistan [9], Pakistan, and Nepal have been linked to municipal and drinking water, with many cases involving multidrug-resistant strains [10]. Studies found *Salmonella* Typhi in drinking water in Africa, with typhoid fever accounting for 39.3 % of waterborne diseases in Nigeria [10]. Poor water quality, untreated drinking water, and seasonal variations play a crucial role in the transmission of typhoid [10,11].

Salmonella is a gram-negative, flagellated, rod-shaped, non-lactose fermenting, facultatively anaerobic bacterium that is typically transmitted through the fecal-oral route [12]. Typhoid fever presents with a range of symptoms, including prolonged fever, diarrhea or constipation, chills, loss of appetite, hepatosplenomegaly, headache, and abdominal pain [12]. In rare cases, additional symptoms such as rash, nausea, anorexia, vomiting, and leukopenia may occur [13].

Antimicrobial resistance is a natural phenomenon that occurs due to the adaptation of infectious agents to the exposure of antimicrobial agents, posing a significant threat to public health [14]. The easy availability of antibiotics at drug stores without a prescription, the use of allopathic drugs by traditional medicine practitioners, and the unregulated use of antibiotics in agriculture, fisheries, and animal husbandry are among the reasons behind the initial development of antimicrobial resistance in *Salmonella* Typhi and other pathogens [15]. The majority of drug resistance in *Salmonella* Typhi is due to genetic alterations in the organism, either through chromosomal mutation or the acquisition of a plasmid or transposon [16].

Recent studies have increasingly reported the prevalence of *Salmonella* Typhi, and multidrug-resistant (MDR) strains isolated from environmental samples, highlighting the severity of this issue. Whole genome sequencing of *Salmonella* Typhi isolates from urban Dhaka revealed a diverse range of genotypes and antimicrobial resistance (AMR) profiles, with a notable decline in H58 genotypes and an increase in non-H58 strains associated with reduced fluoroquinolone susceptibility, emphasizing the need for continuous molecular surveillance to monitor evolving resistance patterns in Bangladesh [17]. Another study highlighted the high prevalence of antibiotic resistance genes (ARGs) in Non-Typhoidal Salmonella (NTS) enterica serovars from chicken processing environments, including plasmid-mediated *blaTEM, tetA, sul1, sul2*, *sul3*, and *strA/B* genes [18].

Numerous genes found in chromosomes and plasmids are responsible for antimicrobial resistance, including *gyrA, tetB, sul2, tem, catP, qnr,* and *int1* against antibiotics [12,14]. In particular, *catP, sul2, tem*, and *gyrA* are linked to resistance against chloramphenicol, co-trimoxazole, ampicillin, and ciprofloxacin, respectively [12], while the *qnr* family genes such as *qnrA, qnrB, and qnrS* are responsible for plasmid-mediated resistance to quinolones [19]. Furthermore, other antibiotic-resistant genes such as *tetB, blt*, and *int1* are responsible for resistance to tetracycline, cephalosporin, chloramphenicol, streptomycin, and sulphonamides [20]. The presence of multiple antimicrobial-resistant genes within the same isolates of *Salmonella* Typhi may be the possible reason for the development of multidrug resistance.

Multidrug-resistant strains of *Salmonella* Typhi are associated with increased morbidity, leading to greater toxicity and higher mortality rates [12]. Such strains diminish the effectiveness of typhoid treatments, further complicating the management of the disease [21]. The spread of *Salmonella* Typhi contamination through water sources [3] is primarily due to poor sanitation, sewage leaks, and inadequate waste management systems [22], which allow human feces carrying the bacteria to enter water supplies [23]. In densely populated areas, especially in developing countries, open drains, insufficient wastewater treatment, and improper disposal of human waste exacerbate the contamination [24]. Seasonal factors like flooding can further contribute by mixing sewage with drinking water sources [25]. This poses a major public health risk in countries like Bangladesh, especially with the spread of antibiotic-resistant pathogens [24].

Given these circumstances, this study aimed to investigate the consequences of *Salmonella* Typhi strains containing multiple antibiotic-resistant genes in the drinking water of Chattogram City, Bangladesh. Understanding the prevalence and impact of these resistant genes is essential for developing effective strategies to mitigate the transmission of multidrug-resistant *Salmonella* Typhi and to preserve the efficacy of typhoid fever treatments. Addressing the challenges associated with contaminated drinking water sources is vital for improving public health in resource-limited settings.

2. Materials and methods

2.1. Sample collection and processing

During this study, a total of 150 portable drinking water samples were collected from various drinking water sources within Chattogram City, Bangladesh. These sources include restaurants/tea stalls (n = 39), hospitals and diagnostic centers (n = 31), residences (n = 50), and vendor shops (n = 30) situated in Chattogram City, Bangladesh. Stringent aseptic measures were employed during the collection process to ensure sample integrity. Collected samples were kept in a sterilized container (ice bag) and carried to the laboratory and all the samples were stored at 4 °C (for 2–24 h) until further use for microbiological analysis. To identify *Salmonella* Typhi, in every case, 250 ml of drinking water was filtrated using a membrane filter through a 47 mm nylon membrane with a pore size is 0.22 μm.

2.2. Selective plating

After filtration, the filter containing the concentrated sample was transferred onto xylose lysine deoxycholate (XLD) agar plates and incubated at 37 °C overnight. Suspected single colonies showing red coloration with a black center on the XLD agar were selected. These colonies were subcultured once again on XLD agar plates and incubated at 37 °C overnight to obtain a pure culture of the bacterium. The streak plate method was employed for sub-culturing the colonies onto fresh XLD agar plates [12].

2.3. Biochemical test (TSI test)

For biochemical identification, the triple sugar iron (TSI) medium was used. A single colony from the pure culture plate was inoculated onto the TSI medium and incubated at 37 °C overnight [26].

2.4. Genomic DNA extraction and Quantification

The genomic DNA of the isolated *Salmonella* Typhi was extracted using the boiling method as previously described [12,26]. The DNA concentration was measured using a Nanodrop machine, and the extracted DNA was stored at −20 °C.

2.5. Plasmid extraction

Fresh bacterial cultures were used to extract plasmid DNA. Thus, the isolated bacteria were cultured on LB media with different antibiotics. Plasmid extraction was performed using AxyPrep Plasmid Miniprep kit (50 rxn) (Prodict code: Ap-MN-P-50) according to the supplied protocol.

2.6. PCR amplification of target genes

Molecular identification of *Salmonella* Typhi was performed using the PCR assay. The flagellin gene, *fliC*, of *Salmonella* Typhi was targeted to confirm the presence of the bacterium. A pair of primers was chosen to amplify the *fliC* gene [12]. Moreover, to identify four antibiotic-resistant genes such as *gyrA*, *sul2, tem,* and *int1,* primers were selected through a literature study [27,28]. Primer sequences are given in Table 1.

The Go Taq 2X Promega green master mix (M7132) was used, and the PCR reaction mixture was prepared in a 1.5 mL sterilized eppendorf tube. Composition of PCR mixture and cyclic condition are represented in table (Table 1). The PCR products were separated on a 1.5 % agarose gel using an electric current of 120 V and 150 mA for 45 min. The bands were visualized on a UV illuminator to determine the presence of the expected bands. In all cases, *Salmonella* Typhi CHM 55 strain was used as positive control (GenBank accession number: MW819865, details in Table S1) collected from the "Laboratory of Microbial and Cancer Genomics", Department of Genetic Engineering and Biotechnology, University of Chittagong, Chattogram, Bangladesh [12].

2.7. Sequencing

The PCR products of the *fliC* gene were sequenced at "First Base Research Laboratory", Malaysia. After sequencing, the quality of the sequences was assessed and corrected manually based on the corresponding electrochromatogram (Chromas software, version 2.6.6). After that sequences were submitted in GenBank using Bankit. BLAST analysis was done to identify the desired isolates. A phylogenetic tree was made using neighbor-joining method.

Table 1
Target genes, primers, cyclic condition, composition of PCR mixture and amplicon size.

Genes	Primers (5′>3′)	cyclic condition	composition of PCR mixture	amplicon size (bp)
fliC	F: ACTGCTAAAACCACTACT R: TGGAGACTTCGGTCG CGTAG	1 min at 94 °C; 36 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min; 10 min for 72 °C.	For 20 μl: 10 μl master mix, 4 μl template, 2 μl^F, 2 μl^R, 2 μl water	367
gyrA	F: TACCGTCATAGTTATCCACGA R: GTACTTTACGCCATGAACGT	5 min at 94 °C,30 cycles of 94 °C for 50 s, 62 °C for 50 s and 72 °C for 1 min; 5 min for 72 °C.	For 20 μl: 10 μl master mix, 4 μl template, 2 μl^F, 2 μl^R, 2 μl water	313
sul2	F: TCAACATAACCTCGGACAGT R: GATGAAGTCAGCTCCACCT	1 min at 94 °C,35 cycles of 94 °C for 1 min, 45 °C for 1 min and 72 °C for 1 min; 7 min for 72 °C.	For 20 μl: 10 μl master mix, 4 μl template, 2 μl^F, 2 μl^R, 2 μl water	707
tem	F: GCACGAGTGGGTTACATCGA R: GGTCCTCCGATCGTTGTCAG	4 min at 94 °C,33 cycles of 95 °C for 40 s, 62 °C for 40 s and 72 °C for 40 s; 7 min for 72 °C.	For 20 μl: 10 μl master mix, 4 μl template, 2 μl^F, 2 μl^R, 2 μl water	311
Int1	F: CCTCCCGCACGATGATC R: TCCACGCATCGTCAGGC	2 min at 94 °C,33 cycles of 94 °C for 30 s, 59 °C for 30 s and 72 °C for 30 s, 7 min for72 °C.	For 20 μl: 10 μl master mix, 4 μl template, 2 μl^F, 2 μl^R, 2 μl water	280

Note: F for Forward primer and R for Reverse primer, s for seconds, min for minute (s).

2.8. Antimicrobial susceptibility test (AST)

To assess antibiotic resistance, an antimicrobial susceptibility test was conducted using the Kirby-Bauer disk diffusion method following the guidelines set by the Clinical and Laboratory Standards Institute (CLSI M100, Ed 31st). For our convenience, we used 150 × 15 mm petri dishes in our experiments. This choice allowed for better handling and ensured sufficient space for antibiotic susceptibility testing. The test utilized the 0.5 McFarland standard to standardize the inoculum (1.5×10^8 CFU/mL). Eight types of antibiotic disks from HiMedia **(**Table 2**)** were used. The susceptibility of the isolated bacteria to these antibiotics was determined based on the zone of inhibition observed around each disk. Sterile disks with no antibiotic were used as negative controls in the AST experiments.

3. Results

3.1. Identification of Salmonella Typhi from potable drinking water

Out of the 150 samples analyzed, 10 (6.67 %) isolates were suspected as *Salmonella* Typhi by selective plating and biochemical test (Fig. 1A–B**).** To confirm the presence of *Salmonella* Typhi in the suspected isolates, PCR amplification of the *fliC* gene was performed. Out of the 10 suspected isolates, 8 isolates showed positive amplification of the *fliC* gene (Fig. 1C, Fig. S1), providing strong evidence for their identification as *Salmonella* Typhi. The expected amplicon size was 367 bp. Subsequently, the PCR products of the 8 identified isolates were sequenced and confirmed as *Salmonella* Typhi through BLAST analysis. The obtained sequences were submitted in GenBank (Table 3). To determine the evolutionary relationship among the identified isolates, a phylogenetic tree was constructed using the Neighbor-Joining method implemented in MEGA X (Fig. 1D).

The tree was drawn to scale, with branch lengths representing the evolutionary distances computed using the Maximum Composite Likelihood method. The analysis included a total of 20 nucleotide sequences, comprising 8 query sequences from this study and 12 reference sequences (Fig. 1D) of previously identified *Salmonella* Typhi obtained from GenBank.

3.2. Antibiotic-resistant genes identification

We identified four antibiotic-resistant genes in the genomic DNA of *Salmonella* Typhi isolates. The *sul2* gene (n = 5) (Fig. 2A) and the *int1* gene (n = 5) (Fig. 2B) were simultaneously detected in different sets of 5 isolates, indicating their co-occurrence. The *tem* gene was exclusively found in the *Salmonella* Typhi PW-97 strain (Fig. 2C), while the *gyrA* gene was present in all isolates (Fig. 2D), (For Raw gel electrophoresis data see Fig. S2).

We also identified antibiotic-resistant genes in the plasmid DNA of *Salmonella* Typhi isolates. The *sul2* gene was identified in the *Salmonella* Typhi PW-97 isolate (Fig. 3A), and the *int1* gene was detected in the *Salmonella* Typhi PW-46 isolate (Fig. 3B). The *tem* gene was observed in four isolates (*Salmonella* Typhi PW-97, PW-46, PW-157, and PW-171 strains) (Fig. 3C), while the *gyrA* gene was present in all isolates (Fig. 3D), (For Raw gel electrophoresis data see Fig. S3).

3.3. Antimicrobial resistance profiling of Salmonella Typhi

In our study, we identified 87.5 % (n = 7) of the isolated *Salmonella* Typhi strains as multidrug-resistant (MDR). These MDR isolates demonstrated resistance to at least three different classes of antibiotics. One isolate, *Salmonella* Typhi PW-171, was classified as Double Drug-resistant (DDR) as it showed resistance to two antibiotics. All isolates (n = 8) were resistant to one antibiotic, Ampicillin. Nalidixic acid and Cefepime showed resistance in 87.5 % (n = 7) of the isolates, except for *Salmonella* Typhi PW-137 and PW-171 which were sensitive to these antibiotics. Conversely, two antibiotics, Ciprofloxacin and Co-trimoxazole, were found to be effective against *Salmonella* Typhi, with 100 % (n = 8) sensitivity observed. azithromycin, chloramphenicol, and ceftriaxone exhibited moderate effectiveness in this study (Table S2) (Fig. 4A–D).

4. Discussion

Contaminated water consumption poses a major public health risk in recent times, particularly concerning water-borne microbial

Table 2
List of antibiotic disks.

No	Abbreviations	Antibiotics name	Concentration (μg)
1	AM	Ampicillin	30
2	C	Chloramphenicol	30
3	FEP/CPM	Cefepime	5
4	SXT/COT	Cotrimoxazole	25
5	CTR/CRO	Ceftriaxone	30
6	CIP	Ciprofloxacin	5
7	NA	Nalidixic acid	30
8	AZM	Azithromycin	15

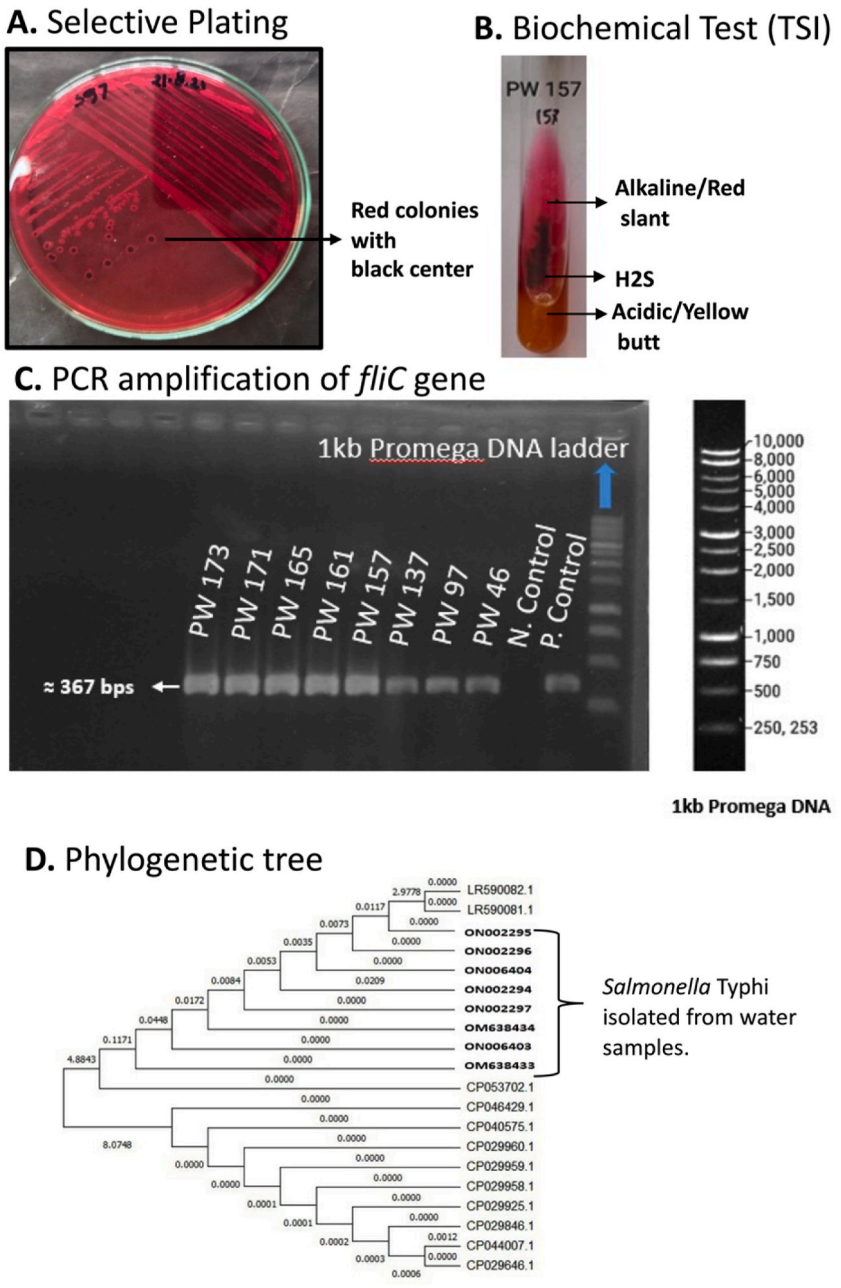

Fig. 1. Identification of *Salmonella* Typhi from Drinking Water. A: XLD agar plate and B: TSI showing representative microorganisms identified. C: Electrophoretic (1.5 %) separation of *fliC* gene confirming the presence of *Salmonella* Typhi (also see Fig. S1). P- Positive; N- Negative. D: Phylogenetic tree of *fliC* gene sequences depicting the evolutionary relationships among the identified isolates.

diseases. Among these, typhoid, caused by *Salmonella* Typhi, stands as the most prevalent bacterial waterborne disease in Bangladesh. According to the World Health Organization (WHO), the global incidence of typhoid reaches an alarming 11–20 million cases annually [24]. Ensuring the safety and purity of drinking water is of paramount importance to prevent such diseases [2].

The findings of the study contribute to the understanding of the prevalence and potential sources of contamination in the region, highlighting the importance of ensuring safe drinking water for public health and provide valuable insights into the identification and antimicrobial resistance profiles of *Salmonella* Typhi isolates. Out of the 150 samples analyzed, 10 (6.67 %) isolates were suspected as *Salmonella* Typhi based on selective plating (Fig. 1A) and biochemical tests (Fig. 1B). To confirm their identity, PCR amplification of the *fliC* gene, a specific marker for *Salmonella* Typhi, was performed (Fig. 1C). This technique confirmed the presence of *Salmonella* Typhi in 8 (5.33 %) out of the 10 suspected isolates, thus validating their identification. The identification of *Salmonella* Typhi in

Table 3
Gene bank accession number and percentage of similarity with reference sequence in BLAST of *fliC* gene sequences of the identified isolates.

Isolate ID	% of similarity with reference sequence in BLAST	Identified isolate	GenBank Accession No.
PW-46	99.61 % [CP053702.1]	*Salmonella* Typhi	OM638433
PW-97	99.41 % [CP053702.1]	*Salmonella* Typhi	ON006403
PW-137	99.11 % [CP053702.1]	*Salmonella* Typhi	ON002294
PW-157	99.11 % [CP053703.1]	*Salmonella* Typhi	ON002295
PW-161	99.11 % [CP053703.1]	*Salmonella* Typhi	OM638434
PW-165	99.41 % [CP053703.1]	*Salmonella* Typhi	ON002296
PW-171	99.41 %[CP053703.1]	*Salmonella* Typhi	ON002297
PW-173	99.41 % [CP053703.1]	*Salmonella* Typhi	ON006404

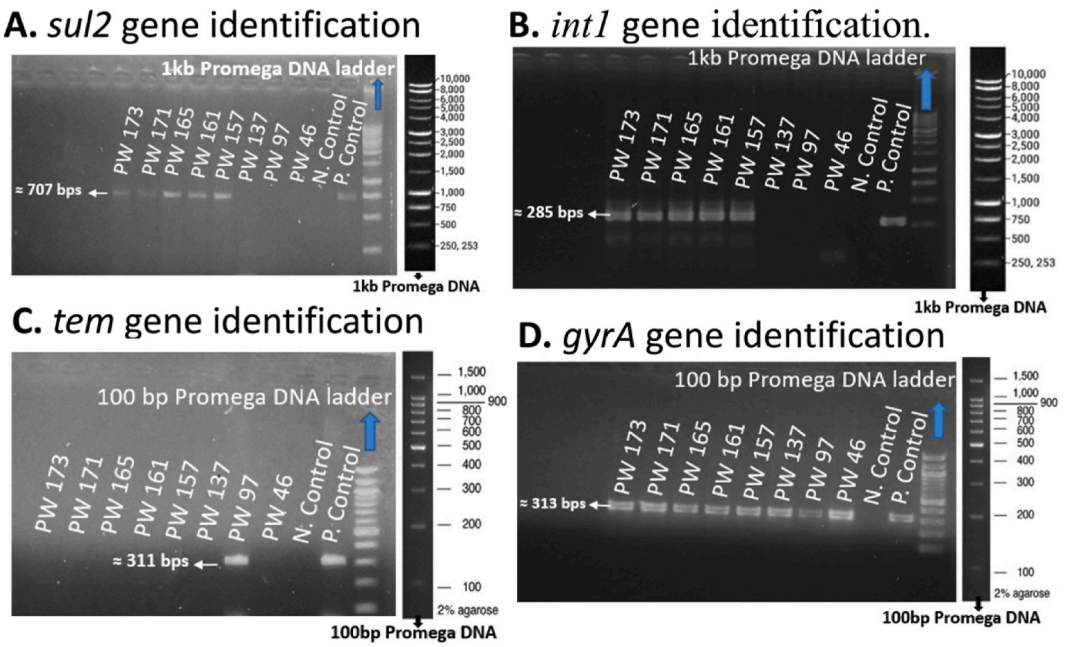

Fig. 2. Molecular identification of antibiotic resistant genes *sul2* (a), *int1* (b), *tem* (c) and *gyrA* (d) from genomic DNA of isolated *Salmonella* Typhi through electrophoretic (1.5 %) separation. Here, *Salmonella* Typhi CHM 55 was used as positive control (GenBank accession number: MW819865) (also see Fig. S2).

drinking water samples from diverse sources, including restaurants, residences, hospitals, and diagnostic centers, highlights a potential public health risk associated with waterborne transmission (Table S3).

The subsequent sequencing of the PCR products and their comparison through BLAST analysis further confirmed these 8 isolates as *Salmonella* Typhi. The obtained sequences were submitted to GenBank, contributing to the existing knowledge of *Salmonella* Typhi genetic diversity.

The construction of a phylogenetic tree using the Neighbor-Joining method revealed the evolutionary relationships among the identified isolates (Fig. 1D). This analysis included 8 sequences from our study and 12 reference sequences of previously identified *Salmonella* Typhi strains. The tree provided insights into the genetic relatedness and evolutionary distances among these isolates, facilitating a better understanding of the population dynamics and genetic diversity of *Salmonella* Typhi.

Antibiotic resistance is a major concern in the management of *Salmonella Typhi* infections [29]. As the acquisition of foreign genes via plasmids and mutations on the chromosome is the two major factors in antibiotic resistance in *Salmonella* Typhi, in this study, we selected four antibiotic resistance genes (*gyrA, sul2, tem, and int1*). These genes were selected for their significant roles in treatment efficacy and prevalence in previous studies. The antibiotics ceftriaxone [30], ampicillin, azithromycin, chloramphenicol [31], ciprofloxacin [30], levofloxacin, co-trimoxazole, and nalidixic acid [30,32] are widely used for typhoid fever treatment. The selection of these four resistance genes reflects their relevance to the resistance mechanisms targeting these commonly used antibiotics. According to a previous study, *gyrA* was only chromosome-origin, while *sul2* was both plasmid and chromosome-borne and identified in *Salmonella* Typhi [12,27,28]. Our study investigated the presence of antibiotic-resistant genes in both genomic DNA and plasmid DNA of the isolates. The detection of the *sul2* and *int1* genes (Fig. 2A & B) in different sets of isolates highlights the diversity of antibiotic resistance mechanisms among *Salmonella* Typhi strains. In our recent study [24], we made a noteworthy investigation regarding the presence of the *int1* gene in coliform bacteria isolated from drinking water sources. This consistent finding of the *int1* gene across

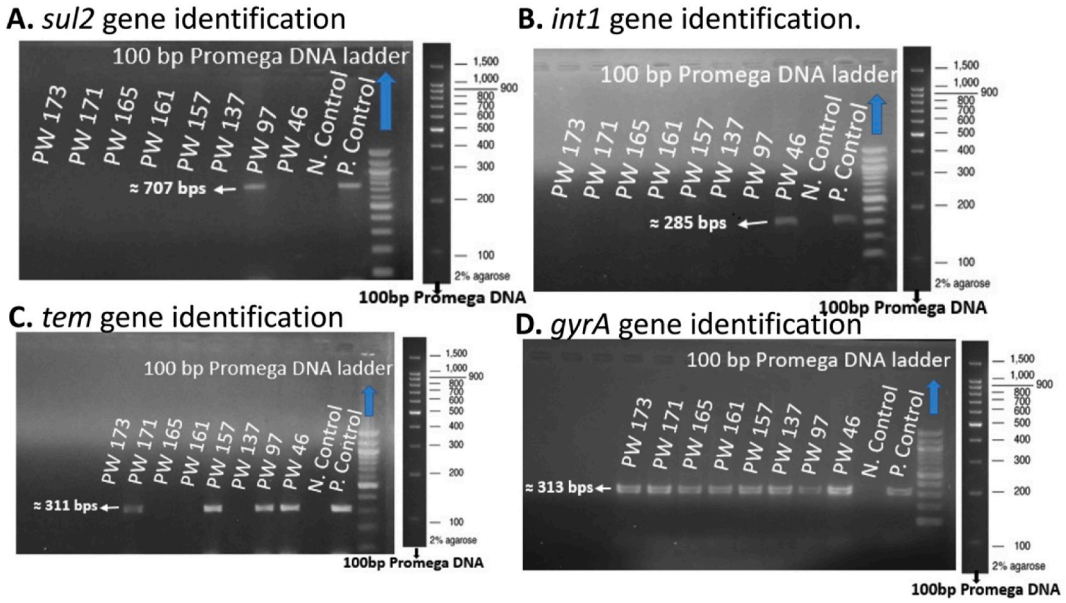

Fig. 3. Molecular identification of antibiotic resistant genes *sul2* (a), *int1* (b), *tem* (c) and *gyrA* (d) from plasmid DNA of isolated *Salmonella* Typhi through electrophoretic (1.5 %) separation. Here, *Salmonella* Typhi CHM 55 was used as positive control (GenBank accession number: MW819865) (also see Fig. S3).

different bacterial species highlights the significance of contaminated drinking water as a potential contributor to the transmission of bacterial resistance among the general population in Bangladesh. The detection of both *sul2* and *int1* genes in the same 5 (62.5 %) isolates is significant, as it suggests the potential horizontal gene transfer, facilitating the spread of multidrug resistance. The *sul2* confers resistance to sulfonamides [33], while *int1* aids in acquiring and spreading multiple resistance genes [14], potentially increasing the challenge of treating *Salmonella* Typhi infections.

The exclusive presence of the *tem* gene (Fig. 2C) in the *Salmonella* Typhi PW-97 strain suggests a unique resistance profile for this particular isolate. Notably, the *gyrA* gene was found in all isolates, indicating its ubiquitous presence in *Salmonella* Typhi strains. However, only one strain was fully resistant to ciprofloxacin (CIP), while four others exhibited intermediate resistance (see Table-S2). This suggests that while gyrA mutations contribute to reduced susceptibility to quinolones, not all mutations result in complete resistance. Further sequencing of the gyrA gene is needed to identify the specific mutations responsible for the resistance phenotype, as mere detection of the gene is insufficient to establish the clinical implications of resistance.

The correlation between antibiotic susceptibility test (AST) results and resistance genes *int1*, *sul2*, and *tem* in *Salmonella* Typhi isolates was also observed. The *sul2* was prevalent in co-trimoxazole-resistant isolates, and the *tem* was often found in ampicillin-resistant ones, suggesting a genetic basis for resistance. The *int1* gene, associated with mobile genetic elements, appeared in several multidrug-resistant isolates, indicating its role in resistance spread.

The presence of antibiotic-resistant genes on plasmids is of particular concern in the context of bacterial resistance. Plasmids are extrachromosomal elements that can transfer genetic material, including antibiotic resistance genes, between bacteria. In this study, we observed the presence of antibiotic-resistant genes, such as *sul2*, *int1*, *tem* and *gyrA* in the plasmid DNA of *Salmonella* Typhi isolates.

Plasmids carrying genes have been associated with resistance to wide range of antibiotics [34], and their presence in bacteria poses a challenge for effective treatment options. It is important to note that the transfer of plasmids harboring antibiotic-resistant genes can contribute to the dissemination of resistance within bacterial populations, making infections caused by these strains more difficult to treat.

Antimicrobial resistance profiling showed that 87.5 % of the isolated *Salmonella* Typhi strains were multidrug-resistant (MDR), exhibiting resistance to at least three different antibiotic classes. One isolate, *Salmonella* Typhi PW-171, was classified as double drug-resistant (DDR) with resistance to two antibiotics. All isolates demonstrated resistance to ampicillin. ciprofloxacin and co-trimoxazole were 100 % effective against all isolates, while moderate effectiveness was observed for azithromycin, chloramphenicol, and ceftriaxone.

The findings of our study are in line with previous research conducted in Chittagong, Bangladesh, by Akter et al.,2021 [35], Chowdhury et al., 2022 [24], and Mina et al., 2018 [36], which have consistently reported the widespread environmental transmission of antibiotic resistance.

These findings highlight the critical need for ongoing surveillance of antimicrobial resistance patterns in *Salmonella* Typhi isolates. The high prevalence of multidrug-resistant (MDR) strains highlights the urgent necessity for alternative treatment strategies and the development of new antimicrobial agents. In addition, the presence of specific antibiotic-resistant genes, such as *sul2*, *int1*, *tem*, and *gyrA*, suggests that molecular characterization is essential to better understand resistance mechanisms and to inform treatment

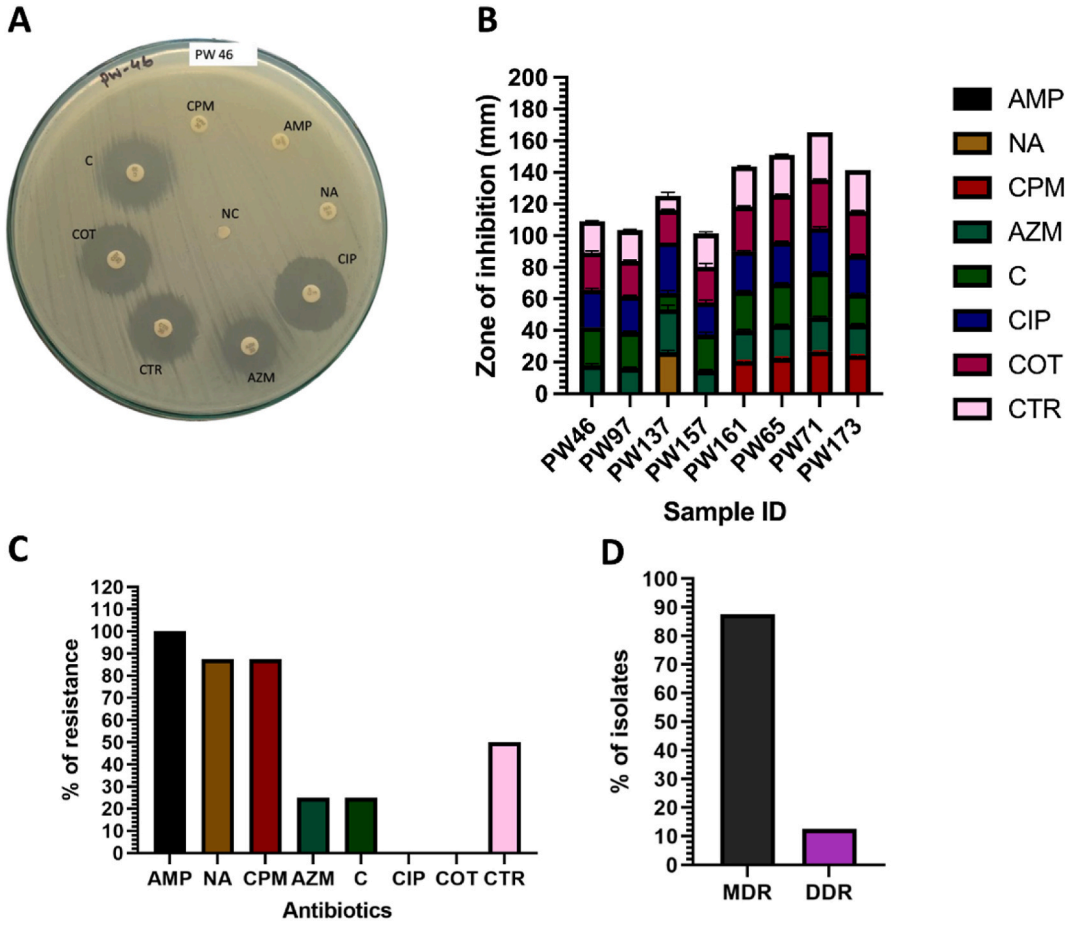

Fig. 4. Antimicrobial susceptibility test of identified *Salmonella* Typhi through disk diffusion method. A. Representative plate of antimicrobial susceptibility test Here; B. Zone of inhibition of the isolated *Salmonella* Typhi against selected antibiotics; C. *Salmonella* Typhi isolates show different percentage of resistance to different types of antibiotics; D. Prevalence rate of Multidrug-resistant (MDR) and Double Drug resistant *of Salmonella* Typhi. AMP: ampicillin, C: chloramphenicol, NA: nalidixic acid, COT: co-trimoxazole, CIP: ciprofloxacin, CTR: ceftriaxone, AZM: azithromycin, CPM: cefepime and NC: negative Control.

decisions. Improving water filtration, sanitation, and sewage management is crucial to prevent *Salmonella* Typhi contamination [37]. Strengthening regulations around antibiotic usage, improving public awareness to reduce misuse are crucial steps to curb the spread of *Salmonella* Typhi through contaminated water sources [38]. Furthermore, expanding vaccination coverage, developing probiotics targeting *Salmonella* Typhi and investing in research for novel treatment options [39] are crucial to mitigating the threat of MDR strains in the long term.

5. Conclusion

Our study successfully identified and confirmed *Salmonella* Typhi isolates using molecular techniques, providing valuable insights into the prevalence of MDR and DDR strains and the effectiveness of various antibiotics. By confirming the presence of *Salmonella* Typhi in drinking water and identifying key antibiotic-resistant genes, this research contributes significantly to understanding the epidemiology and antimicrobial resistance patterns of *Salmonella* Typhi. These findings highlight the urgent need for improved water quality and continuous molecular surveillance to track the spread of resistance. Future research should focus on gene transmission mechanisms through water sources, while public health policies should prioritize stricter antibiotic use regulations and encourage the development of alternative treatments, vaccination and probiotic strategies to better manage and control this infectious disease sources.

CRediT authorship contribution statement

Sohana Akter Mina: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pabitra Debnath:** Writing – review & editing, Writing

– original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A.K.M Zakir Hossain:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Md Zahid Hasan:** Writing – review & editing, Visualization, Investigation, Data curation. **A.M Masudul Azad Chowdhury:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Formal analysis, Conceptualization, Methodology, Data curation.

Limitation

The study's limitations include a relatively small sample size, which may not fully represent the diverse range of *Salmonella* Typhi strains in Chattogram. Additionally, we did not assess other potential pathogens or contaminants that could affect the water quality. The study's cross-sectional design provides a snapshot rather than longitudinal data, limiting our ability to track changes over time. Furthermore, socio-economic factors influencing water quality and resistance patterns were not considered, which could impact the generalizability of the findings.

Data availability

All data are included in this manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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