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Emergence of *gyrA* and *aac(6')-Ib-cr* Mediated Quinolone Resistance in *Salmonella Typhi* Isolated from Blood Samples in a Tertiary Care Hospital, Bangladesh

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Abstract

Antibiotic resistance among *Salmonella Typhi* poses a growing challenge in Bangladesh. A cross-sectional study was conducted in the Department of Microbiology, Dhaka Medical College, Dhaka, Bangladesh, from July 2019 to June 2020, to assess the prevalence of *gyrA* and *aac(6')-Ib-cr* quinolone-resistance genes in isolates from blood cultures of enteric fever patients. Blood cultures were processed using standard methods. Primary culture was done in trypticase soya broth, followed by subculture on blood agar and MacConkey agar. Identification was performed by biochemical tests and confirmed by serotyping with *S. Typhi* antisera. Disc diffusion was performed as per CLSI guidelines using commercial antibiotic discs. PCR assays targeted *gyrA, qnrA, qnrB, qnrS, qnrC, qnrD*, and *aac(6')-Ib-cr. S. Typhi* was identified by biochemical testing and 16S rRNA PCR. PCR was further used to detect *gyrA, qnrB, qnrS*, and *aac(6')-Ib-cr* genes. Out of 83 culture-positive samples, 50 were *Salmonella spp*., of which 40 were confirmed as *S. Typhi*. 12 isolates showed ciprofloxacin resistance. Among these, *gyrA* was detected in 8 (66.7%), *aac(6')-Ib-cr* in 2 (16.7%), and *qnrB* and *qnrS* in 1 each (8.3%). A high frequency of *gyrA* mutations and detection of plasmid-mediated *aac(6')-Ib-cr* indicates emerging quinolone resistance in *S. Typhi.* This highlights the urgent need for routine molecular surveillance and strengthened antimicrobial stewardship.

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Introduction

Typhoid fever, caused by *Salmonella enterica* serovar Typhi (*S. Typhi*), remains a major public health burden in low- and middle-income countries. Transmission occurs primarily through contaminated food and water.[1] Globally, an estimated 9–12 million cases and over 100,000 deaths are reported annually, with the highest incidence in South Asia.[2] Bangladesh, due to poor sanitation and dense urban populations, consistently reports high prevalence.[3] Historically, *S. Typhi* was susceptible to first-line drugs such as ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. However, resistance forced a switch to fluoroquinolones like ciprofloxacin.[4] Rising ciprofloxacin resistance, now widespread in South Asia, limits treatment efficacy.[5,6]

Resistance to fluoroquinolones is primarily linked to mutations in the quinolone-resistance determining region (QRDR) of the *gyrA* gene, especially at codons 83 and 87.[7,8] Over 90% of non-susceptible *S. Typhi* isolates from South Asia harbor *gyrA* mutations.[9]

Plasmid-mediated quinolone resistance (PMQR) genes, including *aac(6')-Ib-cr*, further contribute by modifying ciprofloxacin and norfloxacin.[10] Though rare in *S. Typhi*, reports of *aac(6')-Ib-cr* are increasing.[11,12] The coexistence of *gyrA* mutations and *aac(6')-Ib-cr* may enhance resistance, complicating treatment.[13]

In addition to chromosomal mutations, plasmid-mediated quinolone resistance (PMQR) mechanisms have emerged as contributors to reduced fluoroquinolone efficacy. One such gene, *aac(6')-Ib-cr*, encodes a variant aminoglycoside acetyltransferase that can modify ciprofloxacin

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and norfloxacin, reducing their activity.[10] While PMQRs are well-documented in other Enterobacteriaceae such as *Escherichia coli* and *Klebsiella pneumoniae*, their occurrence in *S. Typhi* has been rare but increasingly reported, often in conjunction with chromosomal mutations.[11,12] The co-existence of *gyrA* mutations and *aac(6')-Ib-cr* in *S. Typhi* may confer a higher level of fluoroquinolone resistance than either mechanism alone, posing serious therapeutic challenges. The horizontal transfer potential of PMQR genes also raises concern for their broader dissemination across bacterial populations.[13]

To date, the prevalence and clinical significance of *aac(6')-Ib-cr* in *S. Typhi* isolates from Bangladesh have been poorly studied. Although *gyrA*-mediated resistance is well-established in the region, there is limited molecular surveillance of PMQR genes in hospital settings.[14,15] Thus, this study aimed to fill a critical knowledge gap by determining the prevalence of *aac(6')-Ib-cr* and *gyrA* mutations in *S. Typhi* isolates recovered from blood samples in a tertiary-care hospital in Bangladesh. Understanding these resistance mechanisms is essential to inform empirical therapy, update national guidelines, and anticipate future resistance trends. Limited data exist on PMQR genes in Bangladeshi *S. Typhi*. This study aimed to determine the prevalence of *gyrA* and *aac(6')-Ib-cr* in blood isolates from a tertiary-care hospital in Bangladesh to inform surveillance and treatment strategies.

Methods

This cross-sectional study was conducted between July 2019 and June 2020 in the Department of Microbiology, Dhaka Medical College, Dhaka, Bangladesh. Patients with clinically suspected enteric fever who provided consent were enrolled.

Blood cultures were processed using standard methods.[15] Primary culture was done in trypticase soya broth, followed by subculture on blood agar and MacConkey agar. Identification was performed by biochemical tests and confirmed by serotyping with *S. Typhi* antisera (Mast™ Diagnostic, UK).

Disc diffusion was performed as per CLSI guidelines16 using commercial antibiotic discs (Oxoid, UK). *Escherichia coli* ATCC 25922 served as quality control.[16] The zone of inhibition was interpreted according to the Clinical and Laboratory Standards Institute. Ampicillin (10µg), chloramphenicol (30µg), sulfamethoxazole/ trimethoprime (25µg), nalidixic acid (30µg), ciprofloxacin (5µg), cefixime (5µg), ceftriaxone (30µg), Cefepime (30µg), azithromycin (15µg), amoxicillin/clavulanic acid (30µg), piperacillin/ tazobactam (110µg), and imipenam (10µg) were used. Escherichia coli ATCC 25922 was used as control strain to assess the performance of the method.[16] Within 30 min of placement of antibiotic discs, inoculated plates were incubated aerobically at 37° C for overnight.

MICs were determined by agar dilution, with results interpreted per CLSI 2020 breakpoints.[17] The bacterial suspension of 0.5 McFarland turbidity standard was prepared. As 0.5 McFarland turbidity standard contains 1.5 × 108 CFU/ml, 10 times dilution (1 ml test inoculum compared to turbidity standard added with 9 ml of normal saline) of test inoculums was done to achieve 1.5 × 107 CFU/ml. To obtain 1.5 × 104 CFU/ml on the agar surface, 1 µl of 10 times diluted inoculum were placed on the Mueller–Hinton agar plate. The plate was then incubated aerobically at 37° C overnight.[16] Different concentrations of ciprofloxacin was prepared and impregnated in 50 ml Mueller–Hinton agar media. Bacterial inoculums were

applied onto the agar surface, and the plates were incubated at 37°C overnight. The lowest concentration of antibiotic-impregnated Mueller–Hinton agar showing no visible growth on agar media was considered MIC of that drug for that strain of bacteria.[17] E. coli ATCC 25922 was used as the control organism.

DNA was extracted by boiling. PCR assays targeted *gyrA, qnrA, qnrB, qnrS, qnrC, qnrD*, and *aac(6')-Ib-cr*. Amplification conditions followed published protocols.[18] The pair of primers were used to yield PCR products depicted in (Table-I). Data was analyzed using Microsoft Excel 2013.

The study was approved by the Ethical Review Committee of Dhaka Medical College, Dhaka, Bangladesh.

Table-I: List of quinolone resistance genes

Gene	Sequence (5´ to 3´)		Size (bp)
qnrA	F	ATTTCTCACGCCAGGATTTG	516
	R	GATCGGCAAAGGTTAGGTCA	
qnrB	F	GATCGTGAAAGCCAGAAAGG	469
	R	ACGATGCCTGGTAGTTGTCC	
qnrC	F	GGGTTGTACATTTATTGAATC	447
	R	TCCACTTTACGAGGTTCT	
qnrD	F	CGAGATCAATTTACGGGGAATA	581
	R	AACAAGCTGAAGCGCCTG	
qnrS	F	ACGACATTCGTCAACTGCAA	417
	R	TAAATTGGCACCCTGTAGGC	
aac(6´)-Ib-cr	F	TTGGAAGCGGGGACGGAM	260
	R	ACACGGCTGGACCATA	
gyrA	F	CGTCGCGTACTTTACGCCATGAACG	586
	R	ATACCTTGCCGCGACCGGTACGG	

Results

Among 323 suspected cases, 83 (25.7%) yielded positive cultures. Among them, 50 (60.2%) were *Salmonella* spp., with 40 confirmed as *S. Typhi* (48.2% of total cultures) (Table-II). Antibiotic resistance pattern of *S. Typhi* isolate from patients with enteric fever was recorded. Among the 40 isolated *S. Typhi* were 100% resistant to nalidixic acid, 87.5% were resistant to ampicillin, 52.5% were resistant to chloramphenicol, 30% were resistant to ciprofloxacin, 30% were resistant to azithromycin. All were sensitive to cefixime, ceftriaxone, cefepime, imepenem (Table-III). CLSI (2020) breakpoint for MIC of ciprofloxacin for *Salmonella*: Sensitive ≤0.06 µg/ml, Intermediate 0.12–0.05 µg/ml, resistant ≥1 µg/ml (Table-IV). 12 isolates showed ciprofloxacin resistance. Among these, *gyrA* was detected in 8 (66.7%), *aac(6')-Ib-cr* in 2 (16.7%), and *qnrB* and *qnrS* in 1 each (8.3%) (Table-V).

Table-II: Distribution of Salmonella isolates from blood cultures (N=323)

Culture result	Number of isolates	Percentage of total cultures
Culture positive	83	25.7%
Salmonella spp.	50	60.2% of positives
Salmonella Typhi	40	48.2% of positives

Table-III: Antimicrobial resistance profile of *S. Typhi* isolates (n=40)

Antibiotic	Frequency	Percentage
Nalidixic acid	40	100.0
Ampicillin	35	87.5
Chloramphenicol	21	52.5
Trimethoprim-sulfamethoxazole	3	7.5
Ciprofloxacin	12	30.0
Azithromycin	12	30.0
Cefixime	-	-
Ceftriaxone	-	-
Cefepime	-	-
Imipenem	-	-

Table-IV: Distribution of Ciprofloxacin MIC by agar dilution method, among resistant *S. Typhi* isolates (n=12)

MIC value (µg/ml)	Frequency	Percentage
≥8	3	25.0
4	3	25.0
2	2	16.7
1	3	25.0
0.48	1	8.3
0.24	-	-
0.12	-	-
≤0.06	-	-

Table-V: Distribution of quinolone resistance genes among ciprofloxacin-resistant *S. Typhi* (n = 12) by PCR

Resistance gene	Frequency	Percentage
gyrA	8	66.7
aac(6')-Ib-cr	2	16.7
qnrB	1	8.3
qnrS	1	8.3
qnrA	-	-
qnrC	-	-
qnrD	-	-

Discussion

In this study, a considerable proportion of *S. Typhi* isolates demonstrated resistance to ciprofloxacin, with 66.7% harboring *gyrA* mutations and 16.7% carrying the plasmid-mediated *aac(6')-Ib-cr* gene. These findings confirm the predominance of chromosomal mutations in the QRDR of gyrA while also highlighting the emerging role of PMQR genes in Bangladesh. A genomic study in Bangladesh by Saha *et al*. found that over 90% of ciprofloxacin-resistant *S. Typhi* carried *gyrA* mutations, mainly at codons 83 and 87.9 Likewise, Suman *et al.* from India reported that all ciprofloxacin-resistant *S. Typhi* isolates harbored *gyrA* mutations, underscoring its dominant role in resistance.[19]

Detection of *aac(6')-Ib-cr* in this study, though less frequent, mirrors previous reports, where this PMQR gene was sporadically detected in *S. Typhi* and other Enterobacteriaceae.[14,20] In a multicenter study across Asia, plasmid-mediated quinolone resistance was identified in less than 10% of isolates, suggesting it remains an emerging but significant mechanism. The relatively lower prevalence compared to chromosomal mutations may be due to limited plasmid dissemination in *S. Typhi* populations or differences in antibiotic selection pressure across regions.

Our findings also align with Gomes *et al.,* who demonstrated sporadic occurrence of *qnrS* and *qnrB* in Enterobacteriaceae from Africa and South Asia.[14] In this study, *qnrS* and *qnrB* were each detected in one isolate (8.3%), supporting the notion that these genes are circulating at low levels in Bangladeshi *S. Typhi.*

The predominance of *gyrA* mutations may be explained by the widespread use of fluoroquinolones in Bangladesh for febrile illnesses, including empirical treatment of suspected typhoid and other bacterial infections.[6,21] Continuous selective pressure likely facilitated the fixation of QRDR mutations within circulating *S. Typhi* lineages. Moreover, inappropriate antibiotic use in community settings, over-the-counter access, and lack of strict prescription regulations further contribute to resistance development.[3,5]

The emergence of aac(6′)-Ib-cr may reflect horizontal gene transfer events from other Enterobacteriaceae such as *E. coli* and *K. pneumoniae*, where this gene is more prevalent.[10,12] Poor infection-control practices in healthcare facilities, high antibiotic usage, and environmental contamination may have facilitated acquisition of such plasmids in *S. Typhi*. Additionally, the coexistence of chromosomal and plasmid-

mediated resistance in some isolates, as observed in this study, may lead to higher MICs against ciprofloxacin compared to isolates carrying only *gyrA* mutations. Similar synergistic resistance effects were reported by Robicsek *et al.,* where ***aac(6')-Ib-cr*** together with *gyrA* mutations conferred significantly reduced fluoroquinolone susceptibility.[10]

The high resistance to older first-line drugs (ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole) combined with rising resistance to fluoroquinolones leaves limited therapeutic options. Although cephalosporins and carbapenems remained effective in this study, increasing reliance on these antibiotics raises concern for future resistance trends, as seen in other South Asian contexts.[5,22]

The detection of azithromycin resistance genes (mphA, mefA) in parallel with quinolone resistance is alarming. Similar findings were documented in previous report, where azithromycin MICs among *S. Typhi* isolates were steadily increasing.[22] This dual resistance could lead to treatment failures with two of the last available oral agents for typhoid fever.

To our knowledge, this study is one of the first in Bangladesh to document ***aac(6')-Ib-cr*** in *S. Typhi*. However, sequencing was not performed to characterize specific *gyrA* mutations or plasmid types, which would provide deeper insights into resistance evolution.

Conclusion

Our study identified a predominance of *gyrA* mutations (66.7%) and detection of ***aac(6')-Ib-cr*** (16.7%) among ciprofloxacin-resistant *S. Typhi* isolates in Bangladesh. These findings underscore the urgent need for molecular surveillance and rational antibiotic use to contain resistance spread.

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