

**Original Article****Diagnosis of Enteric Fever Cases: Current Challenges of Azithromycin Resistant *R717Q* Gene in Rajshahi, Bangladesh**Jarin Sazzad,¹ Md. Shah Alam,² Haimanti Shukla Das,³ Nahreen Rahman,⁴ Quazi Tamanna Haque,⁵ Kazi Mohaimenur Rahman⁶**Abstract**

Salmonella enterica serovars *typhi* is a contributing typhoid agent, leading to untreatable infections based upon the rising prevalence of antimicrobial resistance in the said agent. Consequently, the main concern of the health service providers to treat expansively drug-resistant (XDR) enteric fever has been dependent on the only remaining oral drug, Azithromycin, for creating emerging resistance against it. Therefore, a cross-sectional descriptive study was conducted in the Microbiology Department of Rajshahi Medical College, Rajshahi, to identify *Salmonella* species by Fastidious Antibiotic Neutralization (FAN) method in suspected enteric fever cases along with its antimicrobial susceptibility pattern and molecular detection of Azithromycin resistant *R717Q* gene. Samples were collected purposively. Regarding the population's age distribution, out of 127 specimens, the predominant 37(29.1%) were found within the age group of 35-44 years. Automated blood culture was found positive in 64(50.39%) population; out of them, *Salmonella spp* were 38(59.37%) of the population. Among them 29(76.31%) have travel history, took street food 25(65.7%), used tap water for drinking 21(55.2%) and had kancha latrine 24(63.1%). It was found that 18.43% were sensitive and 81.57% resistant to Azithromycin. Azithromycin-resistant *Salmonella spp.* were detected in 31(81.57%), and among Azithromycin resistant *R717Q* gene was identified in 9(29.03%). The azithromycin-resistant *R717Q* gene was identified by conventional PCR. Because of its oral course of administration, Azithromycin is considered the widely used drug in Bangladesh, which is used once on a dose basis a day. In order to contribute to the development of this drug resistance, there could be many reasons, and among them there are the availability over the counter and the imperfectly done treatment. Thus, this study would be helpful to formulate a regional hospital empirical therapy by antimicrobial sensitivity and resistance pattern of the gene for Azithromycin resistance in Rajshahi Medical College Hospital.

Keywords: Enteric fever, Antimicrobial susceptibility pattern, Azithromycin, *R717Q* gene.

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Introduction

Among the common bacterial diseases in Asia, Africa, and South America, one of the most common is enteric fever, which refers to typhoid and paratyphoid fever. It has also been

accompanied by inadequate proper sanitation and the unavailability of a safe drinking water supply.¹ Considering the death result, globally over 26 million cases of typhoid fever and 5 million cases of paratyphoid infection have been observed, respectively, causing almost 215,000 deaths

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yearly.² If we look over the South Asian subcontinent, we can find that the stretched healthcare scenario has already been aggravated just because of enteric fever being turned into a huge disease draining with a rate of 976 incidences in every 100,000 people per year.³ 394.2 episodes per 100,000 people per year have been observed as the incidence of enteric fever in South Asia.

In contrast, in Bangladesh, during 2003-2004, typhoid fever was reported to be 200 episodes per 100,000 people every year.⁴ The gold standard for diagnosis by blood culture in the separation of the causative bacteria in enteric fever patients remains positive in the first few days of sample collection. 80% can be the limit to reach the sensitivity of automated blood culture.⁵ We may consider the automated blood system Bact/Alert 3D with the use of Fastidious Antibiotic Neutralization (FAN) as an ideal for the isolation of *Salmonella* since the use of antimicrobials is high in the endemic area. The mortality rate can be reduced apparently from supportive care through effective antimicrobial therapy. This therapy can also effectively reduce the illness's complications and squatness. A suitable oral alternative for managing enteric fever is Azithromycin, the best use in recent days.⁶ Azithromycin attains rapid defervescence along with the reduction of relapse rates and eradication of fecal carriage through 72 hours of long elimination and by the feature of its high intracellular concentration.⁷ Due to the spread of multidrug-resistant (MDR) strains of *Salmonella enteric*, the use of many areas of the drugs has been revoked. In the intervention, the resistance to the first-line antimicrobials Ampicillin, Cotrimoxazole and Chloramphenicol are the multidrug-resistant strains of *Salmonella species* are on the intensification worldwide⁸. Besides, from many parts of the world, third-generation Cephalosporins, Fluoroquinolones, Chloramphenicol, Ampicillin and Trimethoprim-Sulfamethoxazole are also being reported.⁹ The spread of the strains with *R717* mutations may

cause the increased use of Azithromycin, and following that, XDR strains are acquired. At the position, the conserved amino acid arginine (R) 717 has been changed to a glutamine (Q) due to the change of mutation. The shifting of enteric fever treatment from outpatient departments, the place where the patients get treated for oral Azithromycin can be done by an Azithromycin-resistant XDR strain. The Bangladeshi adds urgency need to distinguish the local antimicrobial sensitivity and the pattern of resistance caused for the increase of antimicrobial resistance in *Salmonella species*.

Materials and Methods

This was a cross-sectional descriptive study carried out in the department of Microbiology, Rajshahi Medical College, Rajshahi. The study period was from January 2021 to December 2021. Clinically suspected enteric fever cases from inpatients and outpatients Medicine department in Rajshahi Medical College Hospital, Rajshahi, during the study period were considered the study population. A purposive sampling technique was used, and a total of 127 patients were included in this study. Clinically suspected enteric fever patients diagnosed by a physician with a temperature of $>101.8^{\circ}\text{F}$, a step ladder in pattern for ≥ 3 days up to 7 days, was considered. Others symptoms: Headache, myalgia, lethargy, constipation, psychosis, etc. Adult patients (≥ 18 years) of both sexes were included. Patients with apparent symptoms of other infections and unwilling to participate were excluded. Data were collected from the clinically suspected enteric fever patients admitted to Rajshahi Medical College Hospital and seeking attention in inpatient and outpatient departments. Prior to the commencement of the study, the study protocol was approved by the Institutional Review Board and concerned authority, Rajshahi Medical College Hospital.

Results

Regarding age distribution of the population, out of 127 specimens, the predominant 37 (29.1%) were found within the age group of 35-44 years, 33 (26.0%), 32 (25.2%), 25 (19.7%) were within the age group of more than 45 years, 25-34 years and less than 25 years respectively. Automated blood culture was found positive in 64(50.39%) population; out of them, *Salmonella spp* were 38(59.37%) of the population. Regarding food habits, it was observed that among the culture-positive population, 25 (65.7%) of the population took street food, 11 (28.9%) took homemade food and 2 (5.3%) took both types of food. Furthermore, among the culture-positive population, 21 (55.2%) used tap water, 10 (26.4%) used mixed water, and 7 (18.4%) used tube well water. Regarding latrine type, it was found that 24 (63.1%) used kancha latrine, 13 (34.2%) used sanitary latrine, and 1 (2.7%) used open space (Table 1, 2).

Table 1: Distribution of the study population in different age groups (N=127)

The age group of the respondents in years	Respondents	
	No.	%
<25 Years	25	19.7
25-34 Years	32	25.2
35-44 Years	37	29.1
45+ Years	33	26.0
Total	127	100.0

Table 2: Frequency of the culture-positive population based on food habits, source of drinking water, and type of latrine (N=38)

Food habit	Culture-positive respondents (N=38)	
	No.	%
Homemade food	11	28.9
Street food	25	65.7
Both	2	5.3
Source of drinking water		
Tube well water	7	18.4
Tap water	21	55.2
Mixed water	10	26.4
Latrine type		
Sanitary latrine	13	34.2
Kancha	24	63.1
Open space	1	2.7

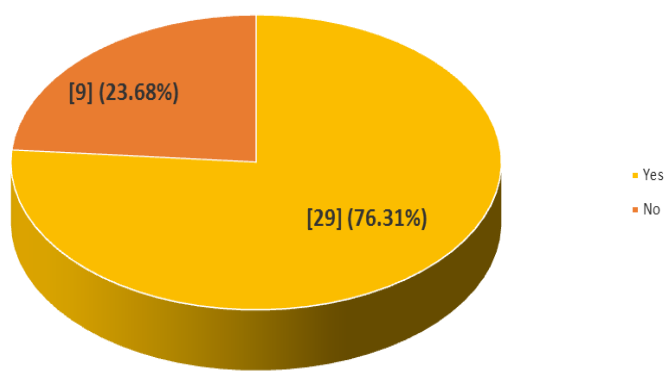
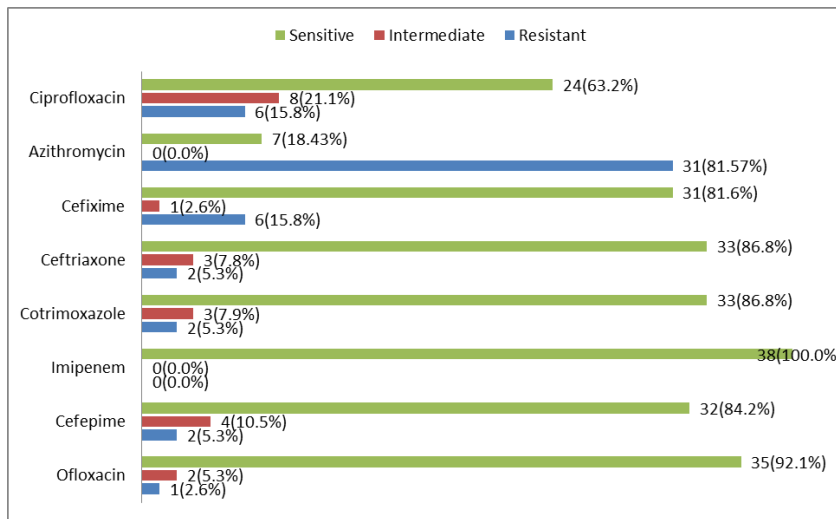


Figure 1: Distribution of the culture-positive population based on travel history (N=38).

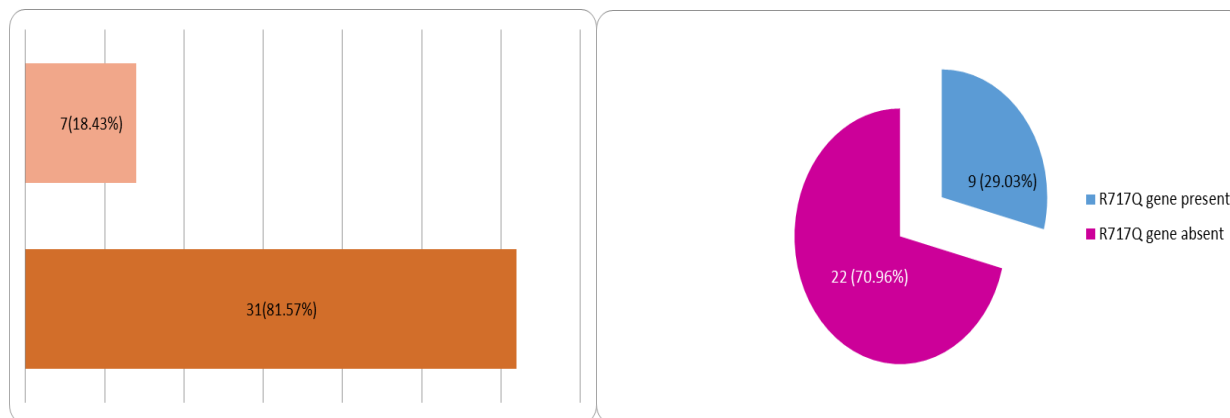
Among the culture-positive population, the majority, 29 (76.31%) of the population had a traveling history.

Figure 2: Antimicrobial susceptibility pattern of *Salmonella* spp (N=38).



It was found that the highest 100.0% sensitive on Imipenem, followed by 92.10%, 86.8%, and 86.8% sensitive Ofloxacin, Cefepime, and Ceftriaxone respectively, and the lowest, 81.57% resistant on Azithromycin.

Figure 3,4: Distribution of the population of Azithromycin-resistant *Salmonella spp* (N=38).



Azithromycin-resistant *Salmonella spp.* were detected in 31 (81.57%), and susceptible *Salmonella spp.* were 7 (18.43%). Among 31 Azithromycin-resistant *Salmonella spp.* 9 (29.03%) Azithromycin resistant *R717Q* gene was identified by PCR.

Discussion

This study was designed to identify *Salmonella* species by the Fastidious Antibiotic Neutralization (FAN) method in suspected enteric fever cases along with its antimicrobial susceptibility pattern and molecular detection of Azithromycin resistant *R717Q* gene in Rajshahi Medical College Hospital. Out of 127 population maximum of 37 (29.1%) were found within the age group of 35-44 years. A study was conducted in Bangladesh, India, and Nepal respectively by Saleh *et al.*, 2008¹⁰ Gautam *et al.*, 2002¹¹ and Maharjan *et al.*, 2008¹² which are similar to this study and respectively found 28.1%, 31.8%, and 28.9%. In the present study, among the culture-positive population, the majority, 29 (76.31%) of the population, had a travel history (Figure I). Because of being traveling in different places by the businessmen, it is among the most. Buckle *et al.*, 2012 in the USA also found a similarity with the study.¹³ Among the culture-positive population, 25 (65.7%) of them took street food. Sharma *et al.*, 2014 in India¹⁴ found that the street food consumption rate was 59.7%, a similarly done study. Among the culture-positive population, 21 (55.2%) used tap water, 10 (26.4%) used mixed water, and 7 (18.4%) used tube well water (Table VI). 51.28%, 49.8%, 43.1%, and 50.9%, respectively, were the findings similar to Bhunia *et*

al., 2019 in India;¹⁵ Nyamusore *et al.*, 2018 in Rwanda;¹⁶ Muti *et al.*, 2014 in Zimbabwe;¹⁷ Srikantiah *et al.*, 2007 in Uzbekistan¹⁸. Regarding the use of latrines among the culture-positive population, it was revealed that 24 (63.1%) used kancha latrines, and 1 (2.7%) used open space. The report of WHO way back in 2008 for Pakistan,¹⁹ was also similar to 58.9% whereas it was 69.2% in India by Patil *et al.*, 2014;²⁰ in East Asia, 55.1% by Clasen *et al.*, 2014;²¹ in Nepal, 60.2% by Karkey *et al.*, 2013.²² In this study *Salmonella spp* showed 92.1%, 10.5% and 63.2% sensitivity to Ofloxacin, Azithromycin, and Ciprofloxacin respectively. In accordance with the present study, Rahat *et al.*, 2018, in Bangladesh²³ observed 87.27%, 18.18%, and 62.72% sensitivity to Ofloxacin, Azithromycin, and Ciprofloxacin, respectively. In this study, *Salmonella spp.* showed 84.2%, 86.8%, 81.6%, and 86.8% sensitivity to Cefepime, Ceftriaxone, Cefixime, and Cotrimoxazole, respectively. In contrast to this study, Hasan *et al.*, 2011²⁴ and Jha *et al.*, 2019,²⁵ showed a higher sensitivity rate of Cefepime, Ceftriaxone, Cefixime, and Cotrimoxazole (79.5%, 85.9%, 78.2%, and 80.9%) in Bangladesh and (75.8%, 81.6%, 75.4%, and 82.5%) India respectively. These indicate that the sensitive pattern of *Salmonella spp* varied with time and geographical locations. Azithromycin-resistant *Salmonella spp.* was detected at 81.57% in the

present study. 70.8% was found in another study conducted in Cambodia by Vlieghe *et al.*, 2012,²⁶. In Bangladesh, Azithromycin resistance was reported by about 95% by Rahman in 2015 and Ahsan & Rahman in 2019,^{27,28} which is similar to the present study. In Figure III, Azithromycin resistant *R717Q* gene was identified in 29.03% of cases. The rate was 20.0% found in the study in Singapur by Octavia *et al.*, 2021²⁹. Finally, the present study describes the sensitivity and resistance pattern of Azithromycin among the *Salmonella spp* isolates in the Rajshahi region due to local indiscriminate use of Azithromycin and others cephalosporins. However, Ofloxacin, Cotrimoxazole, Cefepime, and Ciprofloxacin can be used because of their less utilization. Moreover, Imipenem could be used as a reserved drug for complicated enteric fever cases.

Conclusion

Amongst typhoidal *Salmonella*, the rise of antimicrobial resistance to Azithromycin is gradual in Bangladesh and considered the last presented oral antibiotic for the treatment of enteric fever. Strains with *R717Q* mutations may be increased with the increased use of Azithromycin. As a consequence, in Rajshahi Medical College Hospital, Rajshahi, the suitable use and alternation of antibiotics, in addition to the continuous monitoring of antibiotic susceptibility profiles help to spread of Azithromycin resistant *R717Q* gene of *Salmonella Spp.* and to control the emergence.

Conflict of interest: None declared

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