

Minimal Inhibitory Concentrations Of Azithromycin In *Typhoidal Salmonella* Isolated In Tertiary Care Setting

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ABSTRACT

Objective: To determine the variations in minimal inhibitory concentrations (MIC) of Azithromycin amongst *Salmonella typhi* isolates.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Microbiology, Combined Military Hospital, Lahore Pakistan, from Jan to Dec 2020.

Methodology: Three hundred and eighty-four samples yielding the growth of *Salmonella typhi* were processed. Antibiotic susceptibility testing was done using the Kirby-Bauer Disk Diffusion technique, and the MIC of Azithromycin was determined using the E-strip method. Clinical and Laboratory Standards Institute (CLSI) recommended that MIC breakpoints be used for susceptibility testing of Azithromycin.

Results: Of 384 tested isolates, 103(26.8%) were multidrug-resistant (MDR). Resistance to Ciprofloxacin was as high as 367(95.6%) isolates, whereas extensively drug-resistant (XDR) isolates were calculated to be 204(53.1%). No resistance against Azithromycin was observed. All the isolates were in the susceptible MIC range of 0.5 and 8 µg/ml. The lowest MIC observed was 0.5 µg/ml by 12(5%) of the isolates. The highest MIC value of 8 µg/ml was observed in 6(1.5%) isolates, all of which were XDR. 231(60.2%) isolates had one µg/ml MIC, followed by MIC 4 µg/ml of 72(18.8%) isolates. The mean MIC value of all the isolates was calculated to be 1.82±1.3µg/ml.

Conclusion: The rise in XDR *Salmonella typhi* could lead to Azithromycin resistance. Antimicrobial stewardship is of prime importance, and Azithromycin MICs should be reported to keep the trends in check.

Keywords: Azithromycin, Minimum inhibitory concentration, *Salmonella typhi*.

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INTRODUCTION

Typhoid fever is a systemic infection caused by the gram-negative bacterium *Salmonella enterica*, which includes *Salmonella enterica* subsp., *Enterica* serovars typhi (*S.typhi*) and Paratyphi (*S.Paratyphi*) A, B, and C. Transmission mostly occurs through the fecal-oral route with the consumption of contaminated food and water.¹ Enteric fever remains a leading global cause of morbidity and mortality, particularly in low- and middle-income countries, including Pakistan. The incidence of enteric fever in Southeast Asia is 110 cases/per 100000 population.²

The traditional first-line drugs for treating typhoid fever are ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol. Multidrug-resistant *S.typhi*, resistant to these three drugs, emerged in the 1970s and 1980s, making them ineffective for treatment.³ This led to the usage of

Fluoroquinolones (Ciprofloxacin), but decades later, resistance emerged in this group as well. Fluoroquinolone resistance shifted the treatment options to third-generation Cephalosporins such as Cefixime, Ceftriaxone, and Macrolides, including Azithromycin.⁴ The gradual rise in the Minimum Inhibitory Concentration (MIC) of Ceftriaxone against typhi led to the major catastrophic evolution of extensively drug-resistant (XDR) *Salmonella*, which was resistant to all the first-line drugs, quinolones as well as Cephalosporins. The largest ceftriaxone-resistant *Salmonella* outbreak was reported in Hyderabad, Pakistan, in 2019.^{4,5}

Azithromycin is now commonly used to treat enteric fever as an alternative to Cephalosporins because of its cost-effectiveness, high intracellular concentration, comparatively faster fever clearance time, and good clinical response.^{6,7} Resistance to Azithromycin is infrequent but has been reported from different parts of the world, including India, Nepal, and Bangladesh.^{6,8}

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This study was conducted to observe the values of Azithromycin MICs among different isolates of *S. typhi*. Rising concerns about Azithromycin resistance due to the drug's over-the-counter availability and irrational use during the COVID-19 pandemic highlight the importance of this study. Knowledge of MIC allows us to modify doses to achieve clinical success in certain cases. Moreover, continuous surveillance is necessary to monitor the changing antimicrobial susceptibility trends.

METHODOLOGY

The cross-sectional study was conducted at the Department of Microbiology, Combined Military Hospital, Lahore Pakistan, from January to December 2020 after approval from the Ethical Committee (ERC no 258/2020). The samples were collected using a non-probability convenience sampling technique.

Inclusion Criteria: Blood culture specimens from patients of either gender and all age groups received from the Inpatient and Outpatient Departments of the hospital were included.

Exclusion Criteria: Duplicated samples from the same patient were excluded from the study.

The data was collected daily and analyzed. All the samples received between January and December 2020 (n=384) with the growth of *Salmonella typhi* were processed.

The blood culture specimens were processed using the BACT/ALERT 3D system (BioMérieux). Positive blood cultures showing gram-negative rods were subcultured on Blood agar and MacConkey agar (Oxoid). The isolates were identified using standard microbiological techniques based on colony morphology and biochemical tests using API 20E (BioMérieux).^{7,8} Serotyping was done using group and type-specific antisera (Bio-Rad). The in-vitro susceptibility of the test isolates was determined using the Kirby-Bauer Disk Diffusion method and E strips (BioMérieux) for Azithromycin (AZM). The Muller-Hinton agar plates were incubated aerobically at 35±2 C for 18-24 hours in ambient air. MIC was recorded as the lowest concentration at which there was no visible growth of the isolate. The CLSI recommended MIC breakpoints for AZM (susceptible ≤ 16µg/mL and resistant ≥32 µg/mL) were used for result analysis.^{8,9}

According to standardized definitions, Multi-drug-resistant (MDR) *Salmonella* is a strain resistant to first-line recommended drugs, i.e., Ampicillin, Trimethoprim-Sulfamethoxazole, and Chlorampheni-

col. However, the XDR strain is the one that is resistant to all the first-line drugs as well as Fluoroquinolones and third-generation Cephalosporins.

The statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 28.0. Qualitative variables were summarized as Mean ±SD, while qualitative variables were summarized as frequency and percentages.

RESULTS

Out of the 384 samples processed, 253(65.9%) were males, and 131(34.1%) were females. The maximum number of patients were from the age group 19-59, 179(46.6%). Fifty-two (13.5%) patients were treated outdoors; however, a larger bulk of 332(86.5%) patients were hospitalized, and 27(7%) of the hospitalized patients were admitted to critical care due to complications.

Table-I shows the antimicrobial susceptibility pattern of *Salmonella* isolates. Of 384 tested isolates, MDR isolates were calculated to be 103(26.8%), and resistance to Ciprofloxacin was as high as 367(95.6%) isolates, whereas XDR isolates were calculated to be 204(53.1%). No resistance against Azithromycin was observed. Frequency of MDR and XDR *Salmonella* isolates received in a tertiary care setup is shown in Figure-1.

Table-I: Antimicrobial Susceptibility Pattern of Salmonella Typhi (n=384)

Antimicrobials	Sensitive n(%)	Resistant n(%)
Ampicillin	61 (15.9)	323 (84.1)
Trimethoprim-sulfamethoxazole	57 (14.8)	327 (85.2)
Chloramphenicol	62 (16.1)	322 (83.9)
Ciprofloxacin	17 (4.4)	367 (95.6)
Ceftriaxone	180 (46.9)	204 (53.1)
Azithromycin	384 (100)	-

The distribution of MIC trend against Azithromycin is shown in Figure-2. All the isolates were in the susceptible MIC range of 0.5 and 8 µg/ml. No discordance with disc diffusion was observed for any of these isolates. Although all the isolates were susceptible, higher MICs of 4 µg/ml and eight µg/ml were found in XDR isolates. A large number, i.e., 231(60.2%) isolates, had one µg/ml MIC, followed by MIC 4 µg/ml of 72(18.8%) isolates. The mean MIC value of all the isolates was calculated to be 1.82±1.3µg/ml (Table-II).

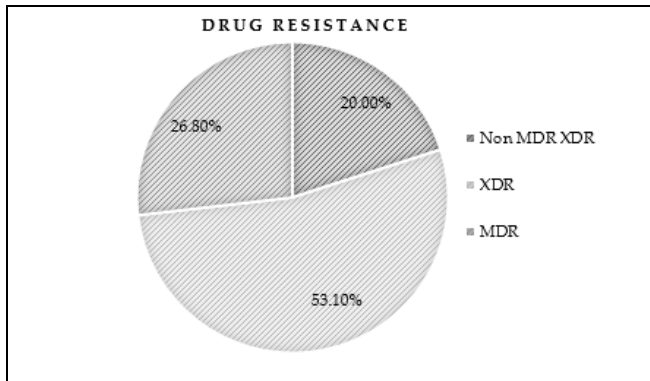


Figure-1: Frequency of Multidrug-Resistant (MDR) and Extensively Drug-Resistant (XDR) *Salmonella* Isolates received in a Tertiary Care Setup (n=384)

Table-II: Minimal Inhibitory Concentrations (MICs) Trend of Azithromycin in Relation to Resistance Pattern (n=384)

Azithromycin Minimal Inhibitory Concentrations (µg/ml)	Resistance		
	Non-MDR XDR n(% of total)	Multidrug-Resistant (MDR) n(% of total)	Extensively Drug-Resistant (XDR) n(% of total)
0.5	2(0.5)	8(2.1)	2(0.5)
1	63(16.4)	79(20.6)	89(23.2)
2	11(2.9)	15(3.9)	37(9.6)
4	1(0.3)	1(0.3)	70(18.2)
8	0(0)	0(0)	6(1.6)
Total n(%)	77(20)	103(26.8)	204(53.1)

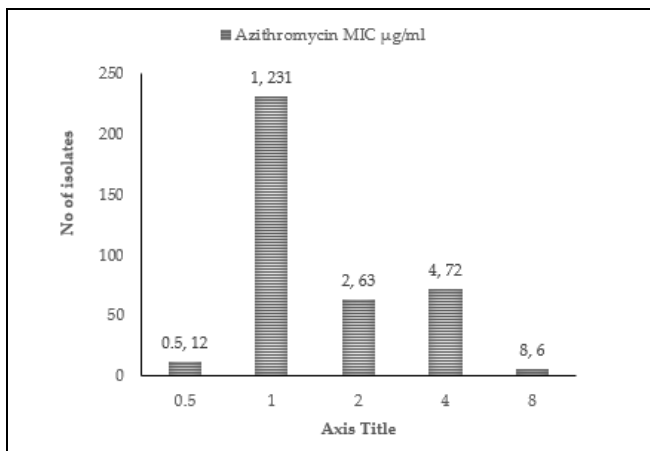


Figure-2: Distribution of Minimal Inhibitory Concentrations (MICs) Trend of Azithromycin Amongst *Salmonella* Isolates of a Tertiary Care Setup (n=384)

DISCUSSION

Enteric fever is a notifiable disease in Pakistan, and the rising trend of XDR *S.typhi* has highlighted the importance of Azithromycin as the only oral option.

According to our study, enteric fever infection was found to be two times more common in males than females. In contrast, the percentage of females was slightly higher than that of males (52% and 48%, respectively) in another study conducted in Sukkur, Pakistan.⁹ However, there seems to be no specific gender association with acquiring the infection.

The larger bulk of patients were adults (46.6%), and this was followed by children (44.5%). The percentage of infants and elderly patients was very low (1.8% and 1.6% respectively). The higher rate of infections in children was in line with multiple previous studies where infections with resistant bugs were associated with children due to poor immune responses.^{10,11} In our study, the ratio between un-hospitalized and hospitalized patients was 1:6. This difference was comparatively less noticeable in another study conducted in Hyderabad, Pakistan, where the percentage of hospitalized patients was 60%, and that of un-hospitalized was 40%.⁴ Although no death was reported among our admitted cases (27), 7% of the patients demanded critical care.

Antimicrobial resistance is an ongoing threat in the treatment of enteric fever. The emergence of resistance to first-line antimicrobials MDR and XDR *Salmonella typhi* isolates has left Azithromycin as the only oral treatment, and we may even run out of this option as well if appropriate actions are not taken in time.¹² More than half of our tested isolates, i.e., 204(53.1%), were XDR. This percentage was near another study by Hussain *et al.* where XDR isolates were calculated to be 48%.¹³

Our study showed 100% susceptibility to Azithromycin, consistent with the findings of another study conducted in Islamabad, Pakistan.¹⁴ Resistance to Azithromycin has been reported in very few studies in Pakistan. One isolate with MICs of 64 µg/ml was reported in a study from Lahore.¹⁵ Another one with MIC 96 µg/ml was reported from a study in Karachi.¹⁶ The highest MIC recorded in our isolates was eight µg/ml, and all 6 of these isolates were XDR. A study by Klemm *et al.* from Hyderabad included over 80 XDR *S. typhi* isolates, and only 1 of these isolates had MIC 8 µg/ml.¹⁷ The larger chunk of isolates, i.e., 231(60%), had MIC 1 µg/ml. The lowest MIC recorded in our study was 0.5 µg/ml, which was contributed by a small percentage of 3%(12) isolates. In contrast, a study conducted in Nepal by Khanal *et al.* included 74 isolates, amongst which 50%(37) had an MIC value of 0.125 µg/ml and 23%(17) had an MIC of 1 µg/ml.¹⁸ In

addition, the mean MIC value of our study was 2.5 µg/ml, which was much higher than this study, which had a mean value of 0.4 µg/ml.

Although we did not encounter any isolate resistant to Azithromycin during the one-year period of this study, the XDR isolates showing higher MICs and the increase in mean MIC value in comparison to older studies mentioned above are alarming and of major significance.

CONCLUSION

Reports of increasing Azithromycin resistance in *Salmonella typhi* worldwide are alarming for endemic countries like ours. Antimicrobial stewardship is of prime importance to prevent this only oral treatment option from developing resistance. Strict antimicrobial susceptibility programs need to be conducted, and MICs of Azithromycin should be monitored to keep the trends in check.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

AT & IAM: Data acquisition, critical review, approval of the final version to be published.

QF & AB: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

SH & CH: Conception, data analysis, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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