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ORIGINAL ARTICLE

Distribution of MDR and XDR typhoidal *Salmonella* and the association of neutrophil-to-lymphocyte ratio in enteric fever

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ABSTRACT

Background: The emergence of extensively drug-resistant *Salmonella* Typhi poses a global threat. The Neutrophil-to-Lymphocyte Ratio (NLR), an inflammatory marker, has shown prognostic value in various infections but its role in typhoidal *Salmonellae* is not well established. The objective of the study is to determine the antimicrobial resistance in typhoidal *Salmonellae* and to evaluate the role of NLR as a clinical biomarker in enteric fever

Material and Methods: Blood cultures from suspected patients of enteric fever were processed from August 2024 to August 2025. Antimicrobial susceptibility was performed by disc diffusion method. NLR was calculated from a complete blood count. ANOVA and Chi-square tests, were used to compare mean NLR across strain types

Results: A total of 450 samples were studied. Among which Typhoidal *Salmonellae* were isolated from 24.4% of samples. *S.* Typhi was the predominant serovar (86.3%). Among 90% of resistant isolates, 64.5% were XDR *S.* Typhi. The overall mean NLR was 2.04 ± 1.14. Statistical analysis revealed no significant difference in mean NLR values (p=0.559) or the distribution of High/Low NLR categories (p=0.87) across the different strain types (MDR, XDR, sensitive).

Conclusion: This study highlights the concerning distribution of drug-resistant *Salmonella* Typhi. Although the neutrophil-to-lymphocyte ratio does not correlate directly with antimicrobial resistance patterns, it may serves as a strong, readily available prognostic indicator of disease severity. We recommend the routine calculation of NLR to facilitate early identification and timely clinical management of the patient

Keywords: Antimicrobial resistance, Extensively drug-resistant, Multidrug resistance, *Salmonella* typhi, Neutrophil-to-lymphocyte ratio, Typhoid fever

BACKGROUND

Enteric fever, a life-threatening systemic infection caused by *Salmonella enterica* serovars Typhi and Paratyphi, remains a significant public health burden in many developing countries. It accounts for approximately 9 million cases and 110,000 deaths annually worldwide.[1,2] Typhoid fever is endemic in several parts of the world, including Southeast Asia and South Asia.[3] The estimated incidence rate of typhoid fever is the highest in Pakistan compared to other countries in South Asia, with a rate of 493.5 cases per 100,000 persons per year.[4]

The disease is predominantly transmitted through the fecal-oral route, facilitated by contaminated food and water.[3] Typhoid fever is characterized by high grade fever, abdominal discomfort and either diarrhea or constipation and rarely rash on upper chest and abdomen.[5] The management of enteric fever has been challenged by the emergence and global spread of antimicrobial resistance. The evolution of resistant strains has evolved from chloramphenicol-resistant isolates in the 1970s, to Multidrug-Resistant (MDR) strains (resistant to ampicillin, chloramphenicol, and co-trimoxazole) in the 1980s-90s. Recently outbreaks of Extensively Drug-Resistant (XDR) strains XDR in South Asia, rendered most first- and second-line therapeutic options ineffective.[6] This escalating resistance crisis underscores the critical need for both novel therapeutic strategies and reliable biomarkers to guide early clinical decision-making.

In this context, there is growing interest in identifying simple, cost-effective, and readily available prognostic indicators. The Neutrophil-to-Lymphocyte Ratio (NLR) has emerged as a potential biomarker of systemic inflammation and stress in various infectious and non-infectious diseases.[7] A elevated NLR has been linked to worse outcomes in sepsis, bacteremia, and

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other acute inflammatory states, reflecting a dysregulated immune response.[8] However, its utility specifically in enteric fever, particularly in distinguishing between infections caused by different drug-resistant strains remains inadequately explored.

Therefore, this study was conducted to investigate the current landscape of antimicrobial resistance in typhoidal *Salmonella* and to evaluate the role of NLR as a clinical biomarker. The primary objectives were to determine the prevalence and serovar distribution of MDR and XDR *Salmonella* strains in study population and to study whether NLR values differ significantly across various drug-resistant profiles and can serve as a predictor of clinical disease severity. The findings from this study aim to provide valuable insights for improving diagnosis, patient risk stratification, and management protocols in an era of increasing antibiotic resistance.

MATERIAL AND METHODS

This cross-sectional study was conducted at united medical and dental college during the study period from August 2024-August 2025. The study was approved by the Institutional Ethical Review Board UMDC/Ethics/2024/30/6/292. The sample size was calculated using the WHO sample size calculator, based on an expected population proportion of *Salmonella* Typhi culture positivity of 25.7% reported in a previous study with a 95% confidence interval and a 5% margin of error. The final sample size was increased to 450 to account for a 20% compliance error.[9]

Blood samples were collected from children aged 0-15 years suffering from fever, abdominal pain discomfort including diarrhea or constipation after taking consent from patient`s parents or guardian. Those who were already taking antibiotics and suffering with any viral and other chronic illness were excluded from the study. Blood samples were collected aseptically and inoculated into blood culture bottles. Positive samples were sub-cultured on MacConkey agar and Blood agar plates and incubated aerobically at 37°C for 18-24 hours. Suspected colonies were further confirmed by using API20E66 and serotyped using commercially available O and H antisera.[10] *Salmonella* poly O and factor 2 antisera (BD-difco™)" were used to identify *Salmonella* Paratyphi A.[11]

Antimicrobial Susceptibility Testing was performed on Mueller-Hinton agar by the Kirby-Bauer disk diffusion method(12). The antibiotic disks (HiMedia, India) used were: ampicillin (10 µg), chloramphenicol (30 µg), co-trimoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), azithromycin (15 µg), and meropenem (10 µg). *E. coli* ATCC 25922 was used as the quality control strain. Zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant based on CLSI breakpoints(13).

Complete blood count (CBC) reports for positive samples were retrieved from the Laboratory Information Management System (LIMS). The differential white blood cell counts from the CBC report taken at the time of admission were used for analysis. The Neutrophil-to-Lymphocyte Ratio (NLR) was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count.

Data were analyzed using IBM Statistical Package for the Social Sciences (SPSS) version 23. Categorical variables, such as gender and *Salmonella* spp were expressed in terms of percentage. Continuous variable NLR was assessed for normality using the Shapiro-Wilk test and presented as mean ± standard deviation One-way Analysis of Variance (ANOVA) was used to compare the mean NLR values across different strain groups. The association between this categorical NLR variable and typhoidal *Salmonella* strains was assessed using a Chi-square test. A p-value of < 0.05 was considered statistically significant for all tests.

RESULTS

A total of 450 blood cultures were analyzed. Typhoidal *Salmonellae* were isolated from 24.4% (n=110) of samples. *Salmonella* Typhi was the predominant pathogen, accounting for 86.3% (n=95) of positive cases, while *Salmonella* Paratyphi was responsible for 13.6% (n=15) of cases.

The study included 110 patients with culture-confirmed enteric fever. The majority were male (61.8%, n=68) compared to female (38.2%, n=42). The age distribution was skewed towards older children, with 73.6% (n=81) of cases belonging to the 5–15 years age group. The remaining 26.4% (n=29) were children under 5 years of age as represent in Table-I.

The susceptibility profile of the 110 *S.* Typhi isolates is summarized in Figure-I. Resistance to first-line antibiotics was very high: ampicillin (83.5%), co-

trimoxazole (76.5%). High rates of resistance were also observed against fluoroquinolones: ciprofloxacin (78.0%) and levofloxacin (71.8%). In contrast, all *S.* Typhi isolates (100%) were susceptible to azithromycin and meropenem.

The distribution of isolates based on antimicrobial resistance profiles revealed a high prevalence of drug-resistant strains. Extensively drug-resistant (XDR) *S.* Typhi was the most common strain, identified in 64.5% (n=71) of all isolates. This was followed by multidrug-resistant (MDR) *S.* Typhi (14.5%, n=16), MDR *S.* ParaTyphi (8.2%, n=9), drug-sensitive S. Typhi (7.3%, n=8), XDR *S.* ParaTyphi (2.7%, n=3), and drug-sensitive *S.* ParaTyphi (2.7%, n=3). Collectively, resistant strains (MDR and XDR) constituted 90% (n=99) of all isolates as shown in Figure -I.

Normality of NLR values was checked using the Shapiro–Wilk tests. In *S.* Typhi, the MDR and sensitive groups showed p-values greater than 0.05, indicating that the data were normally distributed whereas XDR *S.* Typhi group showed p-values less than 0.05, indicating non-normal distribution. For *S.* Paratyphi, all groups (MDR, XDR, and sensitive) had p-values greater than 0.05, showing normal distribution of NLR values. Since majority showed normal distribution so we used parametric tests for further analysis

The overall mean Neutrophil-to-Lymphocyte ratio for the study population was 2.04 ± 1.14. The mean NLR values stratified by specific strain type are summarized in Table No 3. The highest mean NLR values were observed in MDR *S.* Typhi (2.42 ± 1.39) and XDR *S.* Paratyphi (2.41 ± 1.07), while the lowest was observed in XDR *S.* Typhi (1.90 ± 1.16). One-way ANOVA test among mean NLR were not statistically significant (p = 0.559).

When grouped into resistant (MDR + XDR; n = 99) versus sensitive (n = 11) categories, the mean NLR was 2.04 ± 1.17 for resistant isolates and 2.03 ± 0.73 for sensitive isolates.

The distribution of High and Low NLR categories varied among the different *Salmonella* strains (Table-III). The proportion of patients with a High NLR was most prevalent in the MDR *S.* Typhi group 56.3%, followed by the XDR *S.* Para Typhi group (66.7%), and the MDR *S.* Para Typhi group (55.6%). Within the most prevalent strain, XDR *S.* Typhi, the distribution of NLR categories was nearly even, with 46.5% of cases classified as High NLR.

A Statistical test was applied to study significant association between the categorical NLR group and the *Salmonella* strains. The test revealed no statistically significant association (p = 0.69).

Table-I: Distribution of *Salmonella* strains.

Age group	Male	Female	Total
0-5 years	17 (58.6%)	12 (41.3%)	29 (26.36%)
5-15 years	51 (62.9%)	30 (37%)	81 (73.63%)
Total	68 (61.81%)	42 (38.18%)	110

Table-II: ANOVA Mean NLR among *Salmonella* spp.

Parameters	*S.* Typhi			*S.* Para Typhi			*P*-Value
	MDR	XDR	Sensitive	MDR	XDR	Sensitive	
NLR Mean ± Std. Deviation	2.419± 1.388	1.902±1.154	2.067± 0.736	2.380± 0.828	2.410± 1.055	1.932± .874	0.559

Table-III: Distribution of high and low NLR Categories by *Salmonella* Strain type.

Parameters	*S.* Typhi			*S.* Para Typhi			*P*-Value
NLR	MDR	XDR	Sensitive	MDR	XDR	Sensitive	
Low	7 (43.8%)	38 (53.5%)	4 (50.0%)	4 (44.4%)	1 (33.3%)	2 (66.7%)	
High	9 (56.3%)	33 (46.5%)	4 (50.0%)	5 (55.6%)	2 (66.7%)	1 (33.3%)	0.69

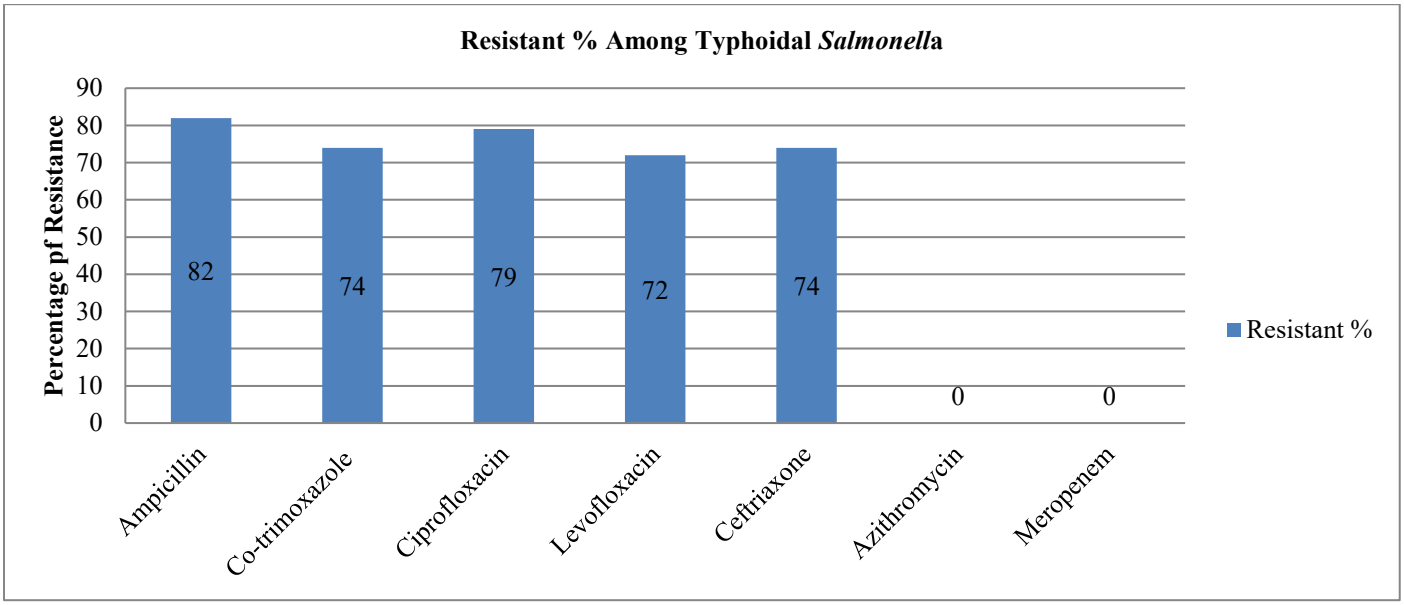

Figure-I: Resistant percentage of Typhoidal *Salmonella.*

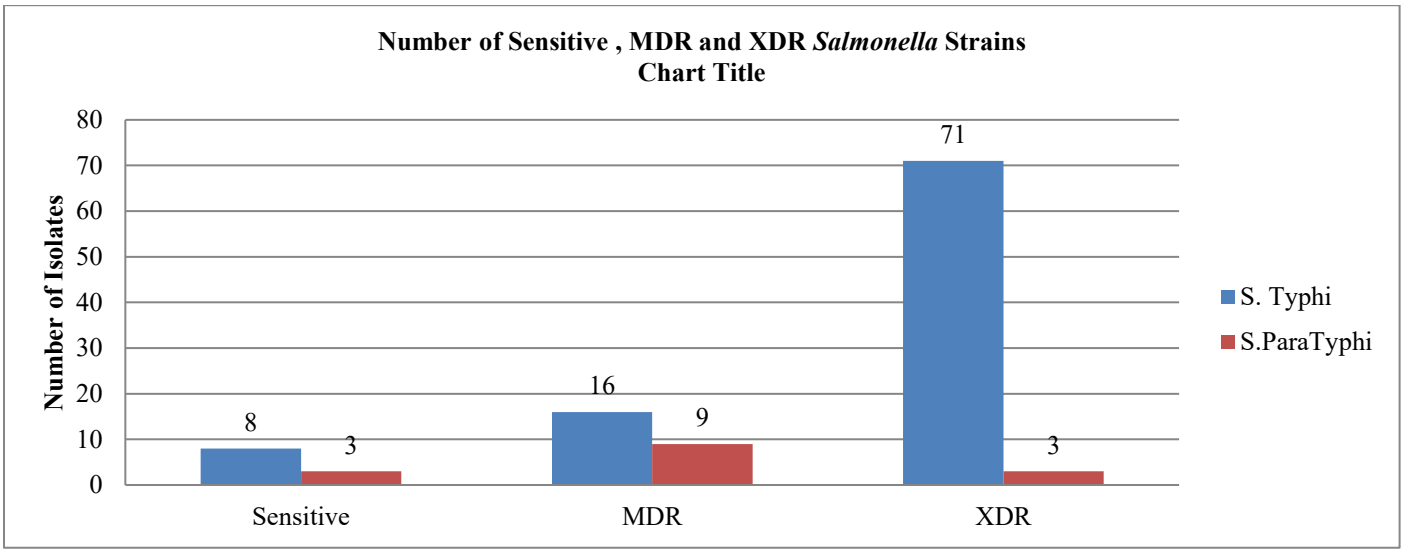

Figure-II: Number of sensitive, MDR and XDR *Salmonella* Strains.

DISCUSSION

This study provides alarming AMR trends in *S.* Typhi circulating in our patient population. The high culture positivity rate (48.3%) highlights the significant burden of enteric fever in our region. The predominance of *S.* Typhi (83.4%) over *S.* ParaTyphi is consistent with reports from other parts of South Asia.[14] The demographic data indicates that school-aged children (61.4%) are the most affected group This is in similar with the known epidemiology of the disease, where this population is at highest risk in endemic areas due to increase exposure.[15]

The striking finding is the profound prevalence of antimicrobial resistance. 90% of isolates were either MDR or XDR underscores a critical public health crisis. The rise of XDR *S.* Typhi, which exhibits resistance to the core first-line antibiotics, fluoroquinolones, and third-generation cephalosporins, drastically narrows the window for effective empirical therapy. This aligns with growing reports from South Asia of XDR typhoid outbreaks necessitating the use of azithromycin and carbapenems.[14,16,17] A recent case report from Pakistan documented the first ceftriaxone-resistant *Salmonella* Paratyphi isolate, highlighting the already worsen resistance condition.[11]

A key objective of this study was to investigate the Neutrophil-to-Lymphocyte Ratio (NLR) as a potential biomarker to differentiate between infections caused

by drug-sensitive and drug-resistant strains. However, our analysis revealed that the mean NLR was remarkably consistent across all strain types, showing no statistically significant variation. The slightly higher mean NLR observed in MDR *S.* Typhi and XDR *S.* Paratyphi groups was not sustained in the larger XDR *S.* Typhi group and was not statistically significant. This lack of differentiation holds true even when comparing the aggregated resistant (MDR+XDR) and sensitive groups.

Furthermore, when patients were categorized into High and Low NLR groups, a Chi-square test also showed no significant association across the various *Salmonella* strain types. This lack of association persists despite the visual variations in proportions, such as a higher rate of High NLR in MDR strains compared to some sensitive strains. This consistent result from two different statistical approaches strongly suggests that the fundamental nature of the systemic inflammatory response is not a unique feature of any particular antimicrobial-resistant strain(18). The systemic inflammatory response, appears to be driven by the host's general reaction to *Salmonella* infection rather than the specific resistance mechanisms of the infecting strain.[19,20] The pathophysiology of fever, inflammation, and endotoxemia might be common across strains with resistance genes being an independent genetic acquisition. Further high NLR value are complex and multifactorial and potentially related to host-specific factors or the timing of presentation rather than the virulence linked to antibiotic resistance genes.[7,18,21] Therefore, while NLR may have prognostic value for severity as indicated elsewhere, it does not serve as a biomarker for distinguishing between drug-resistant and drug-sensitive *Salmonella* infections. This indicates that the fundamental nature of the systemic inflammatory response is not intrinsically different between infections caused by drug-sensitive and drug-resistant *Salmonella*.[22] This suggests that the genetic machinery conferring antimicrobial resistance is not directly related to neutrophilic or lymphocytic response in the host.[23] The immune response appears to be a generic reaction to the infection rather than a specific indicator of the pathogen's resistance profile.[18] This finding determines that NLR cannot differentiate between strain types; it is a powerful indicator of the host's clinical response to the infection across all strains.

LIMITATIONS

This study was conducted at a single center with a specific patient demographic. The NLR is a measure of inflammation, and future research could focus on more specific inflammatory cytokines to study possible correlation with resistance or virulence.

CONCLUSION

The present study demonstrates high prevalence of antimicrobial resistance among typhoidal *Salmonella* isolates, with XDR *S.* Typhi emerging as the dominant strain. The widespread antimicrobial resistance underscores an urgent need for continuous surveillance and antimicrobial stewardship. Although the neutrophil-to-lymphocyte ratio (NLR) did not show a statistically significant association with specific resistance patterns, its elevated values in resistant groups suggest potential as a supportive marker for disease severity assessment. Routine NLR evaluation, alongside microbiological testing, may aid in early clinical risk stratification and improved management of enteric fever cases.

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CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Mehveen Iqbal: Conceived the idea, designed the study and collected data, final approval, accountable for all aspects of publication

Muhammad Hassan: Conceived the idea, designed the study and collected data, final approval, accountable for all aspects of publication

Alishba Shabbir: Manuscript writing, data analysis and result interpretation, final approval, accountable for all aspects of publication

Wajid Hussain: Manuscript writing, result interpretation, corresponding author, final approval, accountable for all aspects of publication

Mahin Shams: Editing and critical revision of manuscript, final approval, accountable for all aspects of publication

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