Beyond Antimicrobial Activity: Immunomodulatory Effects of Antibiotics on Host Defense and Inflammatory Resolution in Enteric Fever

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Abstract

Background: Enteric fever, caused by *Salmonella enterica* serovars Typhi and Paratyphi, remains a major infectious disease burden in low- and middle-income countries, increasingly complicated by multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. Macrolide antibiotics exert immunomodulatory effects beyond bactericidal activity, yet this dimension remains uncharacterized in enteric fever

Objective: To compare immunomodulatory effects of azithromycin versus fluoroquinolone therapy on systemic inflammatory biomarkers, fever clearance, and clinical outcomes in laboratory-confirmed enteric fever.

Methods: A prospective comparative cohort study enrolled 320 laboratory-confirmed enteric fever patients allocated to azithromycin (n = 160) or fluoroquinolone therapy (n = 160). C-reactive protein (CRP), interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and white blood cell (WBC) count were measured at baseline (Day 0) and Day 7. Fever clearance time, treatment failure, and antimicrobial resistance profiles were assessed. Independent samples t-tests, chi-square tests, and multivariable binary logistic regression were applied.

Results: Baseline parameters were comparable across groups (all $p > 0.05$). Azithromycin was associated with significantly greater reductions in CRP (−37.14 vs. −31.39 mg/L), IL-1β (−58.12 vs. −39.60 pg/mL; ~47% greater reduction; Cohen's d = 1.71), IL-6 (−31.03 vs. −23.76 pg/mL), and TNF-α (−46.70 vs. −30.26 pg/mL; all $p < 0.001$). Fever clearance was approximately 13 hours shorter in the azithromycin group (121.86 ± 16.58 vs. 134.73 ± 23.64 hours; $p < 0.001$). WBC reduction was equivalent between groups ($p = 0.736$). Treatment failure rates did not significantly differ (5.6% vs. 8.1%; $p = 0.377$). No independent predictor of treatment failure was identified (Nagelkerke $R^2 = 0.038$).

Conclusion: Azithromycin was associated with superior inflammatory cytokine resolution and accelerated fever clearance compared to fluoroquinolone therapy, without generalized leukocyte suppression or differential cure rates. These findings suggest antibiotic class selection influences host immune recovery beyond antimicrobial activity alone, supporting consideration of azithromycin's immunomodulatory profile in enteric fever management, particularly in MDR-endemic settings.

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1. Introduction

Enteric fever, caused predominantly by *Salmonella enterica* serovar Typhi (*S.* Typhi) and, to a lesser extent, *S.* Paratyphi A, B, and C, remains a major infectious disease burden affecting an estimated 10–20 million individuals annually, with the majority of cases concentrated in South Asia, sub-Saharan Africa, and parts of Southeast Asia. [1,2] Despite advances in vaccine development and sanitation infrastructure, enteric fever continues to cause substantial morbidity and mortality, particularly in settings with limited healthcare resources and widespread antimicrobial resistance. [3] The emergence of multidrug-resistant

(MDR) and extensively drug-resistant (XDR) strains of *S.* Typhi has further complicated therapeutic management, necessitating a deeper understanding of how available antibiotics interact not only with the pathogen but also with the host immune system. [4,5]

The pathophysiology of enteric fever involves complex interactions between *Salmonella* and innate and adaptive immune components of the human host. Upon ingestion, *S.* Typhi invades the intestinal epithelium and is taken up by macrophages and dendritic cells within Peyer's patches, subsequently disseminating via the lymphatic system to achieve systemic infection. [6] This intracellular survival strategy enables the pathogen to evade several host defense mechanisms while simultaneously triggering robust pro-inflammatory signaling cascades. [7] Macrophage activation leads to the elaboration of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α, which collectively orchestrate the febrile response, acute-phase protein synthesis, and systemic inflammation characteristic of the illness. [8,9]

C-reactive protein (CRP) is a prototypical acute-phase reactant synthesized by the liver in response to IL-6 stimulation, and its serum concentration correlates closely with the intensity of systemic inflammation. [10] Elevated CRP concentrations are consistently documented in enteric fever and serve as a surrogate marker of inflammatory activation and disease severity. [11] Similarly, IL-1β plays a central role as an endogenous pyrogen and regulator of acute inflammation, while TNF-α modulates vascular permeability, leukocyte recruitment, and macrophage microbicidal activity during infection. [12,13] Dysregulation of these cytokine networks can result in excessive systemic inflammation, contributing to fever persistence, end-organ stress, and prolonged clinical recovery.

Traditionally, the therapeutic goal in enteric fever has been focused on eradicating the pathogen using antibiotics with documented efficacy against Salmonella. First-line agents have historically included chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole, though widespread resistance has rendered these largely obsolete in many regions. [3] Fluoroquinolones subsequently emerged as agents of choice due to their excellent bioavailability and intracellular penetration; however, the rising prevalence of fluoroquinolone-resistant and ciprofloxacin non-susceptible isolates has significantly curtailed their utility. [14,15] Azithromycin, a macrolide antibiotic with high intracellular concentrations and established efficacy against MDR enteric fever strains, has consequently gained prominence as an alternative therapeutic option. [16]

Beyond their direct antimicrobial properties, there is growing recognition that certain antibiotic classes exert significant modulatory effects on host immune pathways. Macrolides in particular have been extensively studied in the context of immunomodulation, with evidence demonstrating their capacity to suppress pro-inflammatory cytokine production, inhibit neutrophil degranulation, and reduce oxidative burst activity through mechanisms independent of their bactericidal action. [17,18] Fluoroquinolones have also been reported to modulate cytokine networks, though the magnitude and direction of these effects appear to differ from those observed with macrolides. [19] These immunomodulatory properties have been exploited therapeutically in chronic inflammatory pulmonary conditions such as diffuse panbronchiolitis and cystic fibrosis, where long-term macrolide use attenuates airway inflammation independently of antimicrobial activity. [20]

The relevance of antibiotic-mediated immunomodulation to enteric fever management remains underexplored. Given that Salmonella infection induces a potent systemic pro-inflammatory state, the capacity of an antibiotic to attenuate cytokine responses and accelerate inflammatory resolution may represent a clinically meaningful therapeutic attribute beyond pathogen clearance alone. Understanding whether azithromycin and fluoroquinolone therapy differ in their effects on systemic inflammatory markers and clinical recovery indicators could inform therapeutic decision-making, particularly in settings where MDR prevalence is high and treatment options are limited. The present study therefore aimed to assess the broader immunomodulatory effects of antibiotics and their potential role in enhancing host defense mechanisms and inflammatory resolution in a cohort of 320 laboratory-confirmed enteric fever patients treated with either azithromycin or fluoroquinolone therapy.

2. Methods

2.1. Study Design

This was a prospective comparative cohort study designed to evaluate systemic inflammatory resolution and clinical outcomes in patients with laboratory-confirmed enteric fever receiving one of two antibiotic treatment regimens over a seven-day treatment period. The study was conducted at a single tertiary care referral centre. All procedures were conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Institutional Ethics Committee (IEC), RKDF University, with approval reference number 20/RKDF/2025. Written informed consent was obtained from all participants prior to enrollment; for participants below 18 years of age, written consent was obtained from a parent or legal guardian.

2.2. Patient Population and Eligibility

A total of 320 consecutive patients with laboratory-confirmed enteric fever were prospectively enrolled. Diagnosis was established through standard bacteriological methods, specifically blood culture isolation of Salmonella enterica serovar Typhi or Paratyphi from venous blood specimens processed using automated culture systems. Patients were eligible if they met all of the following inclusion criteria: (1) age ≥ 5 years; (2) clinical presentation consistent with enteric fever, including sustained fever, constitutional symptoms, and/or gastrointestinal manifestations; (3) microbiologically confirmed Salmonella infection prior to treatment initiation; and (4) willingness to comply with treatment and scheduled follow-up assessments.

Patients were excluded if they had received any antibiotic therapy within the preceding two weeks, had documented immunosuppressive conditions (including HIV infection, active malignancy, or chronic corticosteroid use), were pregnant or breastfeeding, or had severe hepatic or renal impairment likely to confound inflammatory marker interpretation or alter drug pharmacokinetics.

2.3. Treatment Groups and Allocation

Enrolled patients were prospectively allocated to one of two treatment arms - azithromycin (n = 160) or fluoroquinolone therapy (n = 160) - based on the treating physician's clinical assessment in accordance with institutional antimicrobial stewardship protocols. Allocation was non-randomized and

followed standard therapeutic guidelines applicable to the clinical and microbiological context at the time of enrollment. Azithromycin was administered orally at 20 mg/kg/day (maximum 1 g/day) for 7 days. Fluoroquinolone therapy consisted of oral ciprofloxacin 500 mg twice daily for 10–14 days, or levofloxacin 750 mg once daily for 7–10 days, as determined by susceptibility profile and clinical response. All patients received standard supportive care. No placebo arm was included, consistent with the ethical obligation to treat confirmed bacterial infection. Outcome assessors and laboratory personnel were not formally blinded to treatment allocation, which is acknowledged as a potential source of measurement bias.

2.4. Antimicrobial Resistance Profiling

Antimicrobial susceptibility testing was performed on all confirmed Salmonella isolates using disk diffusion and minimum inhibitory concentration (MIC) broth microdilution methods, interpreted according to Clinical and Laboratory Standards Institute (CLSI) breakpoints (current edition at time of testing). Multidrug resistance (MDR) was defined as acquired resistance to at least one agent in three or more antimicrobial categories, including ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. Fluoroquinolone susceptibility was assessed using nalidixic acid disk screening and ciprofloxacin MIC determination. MDR and nalidixic acid resistance prevalence were documented for each treatment group and compared using the chi-square test.

2.5. Inflammatory Biomarker Measurement

Systemic inflammatory status was assessed at baseline (Day 0, prior to treatment initiation) and at Day 7 of therapy using four primary biomarkers: C-reactive protein (CRP), interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). Additionally, white blood cell (WBC) count and haemoglobin were recorded as haematological parameters.

All ELISA analyses were performed in duplicate; inter-assay and intra-assay coefficients of variation were < 10% and < 8%, respectively. CRP was quantified using high-sensitivity immunoturbidimetric assay on an automated biochemistry analyser. All analyses were conducted in a single certified clinical laboratory under standardized, temperature-controlled conditions, with laboratory personnel blinded to clinical outcome data.

2.6. Clinical Outcome Assessment

The primary clinical outcomes were: (1) fever clearance time, defined as the interval in hours from treatment initiation to the first recording of sustained afebrile status, defined as axillary temperature < 37.5°C maintained for ≥ 24 consecutive hours; and (2) treatment failure, defined as persistence or worsening of clinical signs and symptoms at Day 7, requirement for change of antibiotic regimen due to clinical non-response, or documented microbiological relapse within the 30-day follow-up period. Secondary outcomes included 30-day relapse rate and adverse event profile, both reported by treatment group.

2.7. Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics Version 23.0 (IBM Corp., Armonk, NY, USA). Baseline demographic and clinical comparability between treatment groups was assessed using independent samples t-tests for normally distributed continuous variables and chi-square tests for categorical variables. Normality of continuous variables was assessed using the Shapiro-Wilk test prior to parametric analysis. Changes in inflammatory markers from Day 0 to Day 7 were computed as the absolute difference (Day 7 minus Day 0), and between-group differences in marker changes were compared using independent samples t-tests. Fever clearance duration was compared between groups using an independent samples t-test. Treatment failure rates were compared using the chi-square test with Yates' continuity correction. Multivariable binary logistic regression analysis was performed to identify independent predictors of treatment failure, with antibiotic group assignment, MDR status, sex, age, nalidixic acid resistance, and baseline inflammatory parameters included as candidate covariates. Model fit was evaluated using the omnibus likelihood ratio chi-square test and Nagelkerke R^2 statistic. All tests were two-tailed, and statistical significance was defined at $p < 0.05$.

3. Results

3.1. Study Population Characteristics

A total of 320 laboratory-confirmed enteric fever cases were prospectively enrolled and equally allocated to azithromycin (n = 160) and fluoroquinolone (n = 160) treatment arms. The overall cohort had a mean age of 39.44 ± 12.44 years and mean BMI of 21.93 ± 4.51 kg/m². Sex distribution was approximately balanced, with 52.5% male participants overall. Baseline systemic inflammatory burden was substantial: mean CRP was 69.27 ± 15.56 mg/L, IL-1β was 178.52 ± 28.99 pg/mL, IL-6 was 64.55 ± 13.41 pg/mL, and TNF-α was 169.29 ± 25.68 pg/mL. Antimicrobial resistance was highly prevalent in this cohort, with 60.3% of isolates classified as multidrug resistant (MDR) and 76.6% demonstrating nalidixic acid resistance. Overall cohort characteristics are summarized in Table 1.

Table 1: Overall baseline characteristics of the study cohort (N = 320)

Variable	Mean ± SD / n (%)
Age (years)	39.44 ± 12.44
BMI (kg/m²)	21.93 ± 4.51
Male sex, n (%)	168 (52.5%)
Symptom duration at presentation (days)	7.00 ± 2.08
Haemoglobin (g/dL)	11.46 ± 1.02
WBC count (cells/μL)	9065 ± 1508
CRP (mg/L)	69.27 ± 15.56
IL-1β (pg/mL)	178.52 ± 28.99
IL-6 (pg/mL)	64.55 ± 13.41
TNF-α (pg/mL)	169.29 ± 25.68
MDR isolates, n (%)	193 (60.3%)
Nalidixic acid resistant isolates, n (%)	245 (76.6%)

MDR = multidrug resistant; CRP = C-reactive protein; IL = interleukin; TNF-α = tumor necrosis factor-alpha; WBC = white blood cell; BMI = body mass index.

3.2. Baseline Comparability Between Treatment Groups

Baseline demographic, clinical, haematological, and inflammatory parameters were well-balanced across treatment groups, as shown in Table 2. Sex distribution did not differ significantly between arms (azithromycin: 50.0% male vs. fluoroquinolone: 55.0% male; $\chi 2(1) = 0.802$, $p = 0.370$). No statistically significant between-group differences were identified for age, BMI, symptom duration,

haemoglobin, WBC count, CRP, IL-1β, IL-6, or TNF-α (all p > 0.05), confirming pre-treatment equivalence in disease severity and systemic inflammatory burden. MDR prevalence was numerically higher in the azithromycin group (65.6% vs. 55.0%), though this difference did not reach statistical significance (p = 0.052), making differential baseline resistance an unlikely confound for subsequent between-group comparisons.

Table 2: Baseline demographic, clinical, and inflammatory parameters by treatment group

Variable	Azithromycin (n = 160) Mean ± SD / n (%)	Fluoroquinolone (n = 160) Mean ± SD / n (%)	p-value
Male sex, n (%)	80 (50.0%)	88 (55.0%)	0.370
Age (years)	38.96 ± 12.68	39.93 ± 12.21	0.487
BMI (kg/m²)	22.17 ± 4.81	21.69 ± 4.20	0.343
Symptom duration (days)	7.08 ± 2.14	6.91 ± 2.01	0.467
Haemoglobin (g/dL)	11.50 ± 0.99	11.42 ± 1.06	0.512
WBC count (cells/µL)	8979 ± 1529	9151 ± 1483	0.309
CRP (mg/L)	68.50 ± 15.11	70.05 ± 15.99	0.371
IL-1β (pg/mL)	179.89 ± 27.39	177.15 ± 30.54	0.397
IL-6 (pg/mL)	65.63 ± 13.36	63.48 ± 13.42	0.151
TNF-α (pg/mL)	169.40 ± 25.46	169.18 ± 25.97	0.940
MDR isolates, n (%)	105 (65.6%)	88 (55.0%)	0.052
Nalidixic acid resistant, n (%)	126 (78.8%)	119 (74.4%)	0.346

Independent samples t-test for continuous variables; chi-square test for categorical variables. MDR = multidrug resistant; CRP = C-reactive protein; WBC = white blood cell.

3.3. Inflammatory Marker Resolution (Day 0 to Day 7)

Between-group changes in inflammatory markers over the seven-day treatment period are presented in Table 3. Azithromycin therapy was associated with significantly greater absolute reductions across all four primary inflammatory biomarkers compared with fluoroquinolone therapy (all p < 0.001). Mean CRP reduction was −37.14 ± 9.30 mg/L in the azithromycin group versus −31.39 ± 8.57 mg/L in the fluoroquinolone group (mean difference: −5.75 mg/L; 95% CI: −7.89 to −3.61; Cohen's d = 0.65, medium effect). The IL-1β reduction was the most pronounced differential finding: the azithromycin group achieved a mean decline of −58.12 ± 11.69 pg/mL compared with −39.60 ± 10.21 pg/mL in the fluoroquinolone group, representing approximately 47% greater reduction (mean difference: −18.52 pg/mL; 95% CI: −21.10 to −15.94; Cohen's d = 1.71, large effect). IL-6 and TNF-α reductions were similarly superior in the azithromycin group, with Cohen's d values of 1.06 and 1.74, respectively, indicating large effect sizes for both comparisons (Table 3, Figure 1).

Notably, WBC count reduction did not differ significantly between groups (−1616 ± 456 vs. −1599 ± 473 cells/µL; mean difference: −17 cells/µL; 95% CI: −119 to 85; p = 0.736; Cohen's d = 0.04). This finding is clinically significant: the differential anti-inflammatory effects of azithromycin were selective for cytokine-mediated inflammatory pathways and were not accompanied by generalized leukocyte suppression, a profile consistent with the known immunomodulatory signature of macrolide antibiotics. These findings are illustrated in Figure 2.

Table 3: Absolute change in inflammatory markers from Day 0 to Day 7 by treatment group

Marker	Azithromycin Mean ± SD	Fluoroquinolone Mean ± SD	Mean difference (95% CI)	p-value	Cohen's d
Δ CRP (mg/L)	−37.14 ± 9.30	−31.39 ± 8.57	−5.75 (−7.89 to −3.61)	<0.001	0.65
Δ IL-1β (pg/mL)	−58.12 ± 11.69	−39.60 ± 10.21	−18.52 (−21.10 to −15.94)	<0.001	1.71
Δ IL-6 (pg/mL)	−31.03 ± 7.46	−23.76 ± 6.01	−7.27 (−8.92 to −5.62)	<0.001	1.06
Δ TNF-α (pg/mL)	−46.70 ± 10.35	−30.26 ± 8.42	−16.44 (−18.71 to −14.17)	<0.001	1.74
Δ WBC (cells/µL)	−1616 ± 456	−1599 ± 473	−17 (−119 to 85)	0.736	0.04

Negative values indicate reduction from baseline. Between-group differences assessed by independent samples t-test. 95% CI = 95% confidence interval of the mean difference. Cohen's d interpretation: small ≥ 0.2, medium ≥ 0.5, large ≥ 0.8. Δ = absolute change (Day 7 − Day 0).

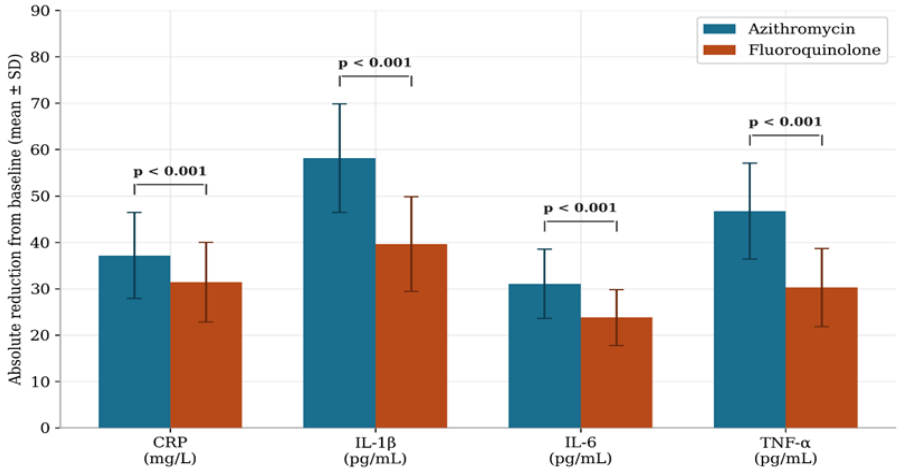

Fig 1: Inflammatory marker reduction from Day 0 to Day 7 by treatment group.

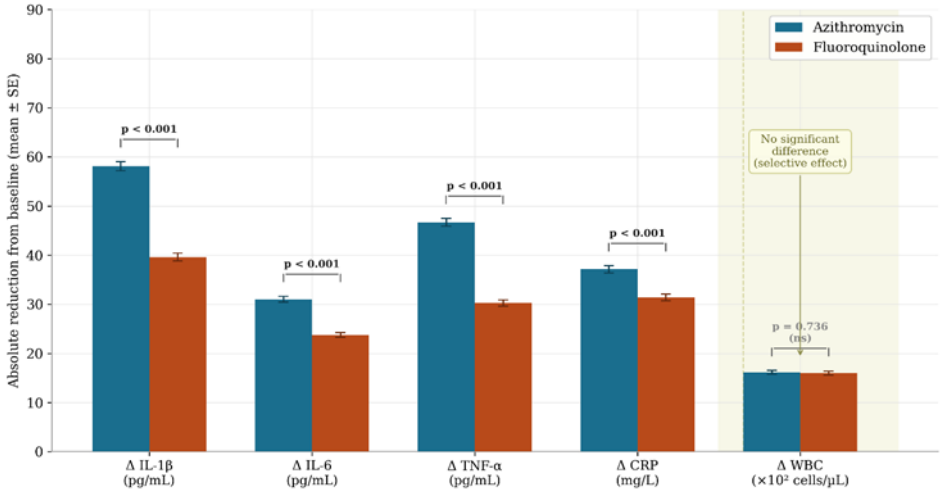

Fig 2: Selective cytokine attenuation by azithromycin - cytokine markers vs. leukocyte count change by treatment group.

3.4. Fever Clearance Time

Fever clearance occurred significantly earlier in the azithromycin group compared with the fluoroquinolone group (121.86 ± 16.58 hours vs. 134.73 ± 23.64 hours; mean difference: −12.87 hours; 95% CI: −17.27 to −8.47; $p < 0.001$; Cohen's d = 0.64), representing approximately 13 hours of accelerated fever resolution (Table 4, Figure 3). This clinically meaningful difference in fever clearance is temporally consistent with and mechanistically supported by the superior cytokine downregulation - particularly IL-1β and IL-6, both established endogenous pyrogens acting on the hypothalamic thermoregulatory centre - observed in Section 3.3.

Table 4: Fever clearance duration by treatment group

Parameter	Azithromycin Mean ± SD (hours)	Fluoroquinolone Mean ± SD (hours)	Mean difference (95% CI)	p-value
Fever clearance time	121.86 ± 16.58	134.73 ± 23.64	−12.87 (−17.27 to −8.47)	<0.001

Between-group comparison by independent samples t-test. 95% CI = 95% confidence interval of the mean difference. Cohen's d = 0.64 (medium-large effect).

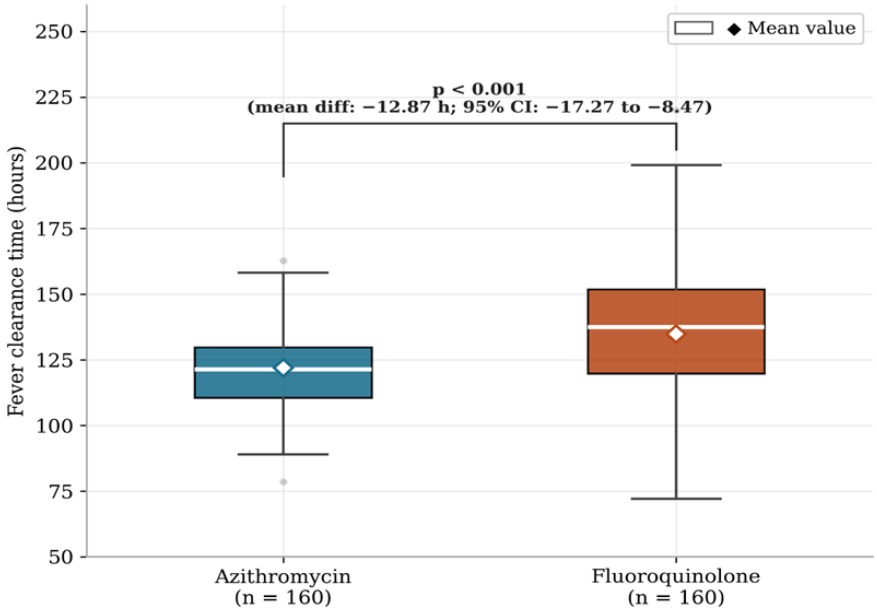

Fig 3: Fever clearance time by treatment group.

3.5. Clinical Outcomes

Treatment failure rates were low in both groups and did not differ significantly between treatment arms (azithromycin: 5.6% vs. fluoroquinolone: 8.1%; $\chi2(1) = 0.793$, $p = 0.377$) (Table 5). Thirty-day relapse was recorded in 2.5% of azithromycin-treated patients and 3.1% of fluoroquinolone-treated patients ($p = 0.754$). Adverse events were reported in 5.0% and 7.5% of azithromycin and fluoroquinolone patients, respectively ($p = 0.341$); no severe adverse events requiring treatment discontinuation were recorded in either arm. Despite clearly superior inflammatory resolution and accelerated fever clearance in the azithromycin group, binary clinical cure rates were statistically equivalent between treatment arms.

Table 5: Clinical outcomes by treatment group

Outcome	Azithromycin n (%)	Fluoroquinolone n (%)	p-value
Treatment failure	9 (5.6%)	13 (8.1%)	0.377
30-day relapse	4 (2.5%)	5 (3.1%)	0.754
Adverse events (any)	8 (5.0%)	12 (7.5%)	0.341

Chi-square test with Yates' continuity correction.

3.6. Multivariable Predictors of Treatment Failure

Multivariable binary logistic regression analysis incorporating treatment group assignment, age, sex, MDR status, nalidixic acid resistance, and baseline inflammatory parameters (CRP, IL-1β, IL-6, TNF-α) as candidate covariates identified no independent predictor of treatment failure (all $p > 0.05$). The overall model demonstrated poor explanatory power (Nagelkerke $R2 = 0.038$; omnibus $\chi2$ $p = 0.311$), indicating that neither antibiotic class, antimicrobial resistance phenotype, nor baseline systemic inflammatory burden independently determined clinical cure probability in this cohort. The odds ratio for treatment group assignment (azithromycin vs. fluoroquinolone: OR = 1.476; 95% CI: 0.603–3.611; $p = 0.393$) confirmed that superior inflammatory resolution in the azithromycin group did not translate into a statistically significant reduction in treatment failure risk under the binary cure/failure classification used in this study.

4. Discussion

4.1. Baseline Inflammatory Activation in Enteric Fever

The overall cohort enrolled in this study demonstrated substantial systemic inflammatory activation at baseline, characterized by markedly elevated CRP and pro-inflammatory cytokine concentrations alongside a high prevalence of multidrug-resistant Salmonella isolates. Mean baseline CRP of 69.27 mg/L, IL-1β of 178.52 pg/mL, IL-6 of 64.55 pg/mL, and TNF-α of 169.29 pg/mL collectively reflect the intensity of innate immune activation that characterizes confirmed enteric fever, wherein bacterial invasion of reticuloendothelial tissues - particularly Peyer's patches and mesenteric lymph nodes - triggers potent macrophage-mediated pro-inflammatory signalling cascades. [6,11] The hepatic acute-phase response, evidenced by markedly elevated CRP, is mechanistically downstream of IL-6 stimulation and serves as a reliable integrative marker of systemic inflammatory burden in this clinical context. [10,21]

The high MDR burden observed in this cohort - with 60.3% of isolates classified as multidrug resistant and 76.6% demonstrating nalidixic acid resistance - is consistent with the global epidemiological trajectory of antimicrobial resistance in Salmonella Typhi. The near-threshold difference in MDR prevalence between groups (65.6% azithromycin vs. 55.0% fluoroquinolone; $p = 0.052$) was not statistically significant and is unlikely to represent a meaningful confound; however, this pattern is consistent with the clinical reality that azithromycin is increasingly preferred in settings with high fluoroquinolone resistance.

4.2. Immunomodulatory Effects of Azithromycin

The principal finding of this study was that azithromycin therapy was associated with significantly greater reductions in CRP, IL-1β, IL-6, and TNF-α over seven days of treatment compared with fluoroquinolone therapy (all $p < 0.001$), with large effect sizes across cytokine outcomes (Cohen's d: IL-1β = 1.71, TNF-α = 1.74, IL-6 = 1.06). Notably, the magnitude of IL-1β reduction was approximately 47% greater in the azithromycin group, suggesting a particularly pronounced modulatory effect on this central mediator of the acute inflammatory response. [12]

Mechanistic evidence from *in vitro* and ex vivo studies demonstrates that macrolides, including azithromycin, attenuate pro-inflammatory cytokine elaboration through inhibition of the nuclear factor kappa-B (NF-κB) transcription factor pathway - a master regulator of gene expression for IL-1β, IL-6, and TNF-α - as well as through suppression of activator protein-1 (AP-1) signalling. [17,18,22] Azithromycin additionally suppresses transcriptional activation of cytokine-encoding genes, thereby attenuating both the magnitude and duration of pro-inflammatory cytokine elaboration during active infection. [23] Importantly, azithromycin achieves intracellular concentrations many-fold higher than corresponding plasma levels within phagocytic cells - including macrophages and neutrophils, the principal cellular compartments of innate immune activation during Salmonella infection - which may enable direct intracellular modulation of cytokine biosynthesis at the site of pathogen-host interaction. [7,18,24]

Fluoroquinolones have also been reported to influence cytokine networks, but the direction and magnitude of their immunomodulatory effects appear context-dependent and are generally less pronounced than those associated with macrolide therapy. [19] Under certain conditions - particularly at sub-inhibitory concentrations - fluoroquinolones may paradoxically stimulate pro-inflammatory cytokine production, a phenomenon that may partially account for the comparatively attenuated inflammatory resolution observed in the fluoroquinolone group. [25]

4.3. Cytokine Regulation and Immune Recovery

The differential cytokine reductions observed between groups encompass a coordinated pattern of broad pro-inflammatory cytokine downregulation in the azithromycin group that extends beyond CRP alone and reflects upstream modulation of the inflammatory cascade. IL-6, which drives hepatic acute-phase protein synthesis including CRP production via the JAK-STAT3 signalling pathway, was significantly more reduced in the azithromycin group. [9,21] This upstream IL-6 attenuation mechanistically accounts for the concurrent and proportionally greater CRP decline, and is consistent with azithromycin suppressing inflammatory signalling at the cytokine transcription level. TNF-α reduction was similarly and substantially greater in the azithromycin group (Cohen's d = 1.74), carrying dual implications: TNF-α is both a potent mediator of systemic vascular inflammation and a critical stimulus for macrophage bactericidal activity against intracellular Salmonella. [7,8,13]

The finding that WBC count reduction did not significantly differ between groups ($p = 0.736$; Cohen's d = 0.04) is of considerable mechanistic importance and is visually demonstrated in Figure 2. The selective attenuation of cytokine-mediated inflammatory pathways without concomitant generalized leukocyte suppression is precisely consistent with the known immunomodulatory signature of

macrolide antibiotics, which modulate cytokine transcription and secretion without broadly impairing leukocyte viability or bactericidal capacity. [17,20]

4.4. Inflammatory Resolution and Clinical Recovery

Accelerated fever clearance in the azithromycin group - approximately 13 hours shorter than in the fluoroquinolone group (mean difference: −12.87 hours; 95% CI: −17.27 to −8.47; $p < 0.001$; Cohen's $d = 0.64$) - provides clinically meaningful and temporally coherent corroboration of the superior inflammatory resolution reflected in cytokine biomarker data. Fever in enteric fever is driven principally by endogenous pyrogens, particularly IL-1β and IL-6, which act on the hypothalamic thermoregulatory centre via prostaglandin E2-mediated pathways to elevate the thermal set-point. [8,26]

This finding is consistent with clinical evidence from the community-acquired pneumonia literature, in which macrolide-containing regimens have been associated with shorter fever duration, faster clinical stabilization, and reduced systemic inflammatory marker concentrations compared to non-macrolide alternatives. [27,28] The present data extend this pattern to the acute enteric fever context, demonstrating that the immunomodulatory benefit of azithromycin is not restricted to pulmonary infection but manifests across distinct systemic bacterial infections characterized by cytokine-driven febrile illness.

4.5. Antibiotics as Immune Modulators: Broader Implications and Novelty

The concept that antibiotics may function as host immune modulators beyond their antimicrobial roles has gained substantial scientific traction over the past two decades. This phenomenon has been most extensively characterized for macrolide antibiotics, where long-term use in chronic inflammatory pulmonary conditions including diffuse panbronchiolitis, cystic fibrosis, non-cystic fibrosis bronchiectasis, and chronic obstructive pulmonary disease has demonstrated sustained reductions in airway inflammatory markers, exacerbation frequency, and disease progression. [20,22,23,24]

The present findings extend this immunomodulatory framework from the domain of chronic pulmonary disease to the acute systemic infectious disease context of enteric fever, demonstrating that even short-course azithromycin therapy is associated with measurably superior inflammatory cytokine resolution compared to fluoroquinolone therapy. To the best of the authors' knowledge, the present study represents one of the first clinical investigations to systematically quantify differential immunomodulatory cytokine trajectories between antibiotic classes specifically in laboratory-confirmed enteric fever, thereby extending the macrolide immunomodulation evidence base from chronic respiratory conditions to acute systemic bacterial infection.

4.6. Treatment Failure and Clinical Outcome Equivalence

Despite the clearly superior inflammatory resolution and accelerated fever clearance associated with azithromycin, binary treatment failure rates did not significantly differ between groups (5.6% vs. 8.1%; $p = 0.377$). Multivariable logistic regression confirmed that neither antibiotic class, MDR status, sex, age, nalidixic acid resistance, nor baseline inflammatory parameters independently predicted treatment failure, with the model demonstrating poor overall explanatory power (Nagelkerke $R2 = 0.038$; omnibus $p = 0.311$). These findings indicate that the two therapeutic regimens achieved statistically equivalent rates of clinical cure, despite differing substantially in their immunomodulatory trajectories.

This apparent paradox - superior inflammatory resolution without differential treatment failure - warrants careful interpretation. The multifactorial determinants of clinical cure in enteric fever extend considerably beyond what biomarker trajectories or antibiotic class alone can capture. It is also plausible that the binary treatment failure endpoint with an overall event rate of only 6.9% across the cohort lacked sufficient statistical power to detect clinically meaningful between-group differences even where such differences existed. A larger sample or a composite endpoint incorporating fever duration, time to functional recovery, and inflammatory normalization would more comprehensively capture the full therapeutic spectrum potentially conferred by azithromycin's immunomodulatory properties.

5. Limitations

Several methodological limitations of this study warrant explicit consideration in the interpretation of findings. First, the observational, non-randomized design precludes definitive causal inference; while baseline characteristics were well-balanced across groups on all measured parameters (all $p > 0.05$), unmeasured confounders inherent to physician-directed treatment allocation cannot be fully excluded. A randomized controlled trial design would be required to establish causality with confidence.

Second, the mechanistic pathways through which azithromycin exerts immunomodulatory effects in this specific clinical context were not directly investigated. The absence of NF-κB activity assays, AP-1 transcriptional analyses, intracellular signalling assessments, or macrophage functional assays limits mechanistic interpretation of the observed cytokine differences.

Third, inflammatory marker follow-up was restricted to Day 7, precluding assessment of longer-term immune recovery trajectories, late-phase cytokine normalization patterns, or sustained anti-inflammatory effects beyond the acute treatment phase.

Fourth, individual host variability in immune responsiveness - including differences attributable to nutritional status, comorbidities, genetic polymorphisms in cytokine-encoding genes (e.g., IL-6, TNF-α, IL-1β gene variants), and baseline immune competence - was not systematically characterized, representing a potential source of residual immunological heterogeneity.

Fifth, this study did not assess Salmonella bacteraemia clearance kinetics or serial intracellular bacterial burden, which would have provided essential complementary microbiological data to contextualize the immunological findings.

Sixth, the fluoroquinolone treatment arm comprised both ciprofloxacin and levofloxacin, and while both agents are classified as fluoroquinolones, their immunomodulatory profiles may not be identical. The heterogeneity within the comparator arm may have introduced variability in observed cytokine responses.

Finally, the single-centre design, while enabling standardized laboratory conditions and consistent clinical management, limits the generalizability of findings to populations with

different baseline resistance profiles, healthcare infrastructure, nutritional contexts, or prevalent Salmonella genotypes.

6. Future Directions

The findings of this study open several important avenues for future investigation. Foremost, a well-powered randomized controlled trial comparing azithromycin and fluoroquinolone therapy in enteric fever - incorporating serial cytokine measurement, mechanistic immune assays, and extended clinical follow-up - is required to establish causal immunomodulatory differences and determine whether the superior inflammatory resolution associated with azithromycin translates into measurable improvements in patient-reported outcomes, quality of life, and longer-term functional recovery.

Future studies should incorporate mechanistic immune analyses including NF-κB and AP-1 pathway activation assays, intracellular cytokine staining, macrophage functional assessments, and transcriptomic profiling of peripheral blood mononuclear cells to delineate the precise intracellular pathways through which azithromycin modulates the host inflammatory response to Salmonella infection.

Extended inflammatory follow-up beyond Day 7 - ideally at Day 14, Day 30, and at the point of complete clinical recovery - would clarify the durability of azithromycin's immunomodulatory effects and the kinetics of cytokine normalization. Simultaneously, studies incorporating composite clinical endpoints combining fever clearance time, time to functional recovery, inflammatory marker normalization, and patient-reported outcomes would provide a more sensitive and clinically meaningful assessment of between-group therapeutic differences than binary cure/failure classifications alone.

Multi-centre studies across geographically and epidemiologically diverse enteric fever settings - including South Asia, sub-Saharan Africa, and Southeast Asia - would establish the generalizability of these immunomodulatory findings across populations with differing Salmonella resistance profiles, host genetic backgrounds, and healthcare contexts. Investigation of host genetic determinants of cytokine response - particularly polymorphisms in IL-1β, IL-6, TNF-α, and NF-κB pathway genes - may further identify subpopulations most likely to benefit from the immunomodulatory properties of azithromycin.

7. Conclusion

This prospective comparative cohort study of 320 laboratory-confirmed enteric fever patients demonstrates that azithromycin therapy is associated with significantly greater and clinically meaningful reductions in systemic pro-inflammatory biomarkers - including CRP, IL-1β, IL-6, and TNF-α - and accelerated fever clearance compared to fluoroquinolone therapy, with large effect sizes across primary cytokine outcomes, despite equivalent binary treatment failure rates. The selective cytokine-attenuating properties of azithromycin, evidenced by differential cytokine reduction in the absence of differential leukocyte count changes, are consistent with the established immunomodulatory pharmacology of macrolide antibiotics and are mechanistically attributable to inhibition of NF-κB and AP-1 inflammatory signalling pathways. [6,17,18,23,20]

These findings provide clinical evidence that antibiotic class selection in enteric fever may influence host immune recovery through mechanisms extending beyond direct antimicrobial activity against Salmonella - a dimension of therapeutic decision-making that has been undercharacterized in this disease context. In settings where MDR and XDR Salmonella prevalence is high and fluoroquinolone utility is progressively curtailed by resistance, the dual antimicrobial and immunomodulatory profile of azithromycin may represent a therapeutically meaningful and currently underutilized advantage in enteric fever management. Randomized controlled trials incorporating mechanistic immune analyses, extended follow-up, and patient-centred outcome measures are warranted to confirm and extend these findings.

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