

Gene Regulation and Innate Immune Evasion in *Salmonella* Paratyphi a Infection

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Abstract

Background: Paratyphoid fever, caused by *Salmonella enterica* serovar Paratyphi A, is an important yet comparatively understudied systemic infection associated with innate immune evasion and dysregulated host gene regulation. The pathogen employs intracellular survival strategies within macrophages, manipulating host transcriptional and inflammatory programs to establish persistent infection. Despite increasing antimicrobial resistance and evolving treatment landscapes, the differential immunomodulatory effects of antibiotic classes on host gene regulatory responses during S. Paratyphi A infection remain incompletely characterized.

Methods: A dual-tiered integrative study design was employed incorporating a mechanistic cohort of 60 participants (30 per treatment arm: azithromycin vs. fluoroquinolone) and a clinical immunomodulatory cohort of 320 participants (160 per arm). The mechanistic cohort underwent *in vitro* infection of human monocyte-derived macrophages with patient-derived S. Paratyphi A isolates. Intracellular colony-forming unit (CFU) quantification, pro-inflammatory cytokine profiling (IL-1β, IL-6, TNF-α), reactive oxygen species (ROS) measurement, phagocytic index assessment, dual RNA-sequencing with differential gene expression analysis, and NF-κB pathway enrichment scoring were performed. Clinical outcomes including fever clearance time, inflammatory biomarker resolution, and treatment failure rates were compared between treatment groups.

Results: Azithromycin-exposed samples demonstrated significantly lower intracellular bacterial burden at 24 and 48 hours post-infection compared to the fluoroquinolone group (CFU 48h: 16,327 ± 4,876 vs. 29,236 ± 4,514; $p < 0.001$). The azithromycin group displayed greater NF-κB pathway enrichment scores (2.68 ± 0.42 vs. 1.59 ± 0.46; $p < 0.001$) and a higher number of differentially expressed genes (851 ± 127 vs. 627 ± 94; $p < 0.001$). NF-κB enrichment score was inversely correlated with intracellular bacterial load ($r = -0.656$; $p < 0.001$). Clinically, azithromycin was associated with greater reduction in IL-1β, IL-6, TNF-α, and CRP, with shorter fever clearance time (121.86 ± 16.58 vs. 134.73 ± 23.64 hours; $p < 0.001$). Treatment failure rates did not differ significantly between groups.

Conclusion: S. Paratyphi A immune evasion operates through transcriptional modulation, selectively attenuating NF-κB pathway activation to reduce antimicrobial gene program execution while sustaining inflammatory signaling. Azithromycin exposure was associated with more robust transcriptional reprogramming and superior bacterial clearance compared to fluoroquinolones. These findings support the role of antibiotic class-specific immunomodulation in the outcome of paratyphoid infection, independent of classical resistance phenotypes.

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Keywords: Salmonella Paratyphi A, innate immune evasion, gene regulation, NF-κB pathway, azithromycin, fluoroquinolone, intracellular survival, paratyphoid fever

1. Introduction

1.1. Global Burden and Clinical Significance of Paratyphoid Fever

Paratyphoid fever, caused primarily by *Salmonella enterica* serovar Paratyphi A, represents a significant yet comparatively understudied component of the enteric fever syndrome. Despite advances in water, sanitation, and hygiene infrastructure globally, enteric fever continues to impose a substantial disease burden across low- and middle-income countries, with paratyphoid fever accounting for a meaningful proportion of cases in several endemic regions [1]. The relative neglect of paratyphoid fever in vaccine development and surveillance programmes, in contrast to typhoid fever, has created critical gaps

in both epidemiological understanding and clinical management.

Enteric fever is a systemic infectious disease caused by host-restricted serovars of *Salmonella enterica* subspecies enterica, encompassing both typhoid fever (S. Typhi) and paratyphoid fever (S. Paratyphi A, B, and C) [2]. Salmonella Paratyphi A and *Salmonella* Typhi are distinguished from non-typhoidal *Salmonella* serovars by their strict host adaptation to humans, a characteristic that fundamentally underpins the transmission dynamics and epidemiology of enteric fever [3]. Critically, S. Paratyphi A lacks the Vi capsular antigen present in S. Typhi, a distinction with direct implications for serodiagnosis and vaccine design [4].

Geographically, paratyphoid fever caused by S. Paratyphi A is predominantly reported from South Asia, particularly India, Nepal, and Pakistan, as well as parts of Southeast Asia and China [5]. In the Kathmandu Valley of Nepal, paratyphoid fever has been documented to approach or exceed typhoid fever in incidence during certain surveillance periods [6]. Antimicrobial resistance represents an increasingly critical dimension of the enteric fever burden, with multidrug resistance (MDR) and reduced fluoroquinolone susceptibility documented in S. Paratyphi A across South Asian endemic settings [7].

1.2. Pathogen Biology and Virulence Determinants

Salmonella enterica serovar Paratyphi A belongs to the phylum Proteobacteria, order Enterobacterales, and family Enterobacteriaceae [8]. McClelland *et al*. [9] sequenced the genome of S. Paratyphi A strain ATCC 9150 and identified a chromosome of approximately 4.58 Mb encoding around 4,120 genes alongside a notable accumulation of pseudogenes. Comparative analysis with S. Typhi CT18 revealed that both organisms share a large proportion of their core genome but differ substantially in gene content relevant to host interaction.

Two type III secretion systems (T3SS) encoded within *Salmonella* Pathogenicity Islands SPI-1 and SPI-2 are most critical to the infectious cycle [10, 11]. SPI-1 mediates invasion of intestinal epithelial cells through translocation of effector proteins that drive cytoskeletal rearrangements and macropinocytic bacterial uptake. SPI-2, expressed primarily following internalisation into the *Salmonella*-containing vacuole (SCV), is essential for intracellular survival and systemic dissemination by preventing phagolysosomal maturation and redirecting vesicular trafficking [11, 12]. The PhoP/PhoQ two-component regulatory system plays a central role in sensing intraphagosomal signals and activating SPI-2 gene expression, thereby coupling environmental sensing to virulence gene induction [13].

1.3. Host Innate Immune Response and Immune Evasion

The innate immune response constitutes the first line of host defence against S. Paratyphi A, with pattern recognition receptors (PRRs) including Toll-like receptor 4 (TLR4) and TLR5 initiating signalling cascades culminating in pro-inflammatory gene transcription. TLR4 recognises the lipid A moiety of *Salmonella* lipopolysaccharide, driving NF-κB activation and production of TNF-α, IL-6, IL-1β, and CXCL8 [14, 15]. IL-1β, processed by caspase-1 following inflammasome activation, exerts synergistic pro-inflammatory effects and potentiates fever generation [16].

The macrophage oxidative burst, mediated by the NADPH oxidase complex, is a primary mechanism of intracellular bacterial killing. Salmonella actively subverts this process through SPI-2-dependent mechanisms that suppress the NADPH oxidase complex at the SCV membrane and prevent phagolysosomal maturation [17, 18]. Modifications to lipid A structure mediated by PhoP/PhoQ-regulated enzymes reduce TLR4-stimulatory potency, providing additional immune attenuation during systemic infection [19].

1.4. Host Gene Regulation During Infection

The transcriptional response of host innate immune cells to *Salmonella* infection is initiated by NF-κB-dependent gene activation and unfolds in temporally distinct waves governed by overlapping regulatory circuits. NF-κB dimers bind to κB-response elements in the promoters of cytokine genes including TNF, IL6, IL1B, and IL8, driving rapid transcriptional activation whose kinetics are modulated by feedback inhibition through re-synthesis of IκBα [15, 20]. Epigenetic mechanisms including histone acetylation (H3K27ac), histone methylation (H3K4me3), and chromatin accessibility govern the permissiveness of gene promoters to transcription factor binding, setting thresholds for innate immune transcriptional response [21].

Studies of peripheral blood transcriptomics in naturally acquired typhoid fever by Ramachandran *et al*. [22] demonstrated suppression of T cell receptor signalling pathways and upregulation of neutrophil-associated gene expression modules during acute disease, with quantitative differences in interferon pathway activation magnitude when comparing S. Typhi and S. Paratyphi A patients. However, systematic characterisation of longitudinal host transcriptional responses specifically during S. Paratyphi A infection, and the differential effects of antibiotic class on transcriptional reprogramming, remained insufficiently characterised prior to the present investigation.

1.5. Antibiotic Therapy: Mechanisms of Action and Immunomodulatory Effects

Fluoroquinolones exert bactericidal activity through inhibition of DNA gyrase and topoisomerase IV, with reduced fluoroquinolone susceptibility mediated primarily by point mutations in the quinolone resistance-determining regions (QRDRs) of gyrA and parC now prevalent in S. Paratyphi A isolates across South Asia [23, 7]. Azithromycin inhibits bacterial protein synthesis through binding to the 23S ribosomal RNA, with marked intracellular accumulation achieving concentrations several hundred-fold greater than extracellular levels, making it pharmacologically relevant for infection of the mononuclear phagocyte system [24]. Beyond direct antimicrobial activity, azithromycin has been shown to exert anti-inflammatory effects through modulation of the p38 MAPK pathway and inhibition of the non-canonical inflammasome, reducing TNF-α and IL-1β production in human monocyte models [25, 26]. In contrast, fluoroquinolones have been reported to paradoxically upregulate pro-inflammatory cytokine expression in infected macrophage models [27].

1.6. Research Gaps and Study Objective

Despite significant advances in understanding S. Paratyphi A pathogenesis, the current literature remained fragmented across host biology, bacterial regulation, and antimicrobial response. Longitudinal host regulatory dynamics, the relationship between NF-κB pathway enrichment and intracellular bacterial burden, the differential transcriptional

reprogramming associated with antibiotic class exposure, and the independence of these effects from classical antimicrobial resistance phenotypes had not been comprehensively investigated. The present study was therefore designed to investigate gene regulatory mechanisms associated with innate immune evasion during S. Paratyphi A infection, and to evaluate the differential immunomodulatory effects of azithromycin versus fluoroquinolone exposure on host transcriptional activation, intracellular bacterial clearance, and systemic inflammatory resolution.

2. Materials and Methods

2.1. Study Design and Overview

This investigation employed a dual-tiered, integrative research design combining mechanistic laboratory experimentation with a prospective comparative clinical cohort study. The study was structured around three discrete but interrelated objectives: (1) elucidation of innate immune evasion mechanisms and intracellular survival kinetics; (2) characterisation of host–pathogen gene regulatory networks and transcriptional modulation; and (3) assessment of the systemic immunomodulatory effects of antibiotic therapy.

The first component-the Mechanistic Cohort-comprised 60 participants (30 per treatment arm) whose biological samples served as the substrate for *in vitro* immune evasion experiments and transcriptomic profiling. The second component-the Clinical Immunomodulatory Cohort-comprised 320 participants (160 per treatment arm) enrolled in a prospective, parallel-arm, randomised comparison of azithromycin versus fluoroquinolone therapy, with serial collection of systemic inflammatory biomarkers and clinical outcome data.

2.2. Study Setting and Ethical Approval

Clinical sample collection was performed in the infectious disease ward of a designated tertiary-care hospital. All microbiological experiments involving viable S. Paratyphi A were conducted in a Biosafety Level 2 (BSL-2) certified laboratory in compliance with WHO Laboratory Biosafety Manual guidance and institutional biosafety committee approval. The study protocol was approved by the relevant institutional review board in accordance with the Declaration of Helsinki [28]. Written informed consent was obtained from all participants prior to enrollment.

2.3. Inclusion and Exclusion Criteria

Participants were eligible if they were aged ≥ 18 years, had laboratory-confirmed paratyphoid fever by blood culture isolation of S. Paratyphi A, presented within 10 days of fever onset, and provided informed consent. Exclusion criteria included prior antibiotic therapy exceeding 48 hours, known hypersensitivity to study antibiotics, concurrent immunosuppressive therapy or immunodeficiency, pregnancy, severe organ dysfunction, co-infection with another confirmed pathogen, and participation in another clinical trial within 30 days.

2.4. Sample Size Determination

Sample size for the mechanistic cohort was based on a two-arm parallel comparison with significance level $\alpha = 0.05$, statistical power 80%, and a standardised effect size (Cohen's d) of 0.8, yielding a minimum of approximately 25 participants per group; this was increased to 30 per group (total n = 60) to accommodate biological variability inherent to primary human cell preparations. For the clinical cohort, using a conservative effect size of d = 0.33 and applying a 5–7% attrition correction, a final enrollment target of 320 participants (160 per arm) was determined.

2.5. Biological Materials and Sample Collection

All microbiological experiments used clinical isolates of S. Paratyphi A obtained from blood cultures of enrolled participants, confirmed by standard biochemical identification and serotyping using *Salmonella* antisera. Peripheral venous blood was collected at Day 0 (baseline, prior to antibiotic administration) and Day 7. Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation using Ficoll-Paque PLUS. Monocytes were differentiated into macrophages over 7 days using 50 ng/mL recombinant human macrophage colony-stimulating factor (rhM-CSF). Differentiation was confirmed by CD14+/CD16+/CD68+ surface marker expression.

2.6. In Vitro Infection and Intracellular Survival Assay

Human monocyte-derived macrophages were infected with S. Paratyphi A clinical isolates at a multiplicity of infection (MOI) of 10:1 using a standardised gentamicin protection assay. Following 30-minute phagocytosis, extracellular bacteria were eliminated by gentamicin treatment (100 µg/mL for 1 hour, then 20 µg/mL). At 0, 24, and 48 hours post-infection, cells were lysed with 0.1% Triton X-100 and lysates were serially diluted and plated on Luria-Bertani agar in triplicate. Colony-forming units (CFU) were counted after 18–24 hours at 37°C and expressed as CFU per 10^5 cells.

2.7. Cytokine Profiling and Functional Immune Assays

Pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) were quantified from infected cell culture supernatants at 24 hours post-infection using validated ELISA kits. Intracellular reactive oxygen species (ROS) generation was quantified using the 2′,7′-dichlorofluorescein diacetate (DCFH-DA) fluorescence assay at excitation/emission wavelengths of 485/530 nm, normalised to protein content by BCA assay. Phagocytic index was determined using IgG-opsonized fluorescent latex beads assessed across a minimum of 200 cells per condition.

2.8. Transcriptomic Analysis

Total RNA was extracted from infected and uninfected macrophages using TRIzol reagent with column-based purification. RNA quality was confirmed by BioAnalyzer (minimum RNA Integrity Number of 7.0). Dual RNA-sequencing was performed on the Illumina NovaSeq 6000 platform (150 bp paired-end reads), simultaneously capturing host and pathogen transcriptomes. Differential gene expression analysis was conducted using DESeq2 in R/Bioconductor, with significance defined as $|\log_2 \text{fold change}| \geq 1.0$ and FDR-adjusted p-value < 0.05. NF-κB pathway enrichment was scored using normalised enrichment scores (NES) derived from Gene Set Enrichment Analysis (GSEA).

Key immune regulatory genes (IL1B, TNFA, NFKB1, TLR4) were validated by quantitative reverse-transcription

PCR (qRT-PCR) using the $2^{-}\Delta\Delta Ct$ method normalised to reference genes GAPDH and ACTB.

2.9. Antibiotic Treatment and Clinical Assessment

Participants in the clinical cohort were allocated 1:1 to azithromycin (Group A) or fluoroquinolone therapy (Group B) using computer-generated block randomisation stratified by MDR status and age group. Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion and broth microdilution in accordance with CLSI standards. MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial categories [29]. Resistance to nalidixic acid served as a surrogate marker for reduced fluoroquinolone susceptibility.

Systemic inflammatory biomarkers (CRP, IL-1β, IL-6, TNF-α, WBC) were measured at Day 0 and Day 7, with change scores (Δ = Day 7 − Day 0) calculated as the primary outcome. Fever clearance time was defined as the number of hours from antibiotic initiation to the first temperature ≤ 37.5°C sustained for a minimum of 24 hours without antipyretic administration. Treatment failure was defined as persistent fever beyond Day 7 with positive blood culture or requirement for antibiotic change.

2.10. Statistical Analysis

Intergroup comparisons of continuous normally distributed variables were performed using independent samples t-tests; Mann-Whitney U tests were used for non-normally distributed data; chi-square tests for categorical variables. Multiple linear regression was used to identify independent predictors of IL1B $\log_2$ fold change. Binary logistic regression was employed for binary outcomes including high-DEG phenotype and treatment failure. Bivariate correlations were computed using Pearson's r or Spearman's ρ as appropriate. All analyses were conducted using IBM SPSS Statistics version 23.0 and R/Bioconductor, with a significance threshold of $\alpha = 0.05$ (two-tailed). FDR correction was applied using the Benjamini-Hochberg procedure.

3. Results

3.1. Intracellular Bacterial Survival Kinetics

A progressive decline in intracellular colony-forming units (CFU) was observed from baseline (0 h) to 48 h post-infection across the total mechanistic cohort (n = 60; mean CFU: 100,261 → 48,255 → 22,781). Despite this reduction, substantial bacterial persistence at 48 hours indicated incomplete macrophage-mediated clearance. The fluoroquinolone group demonstrated significantly higher intracellular bacterial loads at both time points compared to the azithromycin group (Table 1).

Table 1: Intracellular CFU at 24 h and 48 h by Treatment Group

Time Point	Azithromycin (Mean ± SD)	Fluoroquinolone (Mean ± SD)	p-value
CFU 24 h	39,815 ± 8,359	56,695 ± 8,572	< 0.001
CFU 48 h	16,327 ± 4,876	29,236 ± 4,514	< 0.001

The mean difference in intracellular CFU at 48 hours between treatment groups exceeded 12,000 CFU, with a narrow confidence interval confirming a robust effect size. Figure 1 depicts the intracellular bacterial survival kinetics by treatment group.

3.2. Pro-Inflammatory Cytokine Response

Cytokine quantification at 24 hours post-infection revealed significantly elevated levels of IL-1β, IL-6, and TNF-α in the fluoroquinolone group compared to the azithromycin group (Table 2). The significantly heightened cytokine response in the fluoroquinolone-treated samples corresponded with their higher intracellular bacterial burden, suggesting that inflammatory signalling intensity is proportional to pathogen persistence rather than effective bacterial clearance.

3.3. Reactive Oxygen Species and Phagocytic Function

Assessment of functional innate immune responses demonstrated significantly higher ROS generation and phagocytic index in the azithromycin group compared to the fluoroquinolone group (Table 3). Enhanced oxidative burst and phagocytic capacity were strongly associated with reduced intracellular bacterial load. The fluoroquinolone group exhibited diminished ROS production and impaired phagocytic index, potentially facilitating bacterial persistence.

3.4. Correlation Between Bacterial Load and Immune Activation

Correlation analysis demonstrated a strong positive relationship between intracellular bacterial load at 48 hours and IL-1β expression ($r = 0.556$; $p < 0.001$) and IL1B $\log_2$ fold change ($r = 0.625$; $p < 0.001$). Critically, an inverse correlation was identified between CFU burden at 48 hours and NF-κB pathway enrichment score ($r = -0.656$; $p < 0.001$), indicating that higher NF-κB pathway enrichment is associated with lower intracellular bacterial burden.

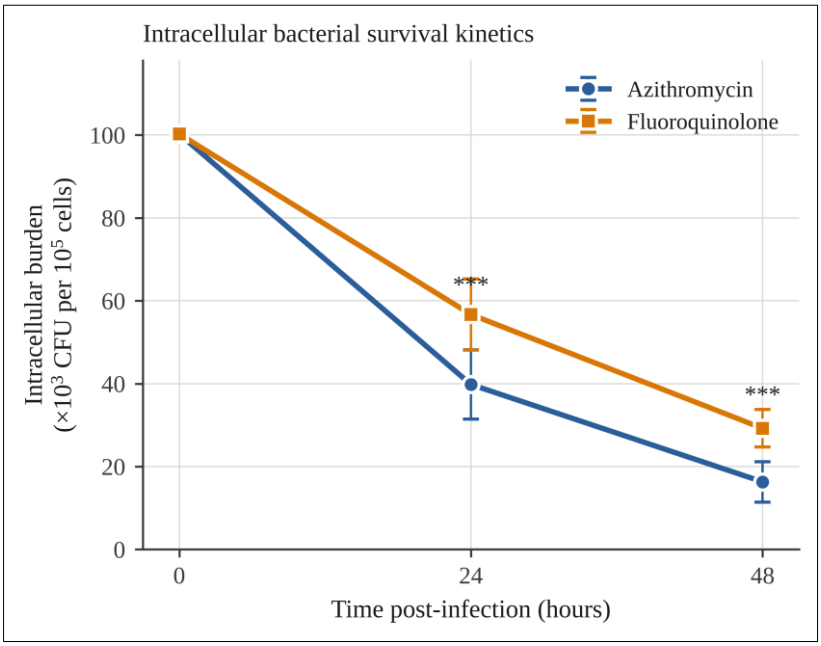

Fig 1: Intracellular Bacterial Survival Kinetics by Treatment Group.

Table 2: Cytokine Levels (IL-1β, IL-6, TNF-α) at 24 Hours by Treatment Group

Cytokine	Azithromycin (Mean ± SD)	Fluoroquinolone (Mean ± SD)	p-value
IL-1β (pg/mL)	124.13 ± 18.69	162.30 ± 22.38	< 0.001
IL-6 (pg/mL)	91.46 ± 16.48	118.99 ± 23.41	< 0.001
TNF-α (pg/mL)	95.38 ± 20.81	137.85 ± 20.91	< 0.001

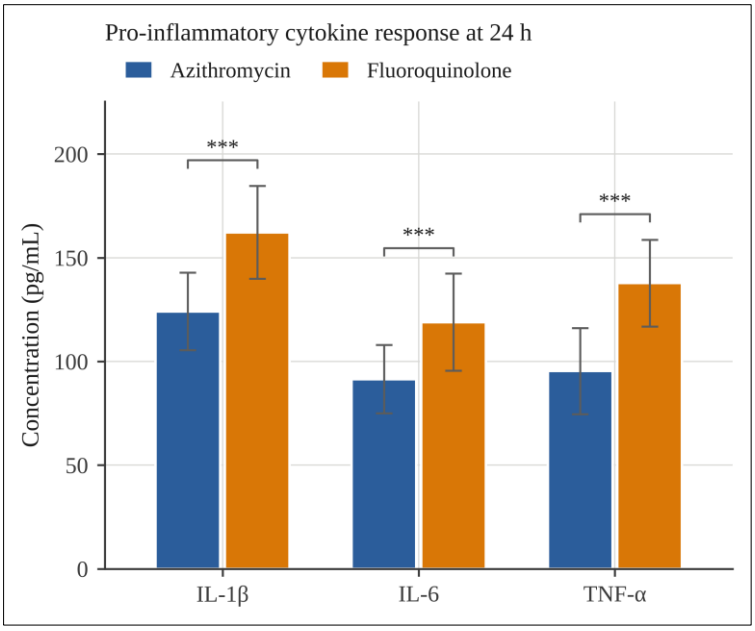

Fig 2: Comparison of Pro-Inflammatory Cytokine Levels (IL-1β, IL-6, TNF-α) at 24 Hours by Treatment Group.

Table 3: Reactive Oxygen Species (ROS) Levels and Phagocytosis Index by Treatment Group

Parameter	Azithromycin (Mean ± SD)	Fluoroquinolone (Mean ± SD)	p-value
ROS Level (RFU)	2.76 ± 0.37	2.19 ± 0.32	< 0.001
Phagocytosis Index	1.61 ± 0.18	1.33 ± 0.22	< 0.001

3.5. Differential Gene Expression Patterns

Log₂ fold change (log_2FC) analysis of key immune regulatory genes revealed distinct transcriptional suppression patterns between treatment groups. The azithromycin group demonstrated greater transcriptional downregulation (more negative log_2FC values) across all evaluated genes, whereas the fluoroquinolone group showed relatively attenuated suppression, consistent with sustained transcriptional activity associated with greater bacterial burden (Table 4).

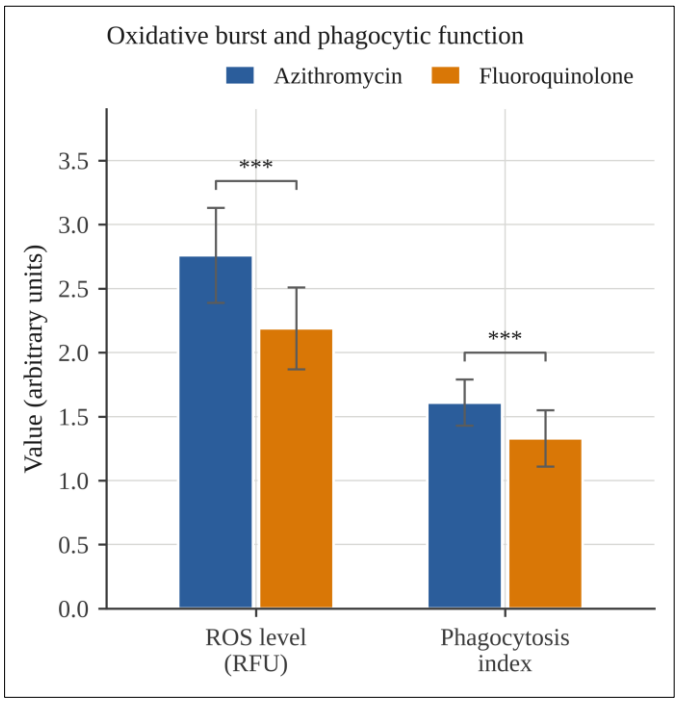

Fig 3: Reactive Oxygen Species (ROS) Levels and Phagocytosis Index by Treatment Group.

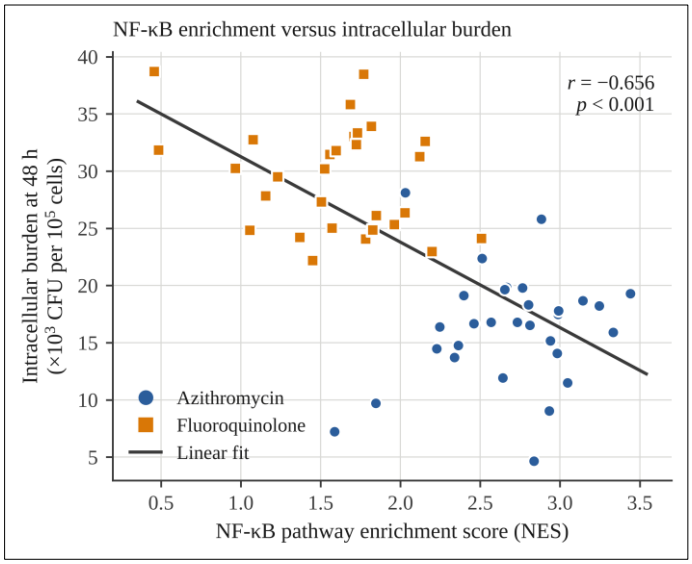

Fig 4: Association Between NF-κB Pathway Enrichment Score and Intracellular Bacterial Load at 48 Hours.

Table 4: Gene Expression (log_2 Fold Change) by Treatment Group

Gene	Azithromycin (Mean ± SD)	Fluoroquinolone (Mean ± SD)	p-value
IL1B	−1.006 ± 0.281	−0.359 ± 0.301	< 0.001
TNFA	−0.836 ± 0.250	−0.234 ± 0.331	< 0.001
NFKB1	−0.915 ± 0.314	−0.526 ± 0.280	< 0.001
TLR4	−0.759 ± 0.303	−0.291 ± 0.263	< 0.001

3.6. NF-κB Pathway Enrichment and Global Transcriptional Burden

Significant intergroup differences were observed in total differentially expressed genes (DEGs) and NF-κB pathway enrichment scores (Table 5). The azithromycin group demonstrated significantly higher total DEGs and stronger NF-κB pathway enrichment, suggesting broader transcriptional reprogramming and more coordinated immune activation. The fluoroquinolone group exhibited reduced global transcriptional responsiveness, consistent with pathogen-driven dampening of transcriptional networks required for effective intracellular clearance.

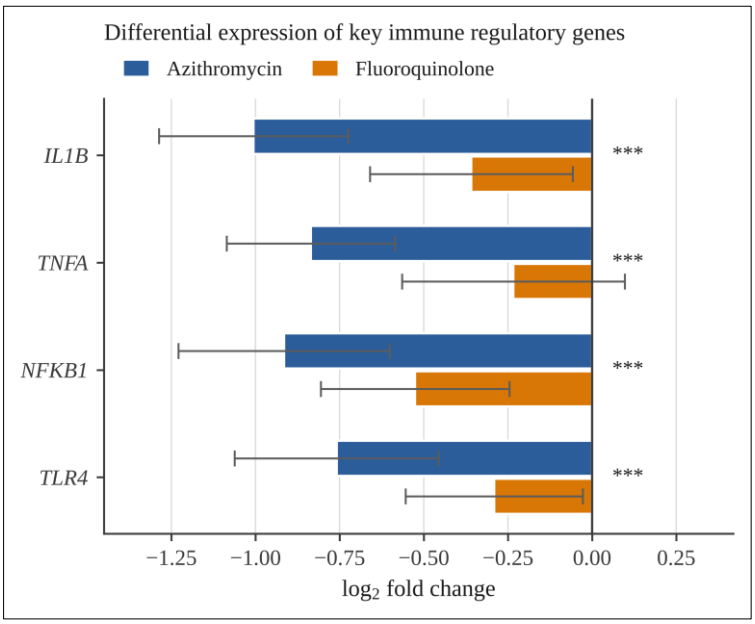

Fig 5: Differential Gene Expression (log_2 Fold Change) of IL1B, TNFA, NFKB1, and TLR4 by Treatment Group.

Table 5: Total Differentially Expressed Genes (DEGs) and NF-κB Pathway Enrichment Score by Treatment Group

Parameter	Azithromycin (Mean ± SD)	Fluoroquinolone (Mean ± SD)	p-value
Total DEGs	851 ± 127	627 ± 94	< 0.001
NF-κB Score (NES)	2.68 ± 0.42	1.59 ± 0.46	< 0.001

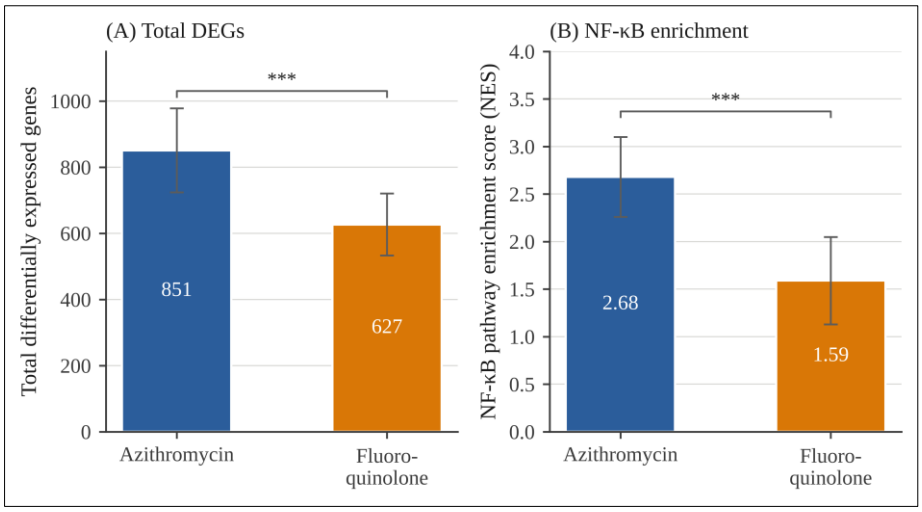

Fig 6: Comparison of Total Differentially Expressed Genes (DEGs) and NF-κB Pathway Enrichment Score by Treatment Group.

3.7. Predictors of IL1B Transcriptional Modulation

Multiple linear regression analysis was performed with IL1B log_2FC as the dependent variable and treatment group, MDR status, nalidixic acid resistance, and age as predictors. The model demonstrated good overall fit ($R = 0.771$; $R^2 = 0.595$; adjusted $R^2 = 0.566$; $F(4,55) = 20.211$; $p < 0.001$), with treatment group emerging as the sole statistically significant predictor ($\beta = 0.769$; $p < 0.001$). MDR status, nalidixic acid resistance, and age were not statistically significant predictors, indicating that transcriptional modulation operates independently of classical resistance phenotypes.

3.8. High Differential Expression Phenotype and Logistic Modelling

A high-DEG phenotype (≥ 701 DEGs) was defined to identify transcriptionally active immune states. Binary logistic regression demonstrated that treatment group was the only significant predictor of high-DEG phenotype (Omnibus $\chi^2 = 29.581$; $p < 0.001$; Nagelkerke $R^2 = 0.520$; Exp(B) = 0.035; $p < 0.001$). Classification accuracy was 83.3%. The odds ratio indicates that fluoroquinolone exposure was associated with markedly reduced likelihood of achieving high transcriptional activation compared to azithromycin.

3.9. Overall Clinical Cohort Characteristics

The clinical cohort (n = 320) demonstrated substantial baseline inflammatory activation (CRP: 69.27 ± 15.56 mg/L; IL-1β: 178.52 ± 28.99 pg/mL; IL-6: 64.55 ± 13.41 pg/mL; TNF-α: 169.29 ± 25.68 pg/mL; Table 6). MDR prevalence was 60.3%, and nalidixic acid resistance was 76.6%. Baseline demographic, clinical, and inflammatory parameters did not significantly differ between treatment groups (all $p > 0.05$), confirming comparability prior to treatment allocation.

Table 6: Overall Baseline Characteristics of the Study Cohort (n = 320)

Variable	Mean ± SD or Prevalence
Age (years)	39.44 ± 12.44
BMI (kg/m²)	21.93 ± 4.51
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 (%)	60.3%
Nalidixic Acid Resistance (%)	76.6%

3.10. Inflammatory Resolution and Clinical Outcomes

Azithromycin therapy resulted in significantly greater reductions in CRP, IL-1β, IL-6, and TNF-α between Day 0 and Day 7 compared to the fluoroquinolone group (all $p < 0.001$; Table 7). The magnitude of IL-1β reduction was approximately 50% greater with azithromycin (ΔIL-1β: −58.12 ± 11.69 vs. −39.60 ± 10.21 pg/mL). White blood cell count reduction did not differ significantly between groups ($p = 0.736$). Mean fever clearance time was significantly shorter in the azithromycin group (121.86 ± 16.58 vs. 134.73 ± 23.64 hours; $p < 0.001$; Table 8).

Table 7: Change in Inflammatory Markers (Day 0 to Day 7) by Treatment Group

Marker	Azithromycin (Mean ± SD)	Fluoroquinolone (Mean ± SD)	p-value
Δ CRP (mg/L)	−37.14 ± 9.30	−31.39 ± 8.57	< 0.001
Δ IL-1β (pg/mL)	−58.12 ± 11.69	−39.60 ± 10.21	< 0.001
Δ IL-6 (pg/mL)	−31.03 ± 7.46	−23.76 ± 6.01	< 0.001
Δ TNF-α (pg/mL)	−46.70 ± 10.35	−30.26 ± 8.42	< 0.001
Δ WBC (cells/μL)	−1,616 ± 456	−1,599 ± 473	0.736

Table 8: Fever Clearance Duration by Treatment Group

Parameter	Azithromycin (Mean ± SD)	Fluoroquinolone (Mean ± SD)	p-value
Fever Clearance (hours)	121.86 ± 16.58	134.73 ± 23.64	< 0.001

Treatment failure rates did not significantly differ between groups (5.6% vs. 8.1%; $p = 0.377$). Overall relapse rate at 30 days was 2.8% and adverse event rate was 6.3% across the full cohort. Multivariable logistic regression confirmed that no evaluated variable-including treatment group (OR: 1.476; 95% CI: 0.603–3.611; $p = 0.393$), age, MDR status, or nalidixic acid resistance-independently predicted treatment failure.

4. Discussion

4.1. Intracellular Persistence and Immune Evasion

The persistence of viable intracellular bacteria at 48 hours post-infection, despite active immune engagement in both treatment groups, reflects a classical immune evasion phenotype consistent with the established capacity of typhoidal *Salmonella* serovars to subvert macrophage microbicidal mechanisms. The significantly higher CFU levels observed in the fluoroquinolone-exposed group suggest that differential modulation of host–pathogen interaction, rather than direct antimicrobial effect alone, determines intracellular bacterial burden. This is consistent with the established pharmacological property of azithromycin to accumulate at intracellular concentrations several hundred-fold greater than extracellular levels, potentially enhancing its activity against intra-macrophage *Salmonella* [24].

The observation of a dissociation between inflammatory cytokine activation and bactericidal efficiency in the fluoroquinolone group is mechanistically significant. Elevated IL-1β, IL-6, and TNF-α in the setting of greater bacterial burden suggests that S. Paratyphi A triggers sufficient inflammatory signalling to sustain host cellular responses while avoiding sterilising immunity. This pattern is consistent with SPI-2-mediated suppression of the NADPH oxidase complex at the SCV membrane described in the *Salmonella* pathogenesis literature, which prevents the conversion of inflammatory signalling into effective bactericidal oxidative burst [17]. The reduced ROS generation and impaired phagocytic index observed in the fluoroquinolone group further support this mechanistic interpretation. These results are consistent with evidence from Anuforom *et al*. [27] demonstrating that ciprofloxacin pretreatment in macrophage models does not necessarily enhance bactericidal function, even while altering cytokine profiles.

4.2. Transcriptional Modulation and NF-κB Regulation

The inverse correlation between NF-κB pathway enrichment score and intracellular bacterial burden ($r = −0.656$; $p < 0.001$) constitutes one of the principal findings of this study, providing quantitative evidence that insufficient transcriptional pathway activation correlates with immune escape by S. Paratyphi A. The higher total DEG count and NF-κB enrichment scores in the azithromycin group indicate that robust transcriptional reprogramming is associated with more effective bacterial control, while the attenuated enrichment in the fluoroquinolone group may represent pathogen-driven dampening of the transcriptional networks required for intracellular clearance.

These findings are conceptually consistent with evidence from transcriptomic studies of typhoidal *Salmonella* infection demonstrating active suppression of NF-κB and MAPK signalling cascades, potentially mediated by T3SS effectors targeting host kinases and ubiquitin ligases [30]. The differential $\log_2$FC suppression observed for IL1B, TNFA, NFKB1, and TLR4 in the azithromycin group suggests that azithromycin-associated reduction in bacterial burden permits a qualitatively different transcriptional resolution compared to the sustained inflammatory activation observed with fluoroquinolone exposure. This is supported by evidence that azithromycin modulates the p38 MAPK pathway, reducing TNF-α production through mechanisms distinct from canonical NF-κB inhibition [25].

The regression analysis identifying treatment group as the dominant predictor of IL1B transcriptional modulation ($\beta = 0.769$; $p < 0.001$), with MDR status, nalidixic acid resistance, and age contributing non-significantly, provides important evidence that transcriptional regulation during S. Paratyphi A

infection operates primarily through the host–pathogen interaction context rather than through classical resistance determinants. This dissociation between immune evasion mechanisms and conventional antimicrobial resistance phenotypes has important implications for the clinical interpretation of resistance surveillance data.

4.3. Antibiotic Immunomodulation and Clinical Outcomes

The greater cytokine resolution observed with azithromycin therapy in the clinical cohort-particularly the approximately 50% greater reduction in IL-1β compared to fluoroquinolone therapy-aligns with the established anti-inflammatory properties of macrolide antibiotics. Evidence from human monocyte models demonstrates that azithromycin impairs caspase-4 activation through non-canonical inflammasome inhibition, thereby reducing IL-1β secretion without completely compromising NF-κB-driven pathogen recognition signalling [26]. The accelerated fever clearance associated with azithromycin (approximately 13 hours shorter mean duration) parallels the greater cytokine decline, consistent with the role of IL-1β-mediated prostaglandin E2 induction in hypothalamic fever generation [16].

The absence of a significant difference in binary treatment failure between groups ($p = 0.377$), despite substantial differences in inflammatory resolution kinetics, indicates that although antibiotic class influenced the quality and velocity of immune resolution, neither regimen was decisively superior in achieving clinical cure within the study time frame. This observation is consistent with evidence that the factors determining treatment failure in enteric fever extend beyond direct antimicrobial activity and include host immune capacity, inoculum size, and bacteraemia density [23]. The lack of independent predictive value for MDR status or nalidixic acid resistance in the treatment failure model (Nagelkerke $R^2 = 0.038$) further suggests that clinical outcome in this cohort was not primarily driven by resistance phenotype.

4.4. Study Limitations

This study was conducted at a single study centre, and findings regarding antimicrobial resistance prevalence and immune response patterns may not be directly generalisable to populations with different demographic profiles or circulating strain genotypes. The open-label clinical design, necessitated by the nature of oral antibiotic regimens, introduced potential performance bias, partially mitigated by blinding of laboratory personnel to treatment allocation during immunological analysis. The inherent inter-individual variability of primary human PBMC preparations represents a limitation of the mechanistic cohort, addressed through adequate sample size and mixed-effects modelling. The abundance imbalance between host and bacterial RNA in infected macrophages may have limited pathogen-side transcriptome depth in some samples, mitigated by minimum bacterial read depth thresholds.

5. Conclusion

This study provides quantitative evidence that S. Paratyphi A immune evasion operates through selective modulation of host transcriptional amplitude, attenuating NF-κB pathway activation to reduce effective antimicrobial gene program execution while sustaining inflammatory signalling. The strong inverse relationship between NF-κB pathway enrichment and intracellular bacterial burden demonstrates that insufficient transcriptional pathway activation is a correlate of immune escape rather than its consequence. Azithromycin exposure was associated with more robust transcriptional reprogramming, superior ROS generation, higher phagocytic index, and lower intracellular bacterial burden compared to fluoroquinolone exposure, alongside greater systemic inflammatory resolution and shorter fever clearance time in the clinical cohort. These immunological differences operated independently of classical antimicrobial resistance phenotypes, with MDR status and nalidixic acid resistance failing to independently predict transcriptional modulation or treatment failure. The findings support consideration of antibiotic class-specific immunomodulatory properties in the therapeutic management of paratyphoid fever, and identify NF-κB pathway enrichment as a potential molecular correlate of effective innate immune response to S. Paratyphi A infection.

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Author Contributions

Hirdesh Gour: Writing – original draft; Investigation; Analysis; Visualization.

Arpit Bhargava: Supervision; Investigation; Review and editing.

Conflict of Interest Statement

The authors declare no conflict of interest.

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