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Plasma concentration of azithromycin and correlation with clinical outcomes in patients with enteric fever

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Background: Azithromycin is the only oral option available for XDR enteric fever. Studies correlating azithromycin levels with treatment success are rare.

Methods: Serum azithromycin levels after administration of a once-daily 20 mg/kg dose for 7 days were measured in a single-centre prospective cohort of 25 consecutive adults with blood culture-positive enteric fever. Five blood samples were collected on Day 2 after starting azithromycin, i.e. 30 min before dosing (trough), and 2, 5, 12 and 24 h after dosing. The MIC was determined for all isolates and azithromycin plasma concentration was determined using LC-MS. Clinical and microbiological outcomes were documented.

Results: *Salmonella enterica* serovar Typhi accounted for 92% ($n=23$) and *Salmonella enterica* serovar Paratyphi 8% ($n=2$). Ten (40%) patients received IV azithromycin, and the rest received oral therapy. The median (IQR, range) MIC for azithromycin was 4 (4–6, 3–12) mg/L. Mean azithromycin plasma concentration ranges were: trough, 0.24 ± 0.19 mg/L; 2 h, 1.24 ± 0.98 mg/L; 5 h, 0.64 ± 0.51 mg/L; 12 h, 0.31 ± 0.16 mg/L; and 24 h, 0.37 ± 0.30 mg/L. The C_{max}/MIC and AUC/MIC for azithromycin were 0.29 ± 0.22 and 2.64 ± 1.64, respectively. The median (IQR, range) fever clearance time was 3 (2–3, 2–5) days and the length of hospital stay was 7 (5.5–12, 4–16) days. There was no clinical or microbiological failure, relapse or mortality.

Conclusions: Azithromycin was effective in treatment of enteric fever, despite low extracellular azithromycin plasma levels.

Introduction

Enteric fever remains a major community-acquired bacteraemic illness in tropical low-resource settings. Typhoidal *Salmonella* strains (*Salmonella enterica* serovar Typhi, Paratyphi A, Paratyphi B and Paratyphi C) are human host-restricted organisms causing typhoid fever and paratyphoid fever, collectively referred to as enteric fever. Increased resistance to commonly available oral agents has been reported over the last two decades. With emergence of MDR, defined as resistance to ampicillin, chloramphenicol and co-trimoxazole, and decreased susceptibility to fluoroquinolones, oral drugs available to treat enteric fever are scarce.[1] Fluoroquinolones were considered as the most effective treatment for typhoid fever in the 1990s, but emergence of nalidixic acid-resistant *S.* Typhi and microbiological failure to ciprofloxacin has led to decreased treatment options.[2,3]

The WHO recommends ceftriaxone and azithromycin as the drugs of choice in the treatment of invasive *S.* Typhi infection. Although ceftriaxone is commonly used for the treatment of MDR *S.* Typhi infection, the cost, parenteral administration and recently reported ESBL production are major limitations, particularly as most enteric fever patients are treated as outpatients in low- and middle-income countries.[4,5] Hence, azithromycin is increasingly used as a well-tolerated, safe and efficacious oral antibiotic in the treatment of uncomplicated typhoid fever in Southeast Asia.[6]

Azithromycin, an azalide macrolide antibiotic, has unique pharmacokinetic and pharmacodynamic (PK/PD) properties compared with other macrolides due to its dibasic structure. It has time-dependent bacteriostatic or bactericidal activity, along with concentration-dependent bactericidal activity.[7] Azithromycin is distributed extensively in various tissues and body fluids with a long serum half-life.[8] The drug concentrates in WBCs and the WBC/plasma ratio is high, even at the end of active treatment, resulting in a prolonged post-antibiotic effect.[9] Azithromycin achieves more than 100-fold higher concentration in the macrophages and leucocytes when compared with serum, with excellent tissue penetration.[10,11] With *S.* Typhi being a predominantly intracellular organism, using azithromycin, which has suitable pharmacokinetic properties and a high intracellular concentration, makes intuitive sense.

Consequently, azithromycin achieves a low plasma (extracellular) concentration, secondary to uptake by fibroblasts and cells like polymorphonuclear leucocytes (PMNLs), monocytes and lymphocytes.[8] The EUCAST (v 14.0)[12] and CLSI 2024 guidelines publish an azithromycin MIC breakpoint of ≤16 mg/L for WT *S.* Typhi isolates, which is extrapolated for use with *S.* Paratyphi isolates.[13,14] However, as the drug concentrates more in tissues and intracellular compartments, the role of the MIC in predicting clinical outcomes becomes questionable. Further, there are concerns that up to one-third of *S.* Typhi may be found in the extracellular compartment and these low levels of azithromycin in plasma may lead to resistance. *Salmonella* isolates with increased azithromycin MICs have been reported from India and in returning travellers.[15] Cases with probable azithromycin resistance and clinical failure have also been reported from the Indian subcontinent and South Asia.[16–18] Mechanisms responsible for azithromycin resistance are the presence of resistance genes (*acrB*, *acrR* and *rplV* mutations) or acquisition of macrolide efflux pump genes (*macA* and *macB*).[17–21] In the USA, *mph*(A) and related phosphotransferase genes are the most common cause of azithromycin resistance observed in *S.* Typhi.[22] Notably, azithromycin-resistant *S.* Typhi isolates have been found to carry a non-synonymous single-point mutation, R717L/Q, in the *acrB* efflux pump gene, which has been reported in strains from Bangladesh, Pakistan, Nepal and India.[23] Presence of efflux pumps might be responsible for the poor clinical outcomes; however, this is not easily picked up via routine antibiotic susceptibility testing or MIC.

Reports of isolates from enteric fever patients with azithromycin MICs of >16 mg/L are emerging.[1,23,24] The PK/PD parameters predictive for the clinical efficacy of azithromycin in enteric fever have not been prospectively studied.[13,14] Moreover, optimal therapeutic extracellular plasma levels of azithromycin that achieve both clinical and microbiological cure remain largely unknown. This pilot study was performed to determine the PK of azithromycin and the role of extracellular plasma levels of azithromycin in predicting clinical and microbiological outcomes in adult patients with uncomplicated enteric fever in South India.

Materials and methods

The study population included a prospective cohort of 25 consecutive adults (>18 years old) admitted with blood culture-positive enteric fever to a tertiary care hospital in South India between August 2020 and March 2022. The study protocol was approved by the Institutional Review Board and Ethics committee of Christian Medical College, Vellore, India (IRB Min. No. 12777 dated 8 April 2020), and informed consent was obtained from the study participants. Patients unwilling to provide serial plasma samples ($n=3$) and those on combination therapy ($n=2$) were excluded. Patients who received initial active therapy other than azithromycin were included in the study, provided it was stopped prior to starting azithromycin monotherapy.

All 25 patients recruited to the study received the recommended dosing of 20 mg/kg once daily[25] oral or IV azithromycin for treatment of enteric fever for a total duration of 7 days. The route of drug administration (oral or IV) was determined by the treating physician based on the clinical condition of the patient. Dosing adjustments were made according to the following weight bands: patients weighing ≤60 kg received 1000 mg of azithromycin; those weighing 60–70 kg received 1250 mg; while those weighing ≥70 kg received 1500 mg.

Five serial blood samples were collected on Day 2 after the start of oral or IV azithromycin at intervals of 30 min before the azithromycin dose (trough), and at 2, 5, 12 and 24 h after the azithromycin dose. Blood samples were collected using K_2EDTA-coated blood collection tubes. Plasma was separated immediately and stored at −80°C until analysis. Plasma concentrations of azithromycin were estimated using a validated LC-MS/MS assay. To 100 µL of patient plasma, 25 µL of roxithromycin (internal standard) and 200 µL of 5% ammonium hydroxide (NH_4OH) were added. They were vortex mixed for 1 min and subsequently 1.5 mL of ethyl acetate was added. The mixture was centrifuged at 13 000 rpm for 5 min and 1400 µL of supernatant was evaporated and reconstituted with 200 µL of 30% methanol. Ten microlitres of the extracted solution was injected into the UPLC system and the analytes were separated using a Luna 5 µm PFP column of diameter 50×2.0 mm. The standards and internal quality controls were prepared from pooled human plasma samples. The calibration curve was linear and was in the range of 0.01–2 mg/L. The low, medium and high quality controls were 0.04, 0.5 and 1.5 mg/L, respectively.

Multiple reaction monitoring (MRM) of the ions, 749.6→158.4 and 837.7→158.3 for azithromycin and roxithromycin, respectively, were used for quantification. Non-compartmental analysis was performed by manual curve stripping. AUC was determined using the trapezoid method. The study outcomes assessed were fever clearance time (FCT), clinical cure, clinical failure, microbiological failure, relapse, complications and PK parameters of azithromycin. Follow-up blood cultures were done after completion of 7 days of antibiotic therapy.

Definitions

C_{max} was defined as the maximum (or peak) serum concentration that azithromycin achieved in plasma after it was administered and before the administration of a second dose.[26]

MIC was defined as the lowest concentration of an antibacterial agent which, under strictly controlled *in vitro* conditions, completely prevented visible growth of the test strain of an organism.[27] MIC was determined for all *S.* Typhi and *S.* Paratyphi isolates by means of ETEST.

AUC_{24}/MIC was defined as the AUC over 24 h divided by the MIC.[26]

FCT was defined as from the start of appropriate antibiotic therapy to the first instance when the oral temperature dropped below 37.5°C and remained below this level continuously for 48 h.

Clinical cure was defined as complete and sustained resolution of fever and other clinical symptoms and signs. Clinical failure was defined as the persistence of fever for >6 days.

Microbiological failure was defined as isolation of *S.* Typhi or *S.* Paratyphi from blood or bone marrow cultures in patients who had been on treatment for enteric fever for >7 days. Relapse was defined as recurrence of clinical symptoms and signs compatible with enteric fever within 6 weeks after completion of a course of antibiotics for this

disease, after having had complete resolution of symptoms and signs during the same treatment episode.

Results

In our study, 25 cases with blood culture-positive uncomplicated enteric fever were included. *S.* Typhi accounted for 92% ($n=23$) of cases while 2 (8%) cases had *S.* Paratyphi. The median (IQR, range) duration of illness was 10 (5–21, 2–45) days. Azithromycin therapy was started within 24–48 h after the blood culture flagged positive for enteric fever. Ten (40%) patients received IV azithromycin while 15 (60%) received oral therapy only. The median (IQR, range) dose of azithromycin that patients received was 1000 mg (1000–1250, 1000–1500) mg. Five (20%) patients received active therapy prior to azithromycin monotherapy for a median (IQR, range) duration of 3 (2–3, 2–4) days. Three (12%) patients received ceftriaxone and 2 (8%) patients received co-trimoxazole as prior active therapy, all of which were susceptible *in vitro* (Table 1). Median (IQR, range) MIC of azithromycin was 4 (4–6, 3–12) mg/L (Table 1). For *S.* Typhi, the azithromycin MIC ranged from 3 to 8 mg/L, with the MIC_{50} and MIC_{90} at 4 and 8 mg/L, respectively. In contrast, the azithromycin MICs for the two *S.* Paratyphi isolates were 6 and 12 mg/L.

Table 1. Demographic, clinical and microbiological characteristics of patients with uncomplicated enteric fever treated with azithromycin

Variable	Values ($N=25$)
Age (years), median (range)	24 (15–56)
Male sex, *n* (%)	15 (60.0)
Patients from urban area, *n* (%)	22 (88.0)
Days of illness, median (IQR)	10 (5–21.0)
Diarrhoea, *n* (%)	14 (56.0)
Vomiting, *n* (%)	10 (40.0)
Abdominal pain, *n* (%)	7 (28.0)
Admission temp (°C), median (range)	102.5 (100–105)
Hepatomegaly, *n* (%)	3 (12.0)
Splenomegaly, *n* (%)	5 (20.0)
S. Typhi, *n* (%)	23 (92.0)
S. Paratyphi A, *n* (%)	2 (8.0)
MDR[a] isolate, *n* (%)	1 (4.0)
Ciprofloxacin intermediate, *n* (%)	23 (92.0)
Chloramphenicol susceptible, *n* (%)	24 (96.0)
Co-trimoxazole susceptible, *n* (%)	24 (96.0)
Ceftriaxone susceptible, *n* (%)	25 (100.0)
Azithromycin susceptible, *n* (%)	25 (100.0)
Azithromycin MIC (mg/L), median (IQR, range)	4 (4–6, 3–12)
Azithromycin dose (mg), median (IQR, range)	1000 (1000–1250, 1000–1500)
LOS (days), median (IQR, range)	7 (5.5–12, 4–16)
Fever clearance time, FCT (days), median (IQR, range)	3 (2–3, 2–5)
Clinical failure, *n* (%)	0 (0)
Microbiological failure, *n* (%)	0 (0)
Complicated disease, *n* (%)	4 (16.0)
Relapse, *n* (%)	0 (0)

[a]MDR, resistant to chloramphenicol, ampicillin and co-trimoxazole.

The median (IQR, range) FCT was 3 (2–3, 2–5) days, and the median (IQR, range) length of hospital stay (LOS) was 7 (5.5–12, 4–16) days. Four (16%) patients developed complications; two (8%) patients developed encephalopathy and two (8%) patients had gastrointestinal bleeding. Treatment was successful in all 25 patients, with no clinical or microbiological failure, relapse or disease-related mortality (Table 1).

PK of azithromycin in typhoid fever

The azithromycin plasma concentration levels over a 24 h time period are displayed in Figure 1. The mean (±SD) azithromycin plasma concentration ranges during the specified sampling times were as follows: trough, 0.24 ± 0.19 mg/L; 2 h after azithromycin, 1.24 ± 0.98 mg/L; 5 h, 0.64 ± 0.51 mg/L; 12 h, 0.31 ± 0.16 mg/L; and 24 h, 0.37 ± 0.30 mg/L.

The mean C_{max} attained by azithromycin was 1.38 mg/L. One patient had a trough plasma concentration below the level of detection and the maximum concentration attained was 3.64 mg/L. Azithromycin mean half-life was 28.2 h. The mean plasma C_{max}/MIC and AUC_{24}/MIC for azithromycin were 0.29 ± 0.22 and 2.64 + 1.64, respectively (Table 2). There was poor inverse correlation (Pearson's product moment correlation = −0.2) between plasma AUC_{24}/MIC ratio and fever clearance ($P=0.32$; 95% CI, −0.44 to 0.15) (Figure 2).

Discussion

Azithromycin is the drug of choice for uncomplicated enteric fever, especially in areas where MDR (resistance to ampicillin, chloramphenicol and co-trimoxazole), XDR (MDR + fluoroquinolone non-susceptible and ceftriaxone resistant) and fluoroquinolone-resistant *S.* Typhi infections are prevalent.[28,29] The azithromycin MICs for *S.* Typhi are usually in the range 0.25–16 mg/L.[30–34] In our study, all of the isolates had azithromycin MICs ranging from 3 to 12 mg/L. MIC values were higher for *S.* Paratyphi than *S.* Typhi, as observed in other studies from India[35] and other parts of the world.[1]

There is scarce PK/PD data on azithromycin in enteric fever. Serum levels of azithromycin after oral administration are usually 0.04–0.4 mg/L.[10] The mean azithromycin plasma concentration levels in our study were lower than the MIC. The dosing used in our study was based on the original article comparing gatifloxacin and azithromycin, which used 20 mg/kg as the standard dose.[25] This is different from the dosing strategies followed elsewhere in the world where a lower dose is used based on a very small paediatric trial done prior to the MDR enteric fever era.[36,37] We followed weight-based dosing, and adjustments were made for oral therapy received by 60% of the study population, which may have resulted in a dose marginally less than 20 mg/kg IV daily. This may have contributed to a lower plasma concentration, rather than a level >4 times the MIC usually expected for therapeutic efficacy.[26,38] We noted only a marginally higher mean 24 h azithromycin concentration compared with trough, suggesting that azithromycin did not achieve a steady state by Day 2 of azithromycin treatment. Azithromycin has a slow elimination rate, resulting in longer plasma half-life of up to 70 h, with a steady state achieved at 14 days and increased

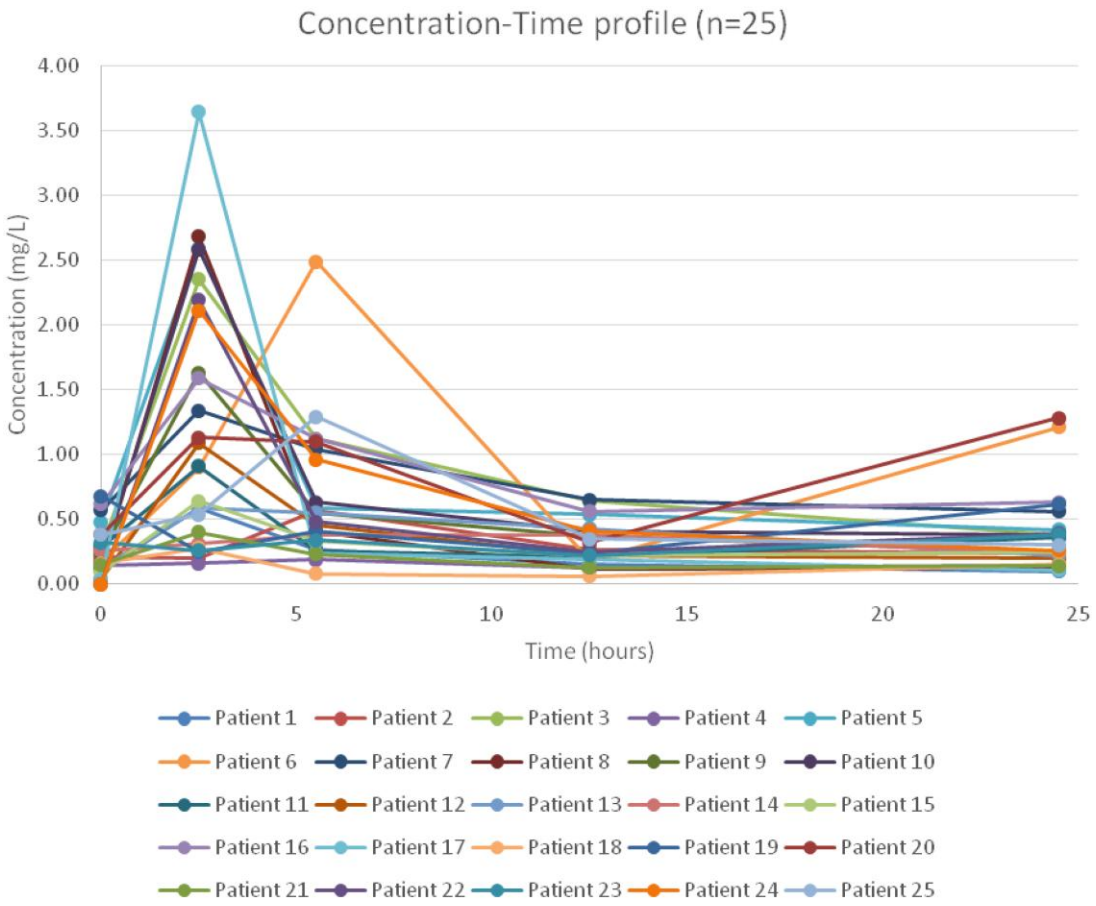

Figure 1. Azithromycin plasma concentration levels over 24 h time period.

Table 2. PK parameters of azithromycin

			Range	
Parameter	Mean ± SD	Median	Min	Max
AUC_{24} (mg·h/L)	12.08 ± 6.00	11.5	2.84	24.11
MIC (mg/L)	5.24 ± 2.01	4	3	12
AUC_{24}/MIC	2.64 ± 1.64	2.19	0.30	6.38
C_{max} (mg/L)	1.38 ± 0.96	1.28	0.19	3.64
C_{max}/MIC	0.29 ± 0.22	0.21	0.02	0.91
Elimination rate constant (h^{-1})	0.04 ± 0.02	0.04	0.01	0.07
Half-life (h)	28.19 ± 27.74	17.22	9.92	120.94
Volume of distribution at terminal phase (L)	1625.88 ± 958.04	1337.53	653.02	4131.21

concentrations in tissues, which may be the reason that we were unable to demonstrate this.[39–41]

The mean plasma concentration for azithromycin was low, with low C_{max}/MIC and AUC/MIC ratios. Hence, the measurable plasma concentration of azithromycin that clears typhoid fever remains unknown. Published data indicate that C_{max}/MIC ratios exceeding 8 and an AUC_{24}/MIC value of >100 are required for successful treatment and prevention of the emergence of resistance in Gram-negative bacterial infections, but this has not been established for macrolides.[42] Macrolide efficacy is determined by the duration of drug concentration above the MIC, and free serum drug levels above the MIC for at least 40%–50% of the dosing interval.[43,44] However, this has been demonstrated only in the treatment of pneumococcal infections, where the azithromycin AUC_{24}/MIC ratio was in the range of 25–35.[42]

In our study there were no clinical or microbiological failures, relapses or disease-related mortality. The median (IQR, range) FCT was 3 (2–3, 2–5) days, and the median (IQR, range) LOS was 7 (5.5–12, 4–16) days. At present, even for antibiotics concentrating intracellularly, we continue to use AUC/MIC as a surrogate PK/PD parameter for efficacy; however, whether this is optimal remains unclear. *In vivo* studies for tigecycline reveal that despite low plasma levels, high concentration in PMNLs occurs.[45] Similar PK/PD assessments for other antimicrobials have shown that even though free-drug exposure in the plasma is below the MIC, *f*AUC/MIC may be a PD parameter that correlates with efficacy, e.g. tigecycline against both *Escherichia coli* and *Klebsiella pneumoniae*.[46] We were unable to measure intracellular concentrations of azithromycin and we were unable to categorically demonstrate if *f*AUC/MIC correlated with efficacy. The extracellular bacteraemia in enteric fever contributing to symptoms in

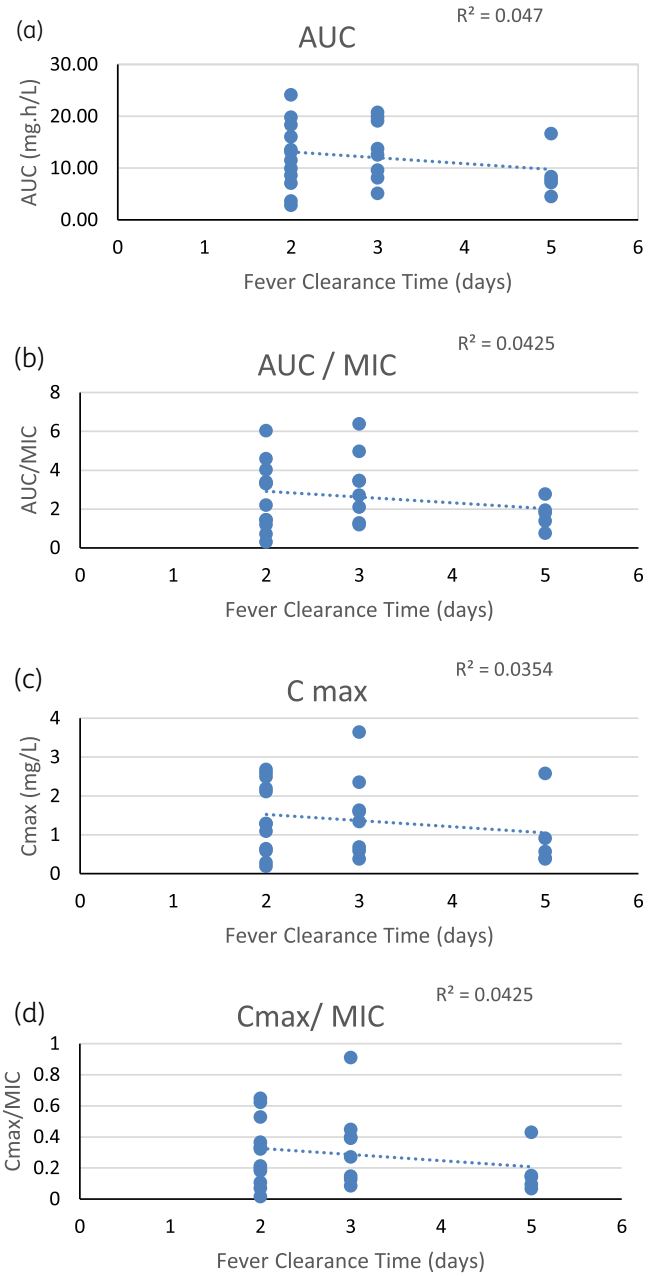

Figure 2. Correlation between azithromycin PK/PD parameters and FCT.

patients[33] seems to be effectively controlled by azithromycin, suggesting that antimicrobial benefits probably occur in the extracellular milieu despite low demonstrable plasma levels.

The low plasma levels of azithromycin, coupled with sporadic reports of azithromycin resistance globally are a cause for concern. Genomic epidemiology studies[47] have shown independent acquisition of plasmids and homoplastic mutations conferring antimicrobial resistance occurring repeatedly in multiple lineages of *S.* Typhi, predominantly arising in South Asia before spreading to other regions. Azithromycin resistance mutations are detected at low prevalence in Southeast Asia, with sequenced isolates containing a mutation exclusively in *acrB*, a gene encoding a component of the AcrAB efflux pump (Table 3).[23,48–52]

Table 3. Azithromycin resistance mechanisms in *S.* Typhi and *S.* Paratyphi A seen in the *acrB* gene in Southeast Asia

Gene mutation	MIC (mg/L)	Country	Reference
R717Q/L	32–64	Bangladesh	Hooda *et al.*[37]
R717L	>256	Nepal	Duy *et al.*[35]
R717Q	>16	India	Carey *et al.*[22]
R717Q	12 (increasing MIC range)	Pakistan	Iqbal *et al.*[34]
R717Q/L	R717Q: 32; R717L: 16	Singapore	Octavia *et al.*[36]

In our study, we noted a number of limitations. Five (20%) study patients received active therapy prior to azithromycin monotherapy for a median (IQR, range) duration of 3 (2–3, 2–4) days and this may have marginally affected the measured clinical outcomes. An inverse relationship between azithromycin AUC/MIC and fever clearance was observed, i.e. a high C_{max} and AUC_{24}/MIC correlated with shorter time to fever clearance (Figure 2); this was not statistically significant, probably due to small sample size and estimation of azithromycin concentrations performed early in the treatment period for a drug with a long half-life and wide tissue distribution. The MIC cut-offs for our study were determined using the ETEST, and it is well known that the elution of the antibiotic from the gradient strip may lead to double zones and trailing edges, which can result in variability of interpretation.[53] This could have also influenced the values of azithromycin AUC_{24}/MIC and C_{max}/MIC. Another limitation of our study is that all *S.* Typhi isolates were susceptible (i.e. MIC < 16 mg/L), hence, it is unclear if outcomes would have differed with resistant isolates. Fifteen patients in our study received oral azithromycin and 10 received an IV formulation; however, there was no demonstrable difference in plasma levels. Literature reveals that due to its stability at low pH, azithromycin has an oral bioavailability of 37%,[54] with low peak plasma concentrations.

Overall, it was heartening to note that despite relatively low concentrations of azithromycin extracellularly, we were unable to demonstrate clinical or microbiological failure.

To conclude, azithromycin was effective in treating patients with susceptible enteric fever infections. The extracellular azithromycin plasma levels were lower than the MIC, but there were no treatment failures. Further larger clinical trials are needed to correlate intracellular azithromycin concentrations with therapeutic efficacy and establish optimal azithromycin plasma level as a determinant of clinical outcomes in typhoid fever. The traditional paradigm of achieving plasma concentration of an antibiotic at least four times the MIC for the infecting isolate seems flawed when considering antibiotics concentrating intracellularly, as was seen here with azithromycin.

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Transparency declarations

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References

1 Hassing RJ, Goessens WH, van Pelt W *et al*. Salmonella subtypes with increased MICs for azithromycin in travelers returned to The Netherlands. *Emerg Infect Dis* 2014; **20**: 705–8. https://doi.org/10.3201/eid2004.131536

2 Rupali P, Abraham OC, Jesudason MV *et al*. Treatment failure in typhoid fever with ciprofloxacin susceptible *Salmonella enterica* serotype Typhi. *Diagn Microbiol Infect Dis* 2004; **49**: 1–3. https://doi.org/10.1016/j.diagmicrobio.2003.12.002

3 Bandyopadhyay R, Balaji V, Yadav B *et al*. Effectiveness of treatment regimens for typhoid fever in the nalidixic acid-resistant *S. typhi* (NARST) era in south India. *Trop Doct* 2018; **48**: 182–8. https://doi.org/10.1177/0049475518758884

4 Al Naiemi N, Zwart B, Rijnsburger MC *et al*. Extended-spectrum-beta-lactamase production in a *Salmonella enterica* serotype Typhi strain from the Philippines. *J Clin Microbiol* 2008; **46**: 2794–5. https://doi.org/10.1128/JCM.00676-08

5 Akinyemi KO, Iwalokun BA, Alafe OO *et al*. *bla*$_{\text{CTX-M-I}}$ group extended spectrum beta-lactamase-producing *Salmonella typhi* from hospitalized patients in Lagos, Nigeria. *Infect Drug Resist* 2015; **8**: 99–106. https://doi.org/10.2147/IDR.S78876

6 Effa EE, Bukirwa H. Azithromycin for treating uncomplicated typhoid and paratyphoid fever (enteric fever). *Cochrane Database Syst Rev* 2008; issue 4: CD006083. https://doi.org/10.1002/14651858.CD006083.pub2

7 Ballow CH, Amsden GW. Azithromycin: the first azalide antibiotic. *Ann Pharmacother* 1992; **26**: 1253–61. https://doi.org/10.1177/106002809202601014

8 Liu P, Allaudeen H, Chandra R *et al*. Comparative pharmacokinetics of azithromycin in serum and white blood cells of healthy subjects receiving a single-dose extended-release regimen versus a 3-day immediate-release regimen. *Antimicrob Agents Chemother* 2007; **51**: 103–9. https://doi.org/10.1128/AAC.00852-06

9 Panteix G, Guillaumond B, Harf R *et al*. In-vitro concentration of azithromycin in human phagocytic cells. *J Antimicrob Chemother* 1993; **31** Suppl E: 1–4. https://doi.org/10.1093/jac/31.suppl_E.1

10 Foulds G, Shepard RM, Johnson RB. The pharmacokinetics of azithromycin in human serum and tissues. *J Antimicrob Chemother* 1990; **25** Suppl A: 73–82. https://doi.org/10.1093/jac/25.suppl_A.73

11 Rakita RM, Jacques-Palaz K, Murray BE. Intracellular activity of azithromycin against bacterial enteric pathogens. *Antimicrob Agents Chemother* 1994; **38**: 1915–21. https://doi.org/10.1128/AAC.38.9.1915

12 EUCAST. *Breakpoint tables for interpretation of MICs and zone diameters*. 2014. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_14.0_Breakpoint_Tables.pdf

13 Parry CM, Thieu NT, Dolecek C *et al*. Clinically and microbiologically derived azithromycin susceptibility breakpoints for *Salmonella enterica* serovars Typhi and Paratyphi A. *Antimicrob Agents Chemother* 2015; **59**: 2756–64. https://doi.org/10.1128/AAC.04729-14

14 Divyashree S, Nabarro LE, Veeraraghavan B *et al*. Enteric fever in India: current scenario and future directions. *Trop Med Int Health* 2016; **21**: 1255–62. https://doi.org/10.1111/tmi.12762

15 Sjölund-Karlsson M, Joyce K, Blickenstaff K *et al*. Antimicrobial susceptibility to azithromycin among *Salmonella enterica* isolates from the United States. *Antimicrob Agents Chemother* 2011; **55**: 3985–9. https://doi.org/10.1128/AAC.00590-11

16 Patel SR, Bharti S, Pratap CB *et al*. Drug resistance pattern in the recent isolates of *Salmonella Typhi* with special reference to cephalosporins and azithromycin in the Gangetic Plain. *J Clin Diagn Res* 2017; **11**: DM01–3. https://doi.org/10.7860/JCDR/2017/23330.9973

17 Manesh A, Balaji V, Kumar DR *et al*. A case of clinical and microbiological failure of azithromycin therapy in *Salmonella enterica* serotype Typhi despite low azithromycin MIC. *Int J Infect Dis* 2017; **54**: 62–3. https://doi.org/10.1016/j.ijid.2016.11.409

18 Karkey A, Thwaites GE, Baker S. The evolution of antimicrobial resistance in *Salmonella* Typhi. *Curr Opin Gastroenterol* 2018; **34**: 25–30. https://doi.org/10.1097/MOG.0000000000000406

19 Vlieghe ER, Phe T, De Smet B *et al*. Azithromycin and ciprofloxacin resistance in *Salmonella* bloodstream infections in Cambodian adults. *PLoS Negl Trop Dis* 2012; **6**: e1933. https://doi.org/10.1371/journal.pntd.0001933

20 Gomes C, Martínez-Puchol S, Palma N *et al*. Macrolide resistance mechanisms in *Enterobacteriaceae*: focus on azithromycin. *Crit Rev Microbiol* 2017; **43**: 1–30. https://doi.org/10.3109/1040841X.2015.1136261

21 Sharma P, Kumari B, Dahiya S *et al*. Azithromycin resistance mechanisms in typhoidal *Salmonellae* in India: a 25 years analysis. *Indian J Med Res* 2019; **149**: 404–11. https://doi.org/10.4103/ijmr.IJMR_1302_17

22 Tagg KA, Kim JY, Henderson B *et al*. Azithromycin–resistant mph(A)–positive Salmonella enterica serovar Typhi in the United States. *J Glob Antimicrob Resist* 2024; **39**: 69–72.

23 Carey ME, Jain R, Yousuf M *et al*. Spontaneous emergence of azithromycin resistance in independent lineages of *Salmonella* Typhi in Northern India. *Clin Infect Dis* 2021; **72**: e120–e7. https://doi.org/10.1093/cid/ciaa1773

24 Rai S, Jain S, Prasad KN *et al*. Rationale of azithromycin prescribing practices for enteric fever in India. *Indian J Med Microbiol* 2012; **30**: 30–3. https://doi.org/10.4103/0255-0857.93017

25 Dolecek C, Tran TP, Nguyen NR *et al*. A multi-center randomised controlled trial of gatifloxacin versus azithromycin for the treatment of uncomplicated typhoid fever in children and adults in Vietnam. *PLoS One* 2008; **3**: e2188.

26 Barger A, Fuhst C, Wiedemann B *et al*. Pharmacological indices in antibiotic therapy. *J Antimicrob Chemother* 2003; **52**: 893–8.

27 Methods for the determination of susceptibility of bacteria to antimicrobial agents. Terminology. *Clin Microbiol Infect* 1998; **4**: 291–6.

28 Parry CM, Ho VA, Phuong Le T *et al*. Randomized controlled comparison of ofloxacin, azithromycin, and an ofloxacin-azithromycin combination for treatment of multidrug-resistant and nalidixic acid-resistant typhoid fever. *Antimicrob Agents Chemother* 2007; **51**: 819–25. https://doi.org/10.1128/AAC.00447-06

29 Crump JA, Sjölund-Karlsson M, Gordon MA *et al*. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clin Microbiol Rev* 2015; **28**: 901–37. https://doi.org/10.1128/CMR.00002-15

30 Metchock B. In-vitro activity of azithromycin compared with other macrolides and oral antibiotics against *Salmonella typhi*. *J Antimicrob Chemother* 1990; **25** Suppl A: 29–31. https://doi.org/10.1093/jac/25.suppl_A.29

31 Gordillo ME, Singh KV, Murray BE. In vitro activity of azithromycin against bacterial enteric pathogens. *Antimicrob Agents Chemother* 1993; **37**: 1203–5. https://doi.org/10.1128/AAC.37.5.1203

32 Tribble D, Girgis N, Habib N *et al*. Efficacy of azithromycin for typhoid fever. *Clin Infect Dis* 1995; **21**: 1045–6. https://doi.org/10.1093/clinids/21.4.1045

33 Butler T, Frenck RW, Johnson RB *et al*. *In vitro* effects of azithromycin on *Salmonella typhi*: early inhibition by concentrations less than the MIC

and reduction of MIC by alkaline pH and small inocula. *J Antimicrob Chemother* 2001; **47**: 455–8. https://doi.org/10.1093/jac/47.4.455

34 Butler T, Sridhar CB, Daga MK *et al*. Treatment of typhoid fever with azithromycin versus chloramphenicol in a randomized multicentre trial in India. *J Antimicrob Chemother* 1999; **44**: 243–50. https://doi.org/10.1093/jac/44.2.243

35 Chatterjee S, Eshwara VK, Tellapragada C *et al*. Azithromycin susceptibility among clinical isolates of *Salmonella*: interfacing guidelines with routine practices. *Indian J Med Microbiol* 2016; **34**: 397–8. https://doi.org/10.4103/0255-0857.188376

36 Frenck RW, Jr., Nakhla I, Sultan Y *et al*. Azithromycin versus ceftriaxone for the treatment of uncomplicated typhoid fever in children. *Clin Infect Dis* 2000; **31**: 1134–8.

37 Frenck RW, Jr., Mansour A, Nakhla I *et al*. Short-course azithromycin for the treatment of uncomplicated typhoid fever in children and adolescents. *Clin Infect Dis* 2004; **38**: 951–7.

38 Ambrose PG, Jr., Owens RC, Grasela D *et al*. Antimicrobial pharmacodynamics. *Med Clin North Am* 2000; **84**: 1431–46.

39 Zheng S, Matzneller P, Zeitlinger M *et al*. Development of a population pharmacokinetic model characterizing the tissue distribution of azithromycin in healthy subjects. *Antimicrob Agents Chemother* 2014; **58**: 6675-84.

40 Choi S, Jeon S, Yim DS *et al*. Contribution of Trough Concentration Data in the Evaluation of Multiple-Dose Pharmacokinetics for Drugs with Delayed Distributional Equilibrium and Long Half-Life. *Drug Des Devel Ther* 2020; **14**: 811–21.

41 Zuckerman JM, Qamar F, Bono BR *et al*. Review of macrolides (azithromycin, clarithromycin), ketolids (telithromycin) and glycylcyclines (tigecycline). *Med Clin North Am* 2011; **95**: 761–91.

42 Craig WA. Does the dose matter? *Clin Infect Dis* 2001; **33** Suppl 3: S233–7. https://doi.org/10.1086/321854

43 Rybak MJ. Pharmacodynamics: relation to antimicrobial resistance. *Am J Infect Control* 2006; **34**(Suppl 1): S38-45.

44 Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clin Infect Dis* 1998; **26**: 1-10.

45 Ong CT, Babalola CP, Nightingale CH *et al*. Penetration, efflux and intracellular activity of tigecycline in human polymorphonuclear neutrophils (PMNs). *J Antimicrob Chemother* 2005; **56**: 498–501.

46 Nicasio AM, Crandon JL, Nicolau DP. In vivo pharmacodynamic profile of tigecycline against phenotypically diverse Escherichia coli and Klebsiella pneumoniae isolates. *Antimicrob Agents Chemother* 2009; **53**: 2756–61.

47 da Silva KE, Tanmoy AM, Pragasam AK *et al*. The international and intercontinental spread and expansion of antimicrobial-resistant *Salmonella* Typhi: a genomic epidemiology study. *Lancet Microbe* 2022; **3**: e567–77. https://doi.org/10.1016/S2666-5247(22)00093-3

48 Carey ME, Dyson ZA, Ingle DJ *et al*. Global diversity and antimicrobial resistance of typhoid fever pathogens: insights from a meta-analysis of 13,000 *Salmonella* Typhi genomes. *Elife* 2023; **12**: e85867. https://doi.org/10.7554/eLife.85867

49 Iqbal J, Dehraj IF, Carey ME *et al*. A race against time: reduced azithromycin susceptibility in *Salmonella enterica* serovar Typhi in Pakistan. *mSphere* 2020; **5**: e00215–20. https://doi.org/10.1128/msphere.00215-20

50 Duy PT, Dongol S, Giri A *et al*. The emergence of azithromycin-resistant *Salmonella* Typhi in Nepal. *JAC Antimicrob Resist* 2020; **2**: dlaa109. https://doi.org/10.1093/jacamr/dlaa109

51 Octavia S, Chew KL, Lin RTP *et al*. Azithromycin-resistant *Salmonella enterica* serovar Typhi AcrB-R717Q/L, Singapore. *Emerg Infect Dis* 2021; **27**: 624–7. https://doi.org/10.3201/eid2702.203874

52 Hooda Y, Sajib MSI, Rahman H *et al*. Molecular mechanism of azithromycin resistance among typhoidal *Salmonella* strains in Bangladesh identified through passive pediatric surveillance. *PLoS Negl Trop Dis* 2019; **13**: e0007868. https://doi.org/10.1371/journal.pntd.0007868

53 Goldblatt J, Ward A, Yusuf M *et al*. Azithromycin susceptibility testing for Salmonella enterica isolates: discordances in results using MIC gradient strips. *J Antimicrob Chemother* 2020; **75**: 1820–3.

54 Fohner AE, Sparreboom A, Altman RB *et al*. PharmGKB summary: Macrolide antibiotic pathway, pharmacokinetics/pharmacodynamics. *Pharmacogenet Genomics* 2017; **27**: 164–7.