A randomised controlled comparison of ofloxacin, azithromycin and an ofloxacin-azithromycin combination for treatment of multidrug-resistant and nalidixic acid resistant typhoid fever.

Running title: Azithromycin and ofloxacin in typhoid fever

Key words: Salmonella Typhi, multidrug resistance, nalidixic acid resistance, ofloxacin, azithromycin

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ABSTRACT

Isolates of *Salmonella enterica* ser. Typhi that are multidrug resistant (MDR, resistant to chloramphenicol, ampicillin and trimethoprim/sulfamethoxazole) and have reduced susceptibility to fluoroquinolones (nalidixic acid resistant, Na\(^R\)) are common in Asia. The optimum treatment for infections caused by such isolates is not established. This study compared different antimicrobial regimens for the treatment of MDR/Na\(^R\) typhoid fever. Vietnamese children and adults with uncomplicated typhoid fever were entered into an open randomised controlled trial. Ofloxacin (20mg/kg/day for 7 days), azithromycin (10mg/kg/day for 7 days) and ofloxacin (15mg/kg/day for 7 days) combined with azithromycin (10 mg/kg/day for the first 3 days) were compared. 187 of the 241 enrolled patients were eligible for analysis (186 *S.enterica* ser.Typhi, 1 ser. Paratyphi A). 87% (163/187) were children, 88% (165/187) of the *S.enterica* ser. Typhi were MDR and 93% (173/187) were Na\(^R\). The clinical cure rate was 64% (40/63) with ofloxacin, 76% (47/62) with ofloxacin/azithromycin and 82% (51/62) with azithromycin (p=0.053). The mean (95% CI) fever clearance time for patients treated with azithromycin [5.8 days (5.1-6.5)], was shorter than with ofloxacin/azithromycin [7.1 days (6.2-8.1)] and ofloxacin [8.2 days (7.2-9.2)], (p<0.001). Positive faecal carriage immediately post treatment was detected in 19.4% (12/62) patients treated with ofloxacin, 6.5% (4/62) treated with the combination and 1.6% (1/62) treated with azithromycin (p=0.006). Both antibiotics were well tolerated. Uncomplicated typhoid fever due to isolates of MDR *S.enterica* ser.Typhi with reduced susceptibility to fluoroquinolones (Na\(^R\)) can be successfully treated with a seven-day course of azithromycin.
INTRODUCTION

Enteric fever, due to infection with *Salmonella enterica* subspecies *enterica* serotype Typhi (S. enterica ser. Typhi) or ser. Paratyphi A, is estimated to cause more than 27 million infections each year worldwide with 216,000 deaths [11]. Multidrug-resistant (MDR) strains of *S. enterica* ser. Typhi and ser. Paratyphi A (resistant to chloramphenicol, trimethoprim/sulfamethoxazole and ampicillin) are endemic in many Asian countries [28]. Third generation cephalosporins and fluoroquinolones are used for treating such infections. Where fluoroquinolones, such as ciprofloxacin and ofloxacin, have become widely used, isolates of *S. enterica* ser. Typhi and ser. Paratyphi A with reduced susceptibility to fluoroquinolones have become common [27,30]. Infections with these isolates have been associated with treatment failures, particularly when very short durations of treatment have been used [31,35]. These isolates are ciprofloxacin susceptible by current disc testing criteria, but are usually resistant to nalidixic acid (Na\(^R\)) [10,35]. In ciprofloxacin susceptible *S. enterica* isolates, nalidixic acid resistance has been proposed as an indicator that infection with such a strain may not respond to fluoroquinolone treatment [23]. In some endemic areas, > 75% of typhoid infections admitted to hospital are Na\(^R\) [27,30] and they are also seen in returning travellers [1].

The choice of oral antimicrobial regimens for uncomplicated typhoid fever caused by isolates of *S. enterica* ser. Typhi that are both MDR and Na\(^R\) is unclear. A fluoroquinolone given in a high dose for seven days is the most affordable first-line option for MDR/Na\(^R\) infections in endemic areas, but at the time this study was planned the efficacy of such a
regimen had not been examined in a randomized controlled trial. The third generation cephalosporins would be effective for the treatment of such infections and resistance to these agents is uncommon [14,32]. However, expense and the need for parenteral therapy limit their usefulness as first line treatment. The azalide antimicrobial azithromycin is a further option. Treatment courses of 500mg a day (10mg/kg/day) for seven days and 1gm a day (20mg/kg/day) for five days have proved successful in adults and children [8,17,18,20] including adults with MDR/NaR infections [25]. A combination of a fluoroquinolone with another antimicrobial directed against a different target is another option that may improve efficacy compared with the fluoroquinolone alone, and potentially reduce the chance of fluoroquinolone resistant mutants emerging. However, there is no controlled trial evidence to support this approach.

We have conducted a three-way comparison of seven-days of ofloxacin (20mg/kg/day), seven-days of azithromycin (10 mg/kg/day) and seven-days of ofloxacin (15 mg/kg/day) combined with azithromycin (10mg/kg/day) for the first three days for the treatment of uncomplicated enteric fever. The combination of ofloxacin and azithromycin was empirically designed to match the different pharmacokinetics of the two antimicrobials. The lower dose of ofloxacin and a shorter duration of azithromycin were chosen to see if it was possible to reduce cost but maintain the efficacy of the regimen.

**MATERIALS AND METHODS**

**Study site and ethical compliance**
The study was conducted on the infection ward at Dong Thap Provincial Hospital, Cao Lanh Town, Dong Thap Province, Vietnam. The hospital is a 300-bed Provincial Hospital for Dong Thap Province in the Mekong Delta. The study had received approval from the Scientific and Ethical Committees of Dong Thap Provincial Hospital and the Hospital for Tropical Diseases, Ho Chi Minh City. Patients, or their parent or guardian for children, gave informed verbal consent before entry into the study. The study was conducted in compliance with ICH and Declaration of Helsinki Guidelines.

**Study population**

Children and adults with the clinical features of enteric fever were enrolled in the study. Eligibility for enrolment required that the patient have a documented fever (temperature ≥ 38°C) and a history of fever for at least four days plus at least one of the following criteria: abdominal pain/tenderness, diarrhoea or constipation, hepatomegaly, splenomegaly and/or rose spots. Patients were excluded if they had evidence of severe or complicated disease (severe gastrointestinal bleeding, intestinal perforation, visible jaundice, myocarditis, pneumonia, renal failure, shock or an altered conscious level), inability to swallow oral medication, a history of significant underlying disease or of hypersensitivity to either of the trial drugs or were pregnant or lactating. Patients who gave a history of treatment with a fluoroquinolone or third generation cephalosporin or macrolide within one week of hospital admission were also excluded.

**Randomization and treatment**
Patients were allocated to one of three treatment groups in an open randomised comparison. The computer generated randomisation list was produced by a member of staff not otherwise involved in the study. The treatment allocations were kept in serially numbered sealed envelopes that were only opened after the patient had been enrolled into the study. Patients were randomised to receive one of the following three regimens.

1. Ofloxacin (Oflocet®, Hoescht Marion Roussel, Paris, France) 20mg/kg/day orally in two-divided dose (maximum 400 mg twice daily) for 7 days.

2. Azithromycin suspension (Zithromax®, Pfizer International) 10mg/kg/day orally once a day (maximum 500 mg daily) for 7 days. Tablets were used for adults.

3. Ofloxacin (Oflocet®, Hoescht Marion Roussel, Paris, France) 15mg/kg/day orally in two divided doses (maximum 300mg twice daily) for 7 days combined with azithromycin suspension (Zithromax®, Pfizer International) 10mg/kg/day orally once a day (maximum 500mg daily) for first 3 days.

Care was taken that calcium containing foods or drugs (e.g. milk, antacids) were not given at the same time as the antimicrobials to avoid problems with ofloxacin absorption.

Laboratory investigations

A haematocrit, white cell count and differential, platelet count, serum aspartate transaminase, alanine transaminase, creatinine and urinalysis were performed before therapy. The aspartate transaminase and alanine transaminase were repeated one day after the end of therapy. The full blood count was repeated if there had been evidence of gastrointestinal bleeding or clinical evidence of anaemia. A chest X-ray and other radiological investigations, including abdominal ultrasound, were performed as clinically
indicated. Blood, bone marrow and fecal cultures were obtained before therapy. A blood
culture was taken in all patients a day after the end of treatment. In addition, three fecal
specimens were cultured between two and four days after the end of treatment.

Isolates of *Salmonella* were identified by standard biochemical tests, and agglutination
with *Salmonella* specific antisera (Murex diagnostics, Dartford, United Kingdom).
Antimicrobial sensitivities were performed by the modified Bauer-Kirby disc diffusion
method with zone size interpretation based on CLSI (formerly NCCLS) guidelines [19].
Antibiotic discs tested were chloramphenicol (30µg), ampicillin (10µg), trimethoprim-
sulphamethoxazole (1.25/23.75µg), ceftriaxone (30µg), ofloxacin (5µg), azithromycin
(15µg) and nalidixic acid (30µg). Isolates were stored in protect beads (Prolabs, Oxford,
United Kingdom) at –20°C for later minimum inhibitory concentration (MIC) testing by
agar plate dilution [24]. Antibiotics powders were purchased from Sigma, UK. The
azithromycin MIC was determined by E-test (AB Biodisk, Solna, Sweeden) according to
the manufacturer's instructions. Escherichia coli ATCC® 25922 and Staphylococcus
aureus ATCC® 25923 were used as control strains for these assays. An isolate was
defined as MDR if it was resistant to chloramphenicol (≥ 32µg/ml), ampicillin (≥
32µg/ml) and trimethoprim/sulfamethoxazole (≥ 8/152 µg/ml). An isolate was defined as
nalidixic acid resistant (NaR) if it was resistant to nalidixic acid (≥ 32µg/ml). The CLSI
breakpoints for ofloxacin were ≤ 2µg/ml susceptible and ≥ 8µg/ml resistant but there are
none for azithromycin [23].

**Definitions**
Patients were examined daily until discharge from hospital, with particular reference to clinical symptoms, side effects of the drug and complications of the disease. Body temperature was measured every six hours. The response to treatment was assessed by clinical parameters (resolution of clinical symptoms and signs), fever clearance time (time from the start of treatment until the body temperature reached ≤ 37.5°C, and remained ≤ 37.5°C for 48 hours), development of complications and evidence of relapse of infection. A clinical treatment failure was defined as the persistence of fever and at least one other typhoid related symptom for more than seven days after the start of treatment or the development of severe complications (severe gastrointestinal bleeding, intestinal perforation, visible jaundice, myocarditis, pneumonia, renal failure, shock or an altered conscious level) during treatment requiring a change in therapy. Microbiological treatment failure was defined as isolation of *S. enterica* ser.Typhi or Paratyphi A from blood or a sterile site after the completion of treatment. Those who failed treatment and, in the opinion of the treating physician, required re-treatment received ceftriaxone 60mg/kg/day for 7 to 10 days. Early fecal carriage was defined as a positive fecal culture, with an isolate having the same susceptibility pattern as the original isolate, after the end of the initial seven day treatment and before hospital discharge.

Patients were requested to return for a follow up assessment at four weeks or earlier if their symptoms recurred. Further follow up was performed at three months and six months post treatment. Those patients who did not return were visited at their home by one of the study team members. At the first follow up, clinical evidence of relapse was sought, the patient was asked about joint symptoms and one stool culture was performed.
A blood culture was performed if the symptoms and signs suggested relapse. A relapse was defined as a recurrence of symptoms and signs suggestive of enteric fever within the four week period after the patient had been discharged well from the hospital accompanied by a blood culture positive for S.enterica ser. Typhi or Paratyphi A.

Sample size and statistical analysis

We assumed the failure rate for the patients treated with azithromycin would be 5%. A sample size of 59 patients in each group would give a power of 80% at a 5% significance level to detect a failure rate of 25% in the patients treated with ofloxacin (ie a difference between the two failure rates of 20%). Detailed analysis of outcome was confined to those patients in whom the pre-treatment culture of blood or bone marrow was positive with S.enterica ser.Typhi or ser. S.Paratyphi A. Proportions were compared with the Chi square test or the Fisher’s exact test. Normally distributed data were compared using a one-way ANOVA with Tukey’s HSD post-hoc multiple comparisons test, non-normally distributed data using the Kruskal-Wallis test. The fever clearance time and duration of admission after the start of treatment were compared using survival analysis and the log rank test. The independent associations of clinical, laboratory and treatment variables with an outcome of clinical failure were determined using multivariable logistic regression analysis, including all variables significantly associated with (p<0.05) on univariante analysis. Statistical analysis was performed using the EpiInfo version 6 (CDC, Atlanta, USA) and SPSS for Windows version 11 (SPSS Inc, Chicago, USA).

RESULTS
Two hundred and forty one patients with suspected enteric fever were entered into the study between 1998 and 2002. Eighty patients were randomised to treatment with ofloxacin, 81 to the ofloxacin and azithromycin combination and 80 to azithromycin. One hundred and ninety three patients were culture positive in blood or bone marrow, including 192 *S. enterica* ser. Typhi and one ser. Paratyphi A. One hundred and thirty seven patients were positive in blood and bone marrow, 32 in bone marrow alone, 24 in blood alone, and 40/161 (24.8%) blood or bone marrow culture positive patients were also fecal culture positive. Four of the culture positive randomised patients had been treated with a fluoroquinolone prior to admission and two self discharged before the completion of treatment, leaving 187 eligible patients with a positive blood or bone marrow culture. Of the remaining 55 patients who completed treatment, all were cured with an average duration of admission of 10 days. One patient randomised to ofloxacin had a prolonged fever clearance lasting 12 days and a further patient randomised to ofloxacin had a positive fecal culture for *S. enterica* ser. Typhi at six-month follow-up.

Sixty three of the eligible patients were randomised to ofloxacin, 62 to ofloxacin and azithromycin and 62 to azithromycin (Figure 1). 163/187 (87%) were children (aged < 15 years), 165/187 (88%) were infected with a MDR isolate and 173/187 (93%) with a Na<sup>R</sup> isolate. All isolates were susceptible to ofloxacin and ceftriaxone by disc test and MIC. The MIC<sub>90</sub> (range) of the isolates for azithromycin was 16 (4-32) µg/ml and ofloxacin 1.00 (0.03-1.00) µg/ml. The MIC<sub>90</sub> (range) of the Na<sup>S</sup> isolates for ofloxacin was 0.06 (0.03-0.125) µg/ml and for the Na<sup>R</sup> isolates was 1.0 (0.25-1.0) µg/ml. The majority of
isolates in the study had an ofloxacin MIC of 0.5 or 1.0 µg/ml. There were no important differences between the admission characteristics of the three groups (Table 1).

There were 49 treatment failures; 23 in the ofloxacin treated patients, 15 in the ofloxacin/azithromycin treated patients and 11 in the azithromycin treated patients (Table 2). The 23 ofloxacin treated patients failed because of persistent fever and symptoms after the end of treatment and repeat blood culture was positive in two. Fourteen patients required re-treatment, while symptoms in the remaining nine resolved during the three day period post treatment. The 15 patients who failed treatment with ofloxacin/azithromycin did so because of persistent fever and symptoms in 14, with a positive repeat blood culture in one, and one developed a gastrointestinal bleed on day seven. Five patients required re-treatment and symptoms resolved in the others in the three days post treatment. All the patients who failed treatment with ofloxacin, or with ofloxacin plus azithromycin, were infected with isolates that had an ofloxacin MIC of 0.5 or 1.0 µg/ml and were nalidixic acid resistant. Nine of the 11 patients who failed azithromycin did so because of persistent fever and symptoms, with a repeat blood culture positive in one, and two developed gastrointestinal bleeding on day four and day six of treatment. Five patients were re-treated while symptoms in the remaining six resolved over the succeeding three days. The post treatment blood culture was positive in one further patient treated with azithromycin. In this patient, the symptoms had already completely resolved and no further treatment was given. Although, the blood culture was not repeated while the patient was still in hospital, at the one, three and six months follow
up the patient was completely well without negative faecal cultures. All the patients requiring retreatment responded promptly.

Table 3 shows the differences between the responses to treatment with each regimen. Clinical failure was more common with ofloxacin treated patients compared with those treated with azithromycin and those treated with ofloxacin/azithromycin. Overall these differences were on the borderline of significance (p=0.053). However, the confidence intervals for the ofloxacin comparison with azithromycin suggests an important difference in this instance. There were no significant differences in the proportion of patients in each group blood culture positive at the end of treatment (p=0.818). Patients treated with ofloxacin were more likely that the patients treated with ofloxacin/azithromycin and those treated with azithromycin to be fecal culture positive immediately post treatment (p=0.006). The detection of positive fecal carriage at any time during the six month follow-up did not differ between the three groups (p=0.908). Figure 2 shows the Kaplan-Meier survival curve for the fever clearance time in the three groups of patients. The average fever clearance time among the patients treated with azithromycin was 1.3 days shorter than that for the patients treated with the ofloxacin and azithromycin combination and 2.4 days shorter than that of the patients treated with ofloxacin (p<0.001). On average, the patients treated with azithromycin were hospitalized for a day less than those treated with ofloxacin although the differences were not significant (p=0.166). On multivariate analysis, the presence of diarrhoea (OR 5.8; 95% CI 1.2-28.3; p=0.029), hepatomegaly (OR 3.7; 95% CI 1.4-9.6; p=0.007) and
randomisation to the ofloxacin treatment arm (OR 3.0; 95% CI 1.4-6.5) p=0.004) were variables independently associated with clinical failure.

A total of 172/187 (92%) of the patients were seen on at least on occasion at follow-up. There were no relapses. Fourteen (8.1%) of 172 patients were fecal culture positive on at least one occasion (Table 2). There were no significant differences in positive fecal carriage between the three groups at each visit (Table 3). No patient was faecal culture positive at more than one visit. For each patient with a positive isolate at follow-up, there was no increase in the ofloxacin MIC of the follow-up isolate compared with the pre-treatment isolate. The two patient’s who were fecal culture positive at the six month visit were lost to further follow up.

Self-limiting gastrointestinal side effects were reported in a small number of patients in each treatment arm but did not require any interruption of therapy. The mean level of AST and ALT fell in all treatment groups during treatment (Table 2). Three patients (aged 5, 11 and 12 years), one in each treatment arm, described joint discomfort during follow-up that had resolved by the next visit. There were no other significant side effects attributable to either antibiotic.

DISCUSSION

Patients with uncomplicated typhoid fever due to infection with S.enterica ser. Typhi fully susceptible to fluoroquinolones (MIC ≤ 0.06 µg/ml) treated with ciprofloxacin for
seven days have a clinical and microbiological success rate approaching 100% [19,20,36]. Such infections even respond well to shorter courses of ofloxacin. In two studies conducted in Vietnam, a five day regimen of ofloxacin at 8-10mg/kg/day cured 100% of 22 adults with a mean fever clearance time of 3.4 days [33] and 97% of 38 children with a mean fever clearance time of 4.4 days [9] with no microbiological failures in either study. In contrast, patients with uncomplicated typhoid fever due to infection with *S. enterica* ser. Typhi with reduced susceptibility to fluoroquinolones (MIC 0.25-1.0 µg/ml) have an impaired response when treated with short courses (< 7 days) of ofloxacin [27,35]. Suggestions that such infections will respond better to longer courses of ofloxacin have not previously been studied in a controlled trial. This study shows that the clinical response to ofloxacin given at 20 mg/kg/day for seven days is significantly impaired in patients infected with isolates of *S. enterica* ser. Typhi with reduced susceptibility to ofloxacin. Only 64% of patients treated with ofloxacin were cured with a mean fever clearance time of 8.2 days. There were two (3.2%) microbiological failures indicated by a positive blood culture post treatment. Subsequent to the period when this study was being conducted, a case series from south India, found eight of 38 patients with *S. enterica* ser Typhi infection had a positive blood culture after 6 days of ciprofloxacin at a dose of 500mg orally or 400mg intravenously twice daily [31]. All of the failure isolates had a ciprofloxacin MIC of 0.25-1.0 µg/ml. These results suggest that fluoroquinolones should only be used to treat typhoid fever caused by isolates of *S. enterica* ser. Typhi with reduced fluoroquinolone susceptibility with considerable caution.
Although there are several potential reasons for treatment failure, pharmacokinetic and pharmacodynamic (PK-PD) parameters are likely to be an important factor. A PK-PD parameter now widely used for fluoroquinolones is the free-drug area under the concentration-time from 0 to 24 hours/MIC ratio (AUC/MIC) [15]. The PK-PD parameters determining the optimum response in fluoroquinolone treated typhoid fever has not been studied in humans. However, one study has attempted to use an in vitro model with *S. enterica* ser. Typhi, and Monte Carlo simulations to explore PK-PD parameters that were predictive of efficacy [6]. This study found that AUC/MIC ratio was the parameter most predictive of efficacy and that a ratio of 105 corresponded to 90% of maximal activity. In a pharmacokinetic study of ofloxacin (7.5 mg/kg body weight twice daily) in the treatment of children, a total area under the concentration-time from 0 to 12 hours of 26.5 mg/h/liter was observed [3]. Using this value, assuming that ofloxacin is approximately 35% protein bound, the calculated AUC/MIC for an isolate with an MIC of ≤ 0.06 µg/mL would be ≥ 574. For an isolate with an MIC of ≥ 0.5 µg/mL, however the calculated AUC/MIC would be ≤ 69. Although the ofloxacin dosage used in the two studies was slightly different, this in vitro data is generally consistent with the good clinical response to ofloxacin with infections caused by isolates with an ofloxacin MIC of ≤ 0.06 µg/ml [9,33] and the poor clinical response to ofloxacin observed in the current study with infections caused by isolates with an ofloxacin MIC of 0.5-1.0 µg/ml. It should be noted that these calculations use a mean AUC value, whereas the degree of exposure to fluoroquinolones in individual patients is variable, and this may explain why not all patients that have an organism with an MIC ≥ 0.5 µg/ml will fail therapy.
There has been ongoing discussion concerning appropriate fluoroquinolone breakpoints for invasive *Salmonella* infections [2,10]. The presence of nalidixic acid resistance has been suggested as a laboratory marker of isolates with reduced susceptibility to fluoroquinolones and an indicator that invasive infections may fail to respond to fluoroquinolone therapy [23]. In this study, isolates with an ofloxacin MIC of 0.25-1.0 µg/mL were detected by the presence of nalidixic acid resistance with a 100% sensitivity and specificity. The ofloxacin MIC of the nalidixic acid susceptible isolates was between 0.03-0.125 µg/mL. However, some recent data highlighted a significant number of *S.enterica* ser. Typhi isolates with reduced susceptibility to fluoroquinolones that were not nalidixic resistant [12]. This suggests that nalidixic acid resistance may no longer be a reliable marker of reduced fluoroquinolone susceptibility.

Nearly 20% of patients treated with ofloxacin had positive fecal cultures immediately post treatment, although convalescent fecal carriage after discharge from hospital was not significantly different from the other treatment arms. This transient fecal carriage post treatment has the potential to allow further transmission of *S.enterica* ser. Typhi among the family and close contacts. A slightly curious feature of this study was the absence of relapses in any of the treatment groups. This cannot be attributed to poor follow up as 86% of patients were seen at the first follow up at one month, and 92% of patients were seen on at least one occasion during the six month follow up.

Azithromycin at 10mg/kg/day for seven days cured more than 80% of patients with an average fever clearance time of 5.8 days in this study. The microbiological failure rate
was 3.2%. In a similar study in Egyptian adults, azithromycin (1gm on day one followed
by 500mg on the succeeding six days) was compared with ciprofloxacin for 7 days [20].
Symptoms and signs had resolved in all patients treated with azithromycin by day 7 with
a mean (SD) fever clearance time of 3.8 (1.1) days. In Egyptian children, azithromycin
(10 mg/kg/day; maximum dose 500mg/day) for seven days was compared with
ceftriaxone for seven days [18]. A total of 91% (31/34) of the azithromycin treated
children were clinically cured by day seven and the mean duration of fever after starting
therapy was 4.1 (1.1) days. Two studies have examined a regimen of azithromycin at a
dose of 20 mg/kg/day (maximum 1 gm/day) in children or 1 gm/day in adults given for
five days. In the Egyptian children 94% (30/32) were cured with a mean duration of fever
of 4.5 days [17]. In Vietnamese adults 96% (42/44) were cured with a mean duration of
fever of 5.4 days [25]. The slightly lower cure rate in this study compared with those in
these other studies may be due to the higher doses of azithromycin used in the other
studies. The response to azithromycin in this study compares favourably with the reported
results for ceftriaxone and cefixime when given for seven days in uncomplicated enteric
fever [5, 9,17,18,36].

The in-vitro activity of azithromycin against S.enterica ser. Typhi in this study (MIC$_{90}$ 16
µg/ml, range 4-32 µg/ml) was similar to other reports [8]. The MIC is above the reported
peak serum level of 0.4 µg/ml following a 500mg oral dose of azithromycin [16].
However, azithromycin achieves intracellular concentrations up to 50-100 times that in
serum and, at an alkaline pH and with a low inoculum, conditions that may reflect the in-
vivo situation, the MIC is lower [7,26,29]. The discordance between in-vitro
susceptibility and \textit{in-vivo} effectiveness is probably explained by the predominantly intracellular location of \textit{S. enterica} ser. Typhi. However, an estimated one third of \textit{S. enterica} ser. Typhi in the blood of patients with typhoid is extracellular [34] and consequently may be exposed to inadequate concentrations of azithromycin that could result in slow clearance of bacteraemia. Of note, therefore, is that one of the patients treated with azithromycin in this study was blood culture positive post treatment despite apparent resolution of symptoms. Exposure of the organism to sub-therapeutic levels of azithromycin may encourage the emergence of resistance and this is an issue that merits further study.

The addition of three days of azithromycin to seven days of ofloxacin improved the overall cure rate compared with ofloxacin alone, despite the use of a lower dose of ofloxacin, although the difference was not statistically significant. There was no evidence that the combination discouraged the emergence of fluoroquinolone resistant strains, although the study was too small to properly address this question. The average fever clearance time with azithromycin was about one and a half days shorter than the ofloxacin/azithromycin combination and two and a half days shorter than ofloxacin alone. Azithromycin alone and the ofloxacin/azithromycin combination were more effective than ofloxacin alone in eradicating the early post-treatment faecal carriage, although there was no difference in faecal carriage rates during convalescence. Both antimicrobials were well tolerated and no significant joint problems were reported in the children treated with ofloxacin in the six-month follow-up. This acceptable safety profile is in keeping with the
observations of other studies in which fluoroquinolones have been used in children [4,13,37].

For a 20kg child in Vietnam, a seven-day course of ofloxacin (20 mg/kg/day) costs $2-10 depending on the manufacturer, the ofloxacin/azithromycin combination used in this study $8-14, seven days of azithromycin $15 and a ten day course of intravenous ceftriaxone $23-93. The use of ofloxacin or ciprofloxacin for seven days as first-line therapy for typhoid infections caused by isolates that are MDR and with reduced susceptibility to fluoroquinolones will result in one third of patients remaining symptomatic at the end of treatment. They will require further treatment and patients will be at increased risk of developing severe or complicated disease. Furthermore, prolonged fecal carriage could lead to increased transmission, and may also encourage the appearance and dissemination of fully resistant isolates [21]. In this study, a seven-day course of azithromycin was more effective as an initial oral treatment for uncomplicated typhoid fever. Whether widespread adoption of azithromycin as first line treatment in areas where MDR strains with reduced susceptibility to fluoroquinolones are common will in turn lead to the emergence of azithromycin resistance, remains to be seen.
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Figure 1
Flow chart for recruitment of patients into the study.

Figure 2.
Kaplan-Meier survival curve showing the percentage of patients still febrile following the start of treatment. Excludes the patients who failed treatment and required re-treatment with a further course of antimicrobial.
TABLE 1
Epidemiological, clinical and laboratory features in the 187 patients with culture-confirmed enteric fever.

<table>
<thead>
<tr>
<th></th>
<th>Ofloxacin</th>
<th>Ofloxacin + Azithromycin</th>
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<tr>
<td>Number of patients</td>
<td>63</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Number of males / females</td>
<td>33 / 30</td>
<td>31 / 31</td>
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<td>Age (mean, 95%CI, range) years</td>
<td>8.8 (7.8-9.7, 3-22)</td>
<td>10.2 (8.4-12.0, 4-36)</td>
<td>10.5 (8.9-12.1, 4-42)</td>
</tr>
<tr>
<td>Weight (mean, 95%CI, range) Kg</td>
<td>20 (19-22, 10-50)</td>
<td>23 (20-26, 10-56)</td>
<td>23 (21-27, 12-58)</td>
</tr>
<tr>
<td>Duration of fever before admission (mean, 95%CI, range) days</td>
<td>9.6 (8.5-10.7, 4-30)</td>
<td>9.9 (8.6-11.1, 4-25)</td>
<td>9.6 (8.1-10.2, 4-30)</td>
</tr>
<tr>
<td>Abdominal pain (n (%))</td>
<td>49 (78)</td>
<td>43 (69)</td>
<td></td>
</tr>
<tr>
<td>Vomiting (n (%))</td>
<td>32 (51)</td>
<td>24 (39)</td>
<td></td>
</tr>
<tr>
<td>Diarrhoea (n (%))</td>
<td>52 (83)</td>
<td>54 (87)</td>
<td>45 (79)</td>
</tr>
<tr>
<td>Hepatomegaly (n (%))</td>
<td>43 (68)</td>
<td>41 (66)</td>
<td>41 (66)</td>
</tr>
<tr>
<td>Splenomegaly (n (%))</td>
<td>3 (5)</td>
<td>5 (8)</td>
<td>4 (6)</td>
</tr>
</tbody>
</table>
### Clinical Parameters

<table>
<thead>
<tr>
<th></th>
<th>Ofloxacin</th>
<th>Ofloxacin + Azithromycin</th>
<th>Azithromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haematocrit (%)</strong></td>
<td>34 (33-35, 26-47)</td>
<td>34 (33-34, 25-45)</td>
<td>34 (33-35, 23-43)</td>
</tr>
<tr>
<td><strong>White cell count (x10^9/L)</strong></td>
<td>7.2 (6.6-7.8, 2.9-14.1)</td>
<td>6.3 (5.9-6.7, 3.2-10.9)</td>
<td>7.2 (6.7-7.8, 1.7-13.4)</td>
</tr>
<tr>
<td><strong>Platelet count (x10^9/L)</strong></td>
<td>191 (171-212, 84-442)</td>
<td>194 (172-217, 50-480)</td>
<td>211 (184-239, 60-477)</td>
</tr>
<tr>
<td><strong>AST (IU/L)</strong> a</td>
<td>165 (132-198, 34-788)</td>
<td>166 (135-197, 37-592)</td>
<td>170 (125-176, 35-443)</td>
</tr>
<tr>
<td><strong>ALT (IU/L)</strong> b</td>
<td>109 (86-132, 16-437)</td>
<td>97 (78-115, 15-477)</td>
<td>113 (84-123, 10-471)</td>
</tr>
<tr>
<td><strong>Organism isolated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. Typhi/S. Paratyphi A</em></td>
<td>63/0</td>
<td>61/1</td>
<td>62/0</td>
</tr>
<tr>
<td>Multi-resistant n (%)</td>
<td>57 (90)</td>
<td>55 (89)</td>
<td>55 (89)</td>
</tr>
<tr>
<td>Nalidixic acid resistant n (%)</td>
<td>62 (98)</td>
<td>55 (89)</td>
<td>55 (89)</td>
</tr>
<tr>
<td><strong>Positive pre-treatment faecal cultures n (%)</strong></td>
<td>13/50 (26)</td>
<td>11/51 (22)</td>
<td>16/60 (27)</td>
</tr>
</tbody>
</table>

**Notes:**

- a. AST (aspartate transaminase), normal range: 20-40 IU/L
- b. ALT (alanine transaminase), normal range: 20-45 IU/L
TABLE 2.
Clinical and microbiological outcomes in 187 patients with culture confirmed uncomplicated typhoid fever.

<table>
<thead>
<tr>
<th></th>
<th>Ofloxacin</th>
<th>Ofloxacin + Azithromycin</th>
<th>Azithromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>63</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>Clinical failures: n (%)</td>
<td>23 (36.5)</td>
<td>15 (24.2)</td>
<td>11 (17.8)</td>
</tr>
<tr>
<td>Persistent fever and symptoms</td>
<td>23 (36.5)</td>
<td>14 (22.6)</td>
<td>9 (14.6)</td>
</tr>
<tr>
<td>Gastrointestinal haemorrhage</td>
<td>0 (0)</td>
<td>1 (1.6)</td>
<td>2 (3.2)</td>
</tr>
<tr>
<td>Blood culture positive post treatment n (%)</td>
<td>2 (3.2)</td>
<td>1 (1.6)</td>
<td>2 (3.2)</td>
</tr>
<tr>
<td>Fecal carriage immediately post treatment: n (%)</td>
<td>12/62 (19.4)</td>
<td>4/62 (6.5)</td>
<td>1/62 (1.6)</td>
</tr>
<tr>
<td>Fever clearance time (days) (mean, 95%CI, range)</td>
<td>8.2 (7.2-9.2, 2-24)</td>
<td>7.1 (6.2-8.1, 1-27)</td>
<td>5.8 (5.1-6.5, 1-13)</td>
</tr>
<tr>
<td>Duration of hospitalization after starting treatment (days) (mean, 95%CI, range)</td>
<td>13.7 (12.7-14.6, 9-32)</td>
<td>12.8 (12.0-13.7, 8-33)</td>
<td>12.6 (12.1-13.2, 10-19)</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td>Ofloxacin + Azithromycin</td>
<td>Azithromycin</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------------</td>
<td>--------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td><strong>Post treatment:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (IU/L, mean, 95% CI, range) $^a$</td>
<td>87 (67-108, 12-489)</td>
<td>111 (76-146, 27-632)</td>
<td>102 (79-125, 27-338)</td>
</tr>
<tr>
<td>ALT (IU/L, mean, 95% CI, range) $^b$</td>
<td>75 (62-88, 26-203)</td>
<td>83 (60-106, 12-426)</td>
<td>92 (73-110, 14-301)</td>
</tr>
<tr>
<td>Relapse: n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Fecal carriage during convalescence</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At the one month visit</td>
<td>2/54 (3.7)</td>
<td>2/54 (3.7)</td>
<td>3/52 (5.8)</td>
</tr>
<tr>
<td>At the three month visit</td>
<td>2/54 (3.7)</td>
<td>3/54 (5.8)</td>
<td>1/48 (2.6)</td>
</tr>
<tr>
<td>At the six month visit</td>
<td>0/48 (0)</td>
<td>1/55 (1.9)</td>
<td>0/48 (0)</td>
</tr>
<tr>
<td>At any time during six month follow up</td>
<td>4/58 (6.9)</td>
<td>6/58 (10.3)</td>
<td>4/56 (7.1)</td>
</tr>
</tbody>
</table>

$a.$ AST (aspartate transaminase), normal range: 20-40 IU/L;  
b. ALT (alanine transaminase), normal range: 20-45 IU/L
Table 3. Differences in clinical and microbiological outcomes between each antibiotic treatment group. Data are differences (95% CI)

<table>
<thead>
<tr>
<th></th>
<th>Ofloxacin compared with Ofloxacin/Azithromycin</th>
<th>Ofloxacin compared with Azithromycin</th>
<th>Ofloxacin/Azithromycin compared with Azithromycin</th>
<th>p-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical failures (%)</td>
<td>12.3 (-47.4, 28.3)</td>
<td>18.8 (3.6, 34.0)</td>
<td>6.4 (-7.9, 20.7)</td>
<td>0.053</td>
</tr>
<tr>
<td>Blood culture positive post treatment (%)</td>
<td>1.6 (-36.6, 39.8)</td>
<td>-0.6 (-41.3, 41.5)</td>
<td>1.6 (-38.6, 38.2)</td>
<td>0.818</td>
</tr>
<tr>
<td>Fecal carriage immediately post treatment (%)</td>
<td>12.9 (1.4, 28.0)</td>
<td>17.8 (7.6, 28.0)</td>
<td>4.9 (-2.0, 11.8)</td>
<td>0.006</td>
</tr>
<tr>
<td>Fever clearance time (days)</td>
<td>1.06 (-0.29, 2.41)</td>
<td>2.44 (1.25, 3.63)</td>
<td>1.38 (0.21, 2.55)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Duration of hospitalization after starting treatment (days)</td>
<td>0.85 (-0.41, 2.11)</td>
<td>1.04 (-0.03, 2.11)</td>
<td>0.19 (-0.81, 1.19)</td>
<td>0.166</td>
</tr>
<tr>
<td>Fecal carriage during convalescence at any time during six month follow up</td>
<td>- 3.4 (-13.2, 6.6)</td>
<td>- 0.2 (-9.1, 8.7)</td>
<td>3.2 (-6.7, 13.1)</td>
<td>0.908</td>
</tr>
</tbody>
</table>

* overall p value using chi squared or log rank test
Figure 1.

241 adults and children with suspected enteric fever

199 patients culture positive (198 S.Typhi, 1 S.Paratyphi A)

80 patients randomised to ofloxacin

67 patients culture positive

3 pretreated
1 self discharged

63 patients completed study

57 (90%) isolates MDR
62 (98%) isolates NaR

81 patients randomised to ofloxacin/azithromycin

63 patients culture positive

1 pretreated

62 patients completed study

55 (89%) isolates MDR
56 (90%) isolates NaR

80 patients randomised to azithromycin

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53 (85%) isolates MDR
55 (89%) isolates NaR

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55 (89%) isolates MDR
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80 patients randomised to azithromycin

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53 (85%) isolates MDR
55 (89%) isolates NaR
Figure 2

Graph showing the percentage of patients still febrile over days after the start of treatment, comparing Azithromycin, Oflox/Azith, and Ofloxacin.