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The Influence of Reduced Susceptibility to Fluoroquinolones in *Salmonella enterica* serovar Typhi on the Clinical Response to Ofloxacin Therapy

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Abstract

**Background:** Infection with *Salmonella enterica* serovar Typhi (*S. Typhi*) with reduced susceptibility to fluoroquinolones has been associated with fluoroquinolone treatment failure. We studied the relationship between ofloxacin treatment response and the ofloxacin minimum inhibitory concentration (MIC) of the infecting isolate. Individual patient data from seven randomised controlled trials of antimicrobial treatment in enteric fever conducted in Vietnam in which ofloxacin was used in at least one of the treatment arms was studied. Data from 540 patients randomised to ofloxacin treatment was analysed to identify an MIC of the infecting organism associated with treatment failure.

**Principal Findings:** The proportion of patients failing ofloxacin treatment was significantly higher in patients infected with *S. Typhi* isolates with an MIC $\geq 0.25$ μg/mL compared with those infections with an MIC of $\leq 0.125$ μg/mL ($p<0.001$). Treatment success was 96% when the ofloxacin MIC was $\leq 0.125$ μg/mL, 73% when the MIC was between 0.25 and 0.50 μg/mL and 53% when the MIC was 1.00 μg/mL. This was despite a longer duration of treatment at a higher dosage in patients infected with isolates with an MIC $\geq 0.25$ μg/mL compared with those infections with an MIC of $\leq 0.125$ μg/mL.

**Significance:** There is a clear relationship between ofloxacin susceptibility and clinical outcome in ofloxacin treated patients with enteric fever. An ofloxacin MIC of $\geq 0.25$ μg/mL, or the presence of nalidixic acid resistance, can be used to define *S. Typhi* infections in which the response to ofloxacin may be impaired.

Introduction

Enteric fever is a systemic infection caused by *Salmonella enterica* serovar Typhi (*S. Typhi*) and *Salmonella enterica* serovar Paratyphi A (*S. Paratyphi A*) [1]. Antimicrobial therapy is critical for the clinical management of enteric fever. The emergence and sustained circulation of antimicrobial resistant organisms have become problematic in many endemic regions [1,2]. Multiple drug resistant *S. Typhi* (MDR: resistance to chloramphenicol, trimethoprim/sulfamethoxazole and ampicillin) have been widespread since the early 1990’s and hinder effective treatment and limit alternatives.

Fluoroquinolones are commonly used for treating enteric fever and have been recommended by the WHO for the treatment of uncomplicated enteric fever caused by fully sensitive and MDR organisms [3]. Ciprofloxacin and ofloxacin were chosen for treating typhoid because of potent bactericidal activity against *S. Typhi* and *S. Paratyphi A*, in *vivo*, both drugs have plasma levels considerably in excess of the prevailing MICs and excellent intracellular penetration [4]. Widespread use of fluoroquinolone therapy for enteric fever has been followed by the emergence of *S. Typhi* and *S. Paratyphi A* isolates with elevated minimum inhibitory concentrations (MIC) to ciprofloxacin and ofloxacin across Asia and in parts of Africa [5,6,7,8,9]. These strains are associated with point mutations in the *gyrA* gene and occasionally the *parC* gene [5,7,9,10,11,12]. To date, there have been few published reports of plasmid mediated quinolone resistance (PMQR) genes, such as *qnr*, *aac(6’)-Ib-cr*, or efflux pumps, in *S. Typhi* as described in some non-typhoidal *Salmonella* serovars [2,13]. The identification of strains with reduced susceptibility to fluoroquinolones is important to guide treatment, yet these strains are categorised as susceptible by the current guidelines for fluoroquinolone disk susceptibility testing [14,15,16]. Such isolates can be identified by using resistance to nalidixic acid as a surrogate marker of fluoroquinolone susceptibility, although this is not completely reliable [17,18]. Enteric fever caused by *S. Typhi* strains with an elevated MIC to ciprofloxacin and ofloxacin have been coupled with the failure of treatment with these antimicro-

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Author Summary

Typhoid fever is an infection of the bloodstream caused by the organism Salmonella Typhi (S. Typhi). Treatment with antimicrobials is critical for preventing severe infection and even death, yet antimicrobial resistant organisms have become a problem in many places where typhoid is common. Fluoroquinolones are a group of antimicrobials that are commonly used to treat typhoid, we analysed data from 540 enteric fever patients treated with ofloxacin (a fluoroquinolone) to identify a level of resistance (minimum inhibitory concentration (MIC)) from the infecting organism which is associated with treatment failure. The proportion of patients failing treatment was higher in those infected with a bacterium with an MIC $\leq 0.125$ µg/mL compared with those infections with an MIC of $\leq 0.125$ µg/mL. Treatment success was 96% when the ofloxacin MIC was $\leq 0.125$ µg/mL, yet only 53% when the MIC was 1.00 µg/mL. Our data demonstrates that an S. Typhi bacterium with an ofloxacin MIC of $\geq 0.25$ µg/mL correlates with a poor outcome when treated with this antimicrobial. Therefore, we propose an amendment in the current MIC guidelines for microbiology laboratories to aid clinicians treating typhoid and suggest the use of alternative therapy in these patients.

Clinical procedures

Patients with suspected uncomplicated enteric fever were allocated to one of the treatment groups in an open randomised comparison. A computer generated randomisation list was produced by a member of staff that was not otherwise involved in the study. The treatment allocations were kept in serially numbered sealed opaque envelopes that were only opened after the patient had been enrolled into the study. The treatment arms were ofloxacin (Olopect, Hoechst Marion Roussel, Paris, France) at a dose that varied between 10 and 20 mg/kg/day orally in two-divided dose (maximum 400 mg twice daily) for between two and seven days (depending on the study, or the comparator). The comparator arms were, either, a different regimen of ofloxacin (in three RCTs) [24,26,28] ceftriaxone [23], cefixime [29], or azithromycin (in two RCTs) [23,27].

Patients were excluded if they refused consent, had evidence of progressive or complicated disease, had inability to swallow oral medication, had a history of significant underlying disease, had hypersensitivity to either of the trial drugs or were pregnant or lactating. Additionally, patients who gave a history of treatment with a fluoroquinolone, a third generation cephalosporin or a macrolide within one week of hospital admission were also excluded.

Methods

Ethics statement

The study was conducted according to the principles expressed in the Declaration of Helsinki and approved by the Institutional Review Board of the Hospital for Tropical Diseases and the additional hospital involved in the studies. All patients provided verbal informed consent (verbal informed consent was provided by the parents or guardian of children under 18 years of age) for the collection of samples and subsequent analysis.

Study setting

We analysed the results of seven open RCTs for enteric fever conducted in southern Vietnam between 1992 and 2001 in which treatment with ofloxacin was used in at least one of the trial arms. All the RCTs were conducted using a standard protocol, except for the dose and duration of ofloxacin treatment and the alternative treatment regimens used. The RCTs were conducted at three study sites in southern Vietnam: The Hospital for Tropical Diseases, Ho Chi Minh City [23,24,25,26]; Dong Thap Provincial Hospital, Cao Lãnh, Dong Thap Province [27,28] and Dong Nai Provincial Hospital, Bien Hoa, Dong Nai Province [29]. All studies were approved by the Ethical Committee of the Hospital conducting the study. The studies were conducted in accordance with ICH and Declaration of Helsinki guidelines.

Laboratory investigations and microbiology

A hematocrit, white cell, platelet count and blood differential count were performed with serum aspartate transaminase, alanine transaminase, creatinine levels and urinalysis before therapy was initiated. Aspartate transaminase and alanine transaminase measurements were repeated one day after the end of therapy.
A full blood count was repeated if there was a suggestion of gastrointestinal bleeding or clinical evidence of anaemia. Blood and fecal cultures were obtained before therapy and in one study a bone marrow was also performed in selected patients [27]. A blood culture was performed on 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 and at the one month follow up visit. Bacterial culturing was performed as previously described [23,24,25,26,27,28,29], colonies presumptive of S. Typhi were identified using standard biochemical tests and serotype-specific antisera (Murex Biotech, Dartford, England).

Antimicrobial sensitivities were performed by the modified Bauer-Kirby disc diffusion method with zone size interpretation based on CLSI guidelines [30]. Antimicrobial discs tested were chloramphenicol (30 μg), ampicillin (10 μg), trimethoprim-sulphamethoxazole (1.25/23.75 μg), ceftriaxone (30 μg), ofloxacin (5 μg), azithromycin (15 μg) and nalidixic acid (30 μg). Isolates were stored in protect beads (Prolabs, Oxford, United Kingdom) at −20°C for later ofloxacin MIC testing by agar plate dilution [31]. Escherichia coli ATCC25922 and Staphylococcus aureus ATCC25923 were used as control strains for these assays. An isolate was defined as MDR if it was resistant to chloramphenicol ($≥$32 μg/ml), ampicillin ($≥$32 μg/ml) and trimethoprim/sulphamethoxazole ($≥$8/152 μg/ml). An isolate was defined as nalidixic acid resistant if it had an MIC of $≥$32 μg/ml [15].

**Statistical analysis**

Analysis was restricted to patients in whom S. Typhi was isolated from blood or bone marrow culture prior to treatment with ofloxacin and in whom the ofloxacin MIC of the original infecting isolate had been determined. The pooled admission and outcome data for individual patients was compiled with respect to the ofloxacin MIC of the original infecting isolate. Proportions were compared with the Chi squared test, Fisher’s exact test or analysis of variance. Normally distributed data were compared using the Student t-test, non-normally distributed data using the Mann Whitney U test or Kruskall Wallis test. The fever clearance time was compared using survival analysis and the log rank test. Independent risk factors for clinical failure in the clinical trials were determined by multivariate logistic regression, a \( p \) value of $<0.05$ was considered significant. Statistical analysis was performed using SPSS for Windows version 10 (SPSS Inc, Chicago, USA).

**Results**

Clinical features of enteric fever patients treated with ofloxacin

A total of 540 patients infected with S. Typhi, treated with ofloxacin and in whom the ofloxacin MIC of the isolate was known from seven RCTs were available for analysis. The distribution of ofloxacin MIC values across the different trials is shown in Figure 1. The clinical features of the patients at the time of admission in relation to the ofloxacin MIC of the infecting S. Typhi isolate are shown in Table 1. There was heterogeneity among the six MIC (to ofloxacin) groups ($≤$0.06, 0.064, 0.125 μg/ml, 0.25 μg/ml, 0.50 μg/ml and 1 μg/ml) with respect to a number of features, including the number of
patients in each group. Abdominal pain, vomiting and positive fecal cultures were more frequently observed in patients infected with an isolate with a higher ofloxacin MIC. Nadolixic acid resistance was observed in none of the 423 S. Typhi isolates with an ofloxacin MIC of $\leq 0.125$ μg/mL and 104/105 (99%) of those with an MIC $> 0.125$ μg/mL (p < 0.001) (Table 1).

Clinical response of enteric fever patients to ofloxacin therapy

Details of the duration and dosages of ofloxacin treatment and the corresponding response to therapy are shown in Table 2. The median duration of therapy for patients infected with S. Typhi isolates with an MIC $< 0.125$ μg/mL was three days at a median dose of 11 mg/kg. In patients infected with S. Typhi isolates with an MIC of between 0.125–0.25 μg/mL the median duration of therapy was three days at a median dose of 13–15 mg/kg. For patients infected with isolates with an MIC $> 0.25$ μg/mL the median duration of therapy was seven days at a dose of 16–18 mg/kg.

We calculated the ratio of the administered dose of ofloxacin (mg/kg) to the MICs of the infecting isolates. The ratio of administered dose to bacterial MIC declined from 370 in patients infected with an S. Typhi isolate with an MIC of $\leq 0.03$ μg/mL to 18 in those infected with an S. Typhi isolate with an MIC of 1.0 μg/mL. Despite a longer duration of treatment at a higher dosage, the proportion of patients failing treatment was significantly higher in the patients infected with S. Typhi isolates with an MIC $> 0.25$ μg/mL compared to those infected with isolates with an MIC of $\leq 0.125$ μg/mL (p < 0.001). Concurrently, the time to fever clearance was significantly longer in the patients with a higher MIC (Figure 2 and Table 2). There was an evident relationship between fever clearance time and the MIC of the infecting organism, as shown in Figure 3. Furthermore, the proportion of patients with a positive faecal culture, immediately post study treatment and at one month follow-up was also significantly greater among the patients infected with an isolate with a higher MIC to ofloxacin, p < 0.001 and p = 0.001 respectively (Table 2).

Of the 540 patients, 15 (2.8%) developed a potentially life threatening complication during the course of treatment (gastro-intestinal bleed requiring transfusion, hemodynamic shock, suspected myocarditis, encephalopathy, pneumonia) and one patient died of suspected myocarditis and shock. There was no significant difference in the rate of life-threatening complications for the patients infected with isolates with different ofloxacin MICs (Table 2).

Predicting clinical failure to ofloxacin therapy in enteric fever patients

Univariate analysis was performed to identify the factors that were associated with clinical failure. The presence of abdominal pain (p < 0.001), diarrrhoea (p = 0.03), vomiting (p < 0.001), lower hematocrit (p = 0.03), lower white cell count (p = 0.04), lower platelet count (p = 0.002), infection with an S. Typhi organism with a higher ofloxacin MIC (p < 0.001), prolonged duration of ofloxacin treatment (p < 0.001) and a higher administered dose of ofloxacin (p = 0.009) were all associated with clinical failure. Duration of therapy was deliberately increased as the MIC increased. If this factor is not included in the model, the independent variables associated with clinical failure with ofloxacin treatment using a multivariate logistic regression model were, abdominal pain, Odds Ratio (OR) 0.461, 95% confidence Interval (95% CI) 0.233–0.969, p = 0.033, vomiting; OR 0.427, 95% CI; 0.208–0.876, p = 0.020, hematocrit; OR 0.924, 95% CI; 0.861–0.992, p = 0.033, platelet count; OR 0.990, 95% CI; 0.984–1.00, p = 0.001 and the ofloxacin MIC of the infecting isolate; OR 17.08, 95% CI; 6.62–44.04, p < 0.001.

To define an appropriate ofloxacin MIC breakpoint and the use of nalidixic acid resistance to define clinical failure to ofloxacin, the clinical success rate in each MIC group was calculated (Table 3). The data stratified by MIC to ofloxacin suggests a treatment
Table 2. Treatment response of 540 ofloxacin treated enteric fever patients recruited to clinical trials.

<table>
<thead>
<tr>
<th>Treatment and outcome*</th>
<th>Ofloxacin MIC (µg/mL) of infecting isolate of S.Typhi</th>
<th>( p^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \leq 0.032 )</td>
<td>0.064</td>
</tr>
<tr>
<td>Number of patients</td>
<td>152</td>
<td>271</td>
</tr>
<tr>
<td>Treatment duration (days)</td>
<td>3 (2–3)</td>
<td>3 (2–3)</td>
</tr>
<tr>
<td>Mean ofloxacin dose/kg</td>
<td>11 (9–14)</td>
<td>11 (10–13)</td>
</tr>
<tr>
<td>Mean ofloxacin dose/MIC</td>
<td>370 (317–489)</td>
<td>175 (167–222)</td>
</tr>
<tr>
<td>Clinical failure [n (%)]</td>
<td>4 (3)</td>
<td>13 (5)</td>
</tr>
<tr>
<td>Microbiological failure [n(%)]</td>
<td>1 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Fever clearance time (days)</td>
<td>3.1 (2.5–4.2)</td>
<td>3.8 (2.8–4.8)</td>
</tr>
<tr>
<td>Life-threatening complication [n(%)]</td>
<td>5 (3.3)</td>
<td>9 (3.3)</td>
</tr>
<tr>
<td>Post study feces positive [n (%)]</td>
<td>1/120 (1)</td>
<td>4/229 (2)</td>
</tr>
<tr>
<td>1 month feces positive [n (%)]</td>
<td>0/61 (0)</td>
<td>0/83 (0)</td>
</tr>
<tr>
<td>Relapse [n (%)]</td>
<td>2/61 (3)</td>
<td>4/87 (5)</td>
</tr>
</tbody>
</table>

*Continuous variables given as medians (interquartile range), and proportions as numbers (%).

**Analysis of variance for proportions, Kruskal Wallis test for continuous variables and Log rank test for fever clearance time.

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Figure 2. The relationship between increasing S. Typhi MIC to ofloxacin and clinical failure. Histogram showing the proportion of enteric fever patients who failed treatment (white columns) or had persistent fever (black columns) (>38°C) for more than seven days after the commencement of treatment. Data was combined from seven randomised clinical trials and is comprised from 540 children and adults recruited with uncomplicated enteric fever. The patients are divided according to the MIC to ofloxacin of the infecting isolate.

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success rate with ofloxacin given for a median duration of 3 days at 11–13 mg/kg of 96% in 435 patients infected with an isolate with an ofloxacin MIC of \(0.125\) mg/mL; 73% in 67 patients infected with isolate with an ofloxacin MIC between 0.25 and 0.5 mg/mL with treatment durations between 3 to 7 days at 15–16 mg/kg; and 53% in 38 patients infected with an isolate with an ofloxacin MIC of 1.00 mg/mL despite a median duration of treatment of 7 days at 18 mg/kg. The success rate in 434 patients infected with a nalidixic acid susceptible isolate was 96% compared with a 65% success rate in the 106 patients infected with a nalidixic acid resistant isolate.

**Discussion**

In this study we used individual patient data from seven randomised controlled trials to characterise the relationship between ofloxacin susceptibility and outcome in ofloxacin treated patients with uncomplicated enteric fever. There was clear relationship between a higher ofloxacin MIC of the infecting isolate and a declining response to ofloxacin. Although the trials were conducted according to a standard protocol, the duration and dosage of ofloxacin treatment was not standard across all patient groups and the duration for some patients was shorter than would be routinely recommended. Despite this, a successful response in 96% of patients to an average duration of treatment of three days among infections with an ofloxacin MIC \(\leq 0.25\) mg/mL indicates the efficacy of ofloxacin when isolates are fully susceptible and justifies comparison with the response to infections where the isolate had a higher MIC and the ofloxacin dose used was higher and duration longer.

Our data suggests an MIC breakpoint for ofloxacin of \(\geq 0.25\) mg/mL or the presence of nalidixic acid resistance could be used to define infections in which the response to ofloxacin is impaired. The ciprofloxacin MIC is usually one dilution less than ofloxacin, and this implies a ciprofloxacin breakpoint of \(\geq 0.125\) mg/mL, a breakpoint already suggested [32,33]. The small number of infections with isolates with an MIC of 0.125 mg/mL is a limitation and more information on the response to treatment at this MIC would be valuable. As nalidixic acid resistance is not a reliable proxy marker for reduced fluoroquinolone susceptibility.
Table 3. Ofloxacin MIC breakpoints for S. Typhi infection which predict ofloxacin treatment success.

<table>
<thead>
<tr>
<th>Breakpoint value</th>
<th>Number successfully treated/total number treated (%)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin MIC&lt;0.06 μg/mL</td>
<td>148/152 (97.4%) 144</td>
<td>5.47 (1.95–21.20)</td>
</tr>
<tr>
<td>Ofloxacin MIC=0.06 μg/mL</td>
<td>338/388 (87.1%) 314</td>
<td>10.00 (4.08–24.29)</td>
</tr>
<tr>
<td>Ofloxacin MIC=0.12 μg/mL</td>
<td>406/423 (96.0%) 11.00 (5.71–21.88)</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin MIC=0.25 μg/mL</td>
<td>69/105 (65.7%) 12.09 (6.24–23.81)</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin MIC=0.50 μg/mL</td>
<td>432/455 (94.9%) 10.78 (5.6–20.77)</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin MIC=1.00 μg/mL</td>
<td>47/45 (52.6%) 11.65 (5.26–25.36)</td>
<td></td>
</tr>
<tr>
<td>Nalidixic acid susceptible</td>
<td>417/434 (96.1%)</td>
<td>13.15 (6.74–26.20)</td>
</tr>
<tr>
<td>Nalidixic acid resistant</td>
<td>69/106 (65.1%)</td>
<td>10.46 (4.01–27.06)</td>
</tr>
</tbody>
</table>

References


