Highly resistant *Salmonella Typhi* with a novel *gyrA* mutation questions the long-term efficacy of older fluoroquinolones for treating typhoid fever

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**Running title**
Fluoroquinolone resistant *Salmonella Typhi*

**Abstract**
As a consequence of multi-drug resistance, clinicians are highly dependent on fluoroquinolones for treating the serious systemic infection typhoid fever. Whilst reduced susceptibility to fluoroquinolones, which lessens clinical efficacy, is becoming ubiquitous, comprehensive resistance is exceptional. Here we report ofloxacin treatment failure in typhoidal patient infected with a novel, highly fluoroquinolone resistant *Salmonella Typhi*. The isolation of this organism has serious implications for the long-term efficacy of ciprofloxacin and ofloxacin for typhoid treatment.
Antimicrobial therapy is critical for typhoid treatment, but the circulation of antimicrobial resistant organisms has become ubiquitous in many endemic regions and the presence of multi-drug resistant *Salmonella Typhi* and *Salmonella Paratyphi A* (resistance to chloramphenicol, trimethoprim/sulfamethoxazole and ampicillin) precludes treatment with these antimicrobials (7). Clinicians are now highly dependent on the fluoroquinolones for typhoid therapy. Yet, predictably, widespread fluoroquinolone usage has been followed by the emergence of isolates with elevated minimum inhibitory concentrations (MICs) (1). These isolates are characterized by point mutations within the *gyrA* (DNA gyrase) gene and occasionally an additional nucleotide substitution in the *parC* gene (8). The propagation of these organisms is particularly concerning when one considers a lack of feasible alternatives and an explicit correlation between increasing MIC to fluoroquinolones and treatment failure (9). Yet, despite the widespread dissemination of such organisms, the isolation of organisms that exhibit MICs of > 1.0 µg/mL to ofloxacin has been, until now, negligible.

In June 2011, a 13-year-old male presented to the outpatients department at Patan hospital in Kathmandu with a fever that had started 10 days previously, peaking at around 39°C daily. The patient also developed a headache and mild abdominal discomfort with nausea. On the eighth day of fever, the patient was taken to a local medical store where he was recommended 200mg of ofloxacin to be taken twice daily. After two days of ofloxacin the patient’s symptoms became more pronounced with increased restlessness and a temperature in excess of 40°C. He presented to the outpatients department on the tenth day of illness but had no signs of icterus, anemia, lymphadenopathy, cyanosis, edema or dehydration and no rash on the trunk. His temperature was recorded at 38.8°C, with a pulse of 104/min and respiratory rate of 24/min. A clinical diagnosis of typhoid was made and a complete blood count and culture and sensitivity were requested. The treating clinician increased the ofloxacin dosage to 300mg (20mg/kg/day) twice daily for seven
days. However, the patient returned to the outpatients department after these additional seven
days of ofloxacin, without improvement. In the intervening period, his blood culture had yielded
*Salmonella Typhi*, which was highly resistant to ofloxacin (zone size; 11 mm), ciprofloxacin
(zone size; 11 mm) and nalidixic acid (zone size; 0 mm) a secondary blood culture was not taken.
On these findings, he was prescribed oral azithromycin (20mg/kg/day) once daily for seven days.
The fever declined on the third day of the azithromycin treatment (the twentieth day of fever)
and, ultimately, the patient made a fortuitous and uneventful recovery.

After the patient had recovered from the infection, and with written consent, we investigated the
*Salmonella* Typhi isolate. The resulting fluoroquinolone MICs were exceptional; in excess of 256
µg/ml against nalidixic acid, greater than 32 µg/ml against ofloxacin and ciprofloxacin, 6 µg/ml
against levofloxacin and 2 µg/ml against gatifloxacin. The *Salmonella Typhi* isolate, was not,
however, multi-drug resistant. We purified DNA from the *Salmonella Typhi* isolate, aiming to
define the molecular basis of the fluoroquinolone resistance. We PCR amplified the *gyrA*, *gyrB*
and the *parC* genes and sequenced the resultant PCR amplicons (1). We also attempted to amplify
the common Gram-Negative plasmid mediated quinolone resistance (PMQR) determinants; *qnrA,*
*qnrB*, *qnrS*, *aac(6)lb-cr* and *qepA* (5). We were unable to detect any of the five common PMQR
genes. Yet, DNA sequencing of the three fluoroquinolone target loci identified a single
previously described mutation in *parC* gene, changing serine to isoleucine at codon 80 (S80I),
and a double mutation in the *gyrA* gene (Figure 1). The two-*gyrA* mutations were both within the
quinolone resistance-determining region (QRDR). The primary *gyrA* substitution was common,
inducing a replacement of serine to phenylalanine at codon 83. However, the second mutation
was novel, the substitution of a Cytosine to Thymine at nucleotide 248 had the effect of changing
aspartic acid to valine at codon 87 (Figure 1). The resulting DNA sequence was submitted to
EMBL (Accession number: HE588040).
Antimicrobials are crucial for treating typhoid and resistance is the main constraint that compels antimicrobial therapy preferences to change with time. The current WHO guidelines suggest that the fluoroquinolones are the optimal group of antimicrobials uncomplicated typhoid treatment in adults (6). However, *Salmonella Typhi* and *Salmonella Paratyphi A* isolates with reduced susceptibility to fluoroquinolones are now common in Asia and are becoming increasingly common in Africa (2, 10), yet complete resistance is rare (4). Here we report an isolate exhibiting extensive resistance against fluoroquinolones, marked by a novel *gyrA* mutation and no additional PMQR sequences. Clearly, additional characterization of this isolate is required to precisely understand the basis of its phenotype, yet its isolation should ring alarm bells within the typhoid community. Indeed, one may speculate that other such strains will emerge rapidly, should the organism have acceptable biological fitness and favorable dissemination conditions. Anecdotally, we find that chloramphenicol is making a comeback in the community in Nepal as a consequence of reducing resistance levels; yet, azithromycin will likely become the preferred drug as ofloxacin and ciprofloxacin become ineffective (3).

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**References**


Figure 1. DNA and predicted Amino acid alignments of the DNA gyrase gene (gyrA) from Salmonella Typhi isolates with reduced susceptibility to fluoroquinolones

Outline of seven identified conformations of the QRDR region of the DNA gyrase gyrA in clinical Salmonella Typhi isolates from Parry et al. 2010, and the novel S83F/D87V mutation isolated here. The DNA and the corresponding Amino acid consensus are shown in the first and second rows of the figure, respectively, with the DNA identity between sequences shown beneath. For each conformation the DNA sequence is shown at the top and the predicted Amino
acid sequence is shown beneath. The median MIC to ofloxacin for each of the mutations (with and without a tertiary S80I mutation in the ParC topoisomerase) is shown to the right, data taken from this report and reference 3.