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Molecular characterization of ciprofloxacin-resistant *Salmonella enterica* serovar Typhi and Paratyphi A causing enteric fever in India

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***Objectives*: To define the genetic characteristics and resistance mechanisms of clinical isolates of *Salmonella enterica* serovar Typhi (*S.* Typhi) and *S. enterica* serovar Paratyphi A (*S.* Paratyphi A) exhibiting high-level fluoroquinolones resistance.**

***Methods*: Three *S.* Typhi and two *S.* Paratyphi A ciprofloxacin-resistant isolates (MICs > 4 mg/L) were compared with isolates with reduced susceptibility to ciprofloxacin (MICs 0.125–1 mg/L) by PFGE, plasmid analysis, presence of integrons and nucleotide changes in topoisomerase genes.**

***Results*: In *S.* Typhi and Paratyphi A, a single *gyrA* mutation (Ser-83→Phe or Ser-83→Tyr) was associated with reduced susceptibility to ciprofloxacin (MICs 0.125–1 mg/L); an additional mutation in *parC* (Ser-80→Ile, Ser-80→Arg, Asp-69→Glu or Gly-78→Asp) was accompanied by an increase in ciprofloxacin MIC (≥ 0.5 mg/L). Three mutations conferred ciprofloxacin resistance: two in *gyrA* (Ser-83→Phe and Asp-87→Asn or Asp-87→Gly) and one in *parC*. This is the first report of *parC* mutations in *S.* Typhi. Ciprofloxacin-resistant *S.* Typhi and *S.* Paratyphi A differed in their MICs and mutations in *gyrA* and *parC*. Moreover *S.* Typhi harboured a 50 kb transferable plasmid carrying a class 1 integron (*dfrA15/aadA1*) that confers resistance to co-trimoxazole and tetracycline but not to ciprofloxacin. PFGE revealed undistinguishable *Xba*I fragment patterns in ciprofloxacin-resistant *S.* Typhi as well as in *S.* Paratyphi A isolates and showed that ciprofloxacin-resistant *S.* Typhi have emerged from a clonally related isolate with reduced susceptibility to ciprofloxacin after sequential acquisition of a second mutation in *gyrA*.**

***Conclusions*: To our knowledge this is the first report of molecular characterization of *S.* Typhi with full resistance to ciprofloxacin. Notably, the presence of a plasmid-borne integron in ciprofloxacin-resistant *S.* Typhi may lead to a situation of untreatable enteric fever.**

Keywords: *Salmonella enterica* serovar Paratyphi A, DNA gyrase, topoisomerase IV, integrons, high-level fluoroquinolone resistance

Introduction

Typhoid fever is a major cause of morbidity and mortality with an estimated global incidence of 21.6 million cases and 216 510 deaths per year.[1] In developing countries, its annual incidence ranges from 12 to 622/100 000 persons.[2] *Salmonella enterica* serovar Typhi (*S.* Typhi) is responsible for the majority of cases followed by *S. enterica* serovar Paratyphi A (*S.* Paratyphi A) that causes 20% of the cases. In the last two decades, the worldwide emergence of multidrug-resistant strains of *Salmonella* has led to virtual withdrawal of chloramphenicol and its replacement with fluoroquinolones and third-generation cephalosporins.[2] However, in 1997 the first major outbreak of typhoid fever with strains resistant to nalidixic acid was reported from Tajikistan.[3] Nalidixic-acid-resistant strains exhibiting reduced susceptibility to ciprofloxacin (MICs 0.125–1 mg/L) have become endemic in

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several geographical areas of the Indian subcontinent and have also been reported in the US, in the UK and in other developed countries, reflecting the emergence of a global problem.[4–6] Clinical treatment failures after the administration of ciprofloxacin and other fluoroquinolones to patients with typhoid fever attributable to these strains have been reported.[4,5]

The emergence of complete resistance to ciprofloxacin in *S*. Typhi or *S.* Paratyphi A would severely limit the choice of antimicrobial therapy for treating enteric fever. Recent reports of infections because of strains of *S*. Paratyphi A with high-level resistance to fluoroquinolones are therefore particularly worrying.[7–9] The targets of fluoroquinolones are the two enzymes DNA gyrase and topoisomerase IV, whose subunits are encoded respectively by *gyrA* and *gyrB* and the *parC* and *parE* genes. The alteration caused by single point mutations within the quinolone resistance-determining region (QRDR) of the DNA gyrase subunit *gyrA* gene leads to quinolone resistance (i.e. decreased susceptibility to ciprofloxacin).[4] In *Salmonella*, the most common residues associated with mutation leading to quinolone resistance have been Ser-83 and Asp-87 in the *gyrA* gene, either alone or together.[4,10–12] Additional mutations may be required to attain high-level fluoroquinolone resistance.[13,14] Complete fluoroquinolone resistance in the Enterobacteriaceae usually results from two or more point mutations within the QRDRs of the DNA gyrase and topoisomerase IV genes.[13,14]

Here, we report five cases of enteric fever caused by strains of *S*. Typhi and *S*. Paratyphi A with complete resistance to ciprofloxacin (MICs > 4 mg/L). Molecular characterization of *S*. Paratyphi A with fluoroquinolone resistance has been described previously.[9] To our knowledge this is the first report of molecular characterization of *S.* Typhi showing a full fluoroquinolone resistance phenotype causing enteric fever. The molecular characteristics of ciprofloxacin-resistant isolates of *S*. Typhi and Paratyphi A were compared with those of strains fully susceptible to ciprofloxacin and with reduced susceptibility to ciprofloxacin. PFGE analysis was performed, the presence of class 1 and 2 integrons and mutations in the genes encoding topoisomerases was determined, and the transfer of antibiotic resistance was studied.

Materials and methods

A total of 377 blood culture positive cases of enteric fever were diagnosed between 2001 and 2003 at Safdarjung Hospital, a tertiary care centre in New Delhi, Northern India. During this period, a significant increase in infections caused by *S*. Typhi and Paratyphi A with reduced susceptibility to fluoroquinolones was observed, from 56.9% in 2001 to 88.9% in 2003.[7] In May 2003 the first case of enteric fever attributable to *S*. Paratyphi A with resistance to ciprofloxacin (MIC 8 mg/L) was reported from the paediatric outpatient department. Since then four further cases of enteric fever attributable to ciprofloxacin-resistant strains of *S*. Typhi and Paratyphi A have been diagnosed.

Bacterial strains

A total of 12 isolates, which included 8 *S*. Typhi and 4 *S*. Paratyphi A strains isolated from blood cultures of patients suffering from enteric fever between May 2003 and December 2004, were studied. These included five ciprofloxacin-resistant strains, three *S.* Typhi and two *S*. Paratyphi A strains with reduced susceptibility to ciprofloxacin and two antimicrobial-susceptible *S.* Typhi strains. The isolates were identified by standard biochemical tests and agglutination using specific antisera (Murex Diagnostics Ltd, UK).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using a disc diffusion method according to NCCLS (now CLSI) guidelines.[15] Chloramphenicol, ampicillin, co-trimoxazole, ceftriaxone, ciprofloxacin, nalidixic acid, streptomycin and tetracycline were tested. MICs of ciprofloxacin were determined by agar dilution and final analysis was done using an Etest kit (AB Biodisk, Solna Sweden). The readings were interpreted using NCCLS breakpoint criteria. Inhibition zone diameters $\leq$13 mm, $\geq$19 mm and 14–18 mm around the nalidixic acid disc were used to define nalidixic-acid-resistant, -susceptible and -intermediately-susceptible strains, respectively. Reduced ciprofloxacin susceptibility was defined as isolates with MICs of 0.125–1 mg/L. Strains with MICs >4 mg/L were defined as ciprofloxacin resistant. Strains resistant to ampicillin, chloramphericol and co-trimoxazole with or without resistance to tetracycline and streptomycin were defined as multidrug resistant (MDR).

Plasmid profile typing

Plasmid DNA was extracted from all isolates and from transconjugants by the alkaline lysis method with minor modifications.[16] The *Escherichia coli* reference strains V517 and 39R861 were used as molecular standards for the determination of plasmid sizes.

Mating experiments

To test the transmissibility of resistance, mating experiments were performed using an *E. coli* K-12 strain resistant to ampicillin and kanamycin as recipient.[17] Putative transconjugants on agar plates were confirmed by lactose fermentation and/or failure to agglutinate Omni-O antisera. *E. coli* K-12 transconjugants were tested by disc diffusion for antibiotic susceptibility, analysed for the presence of the transferable resistance-encoding plasmid and subjected to PCR to amplify the *intI1* gene.

PCR amplification of integrons

Strains were screened for the presence of integrons with specific primers for the integrase genes *intI1* and *intI2* as described previously by Ploy *et al*.[18] Analysis of the class 1 integron variable region was performed on *intI1*-positive strains by PCR amplification with primers 5′-CS and 3′-CS,[19] followed by restriction fragment length polymorphism (RFLP) analysis with *Hin*fI (Promega). Strains with identical RFLP were considered identical and one representative sample was subjected to sequencing (CRIBI, Padova).

Amplification of QRDR sequences of gyrA, gyrB, parC and parE genes

All PCRs were performed on a PCR Express, Hybaid cycler using as a template total DNA prepared according to Ausubel *et al*.[20] Four primer pairs described by Kariuki *et al*.[21] were used. Purified PCR products of ciprofloxacin-resistant and nalidixic-acid-susceptible, -resistant and -intermediately-susceptible strains were sequenced to determine whether mutations had occurred in these genes (BMR, University of Padova). NCBI BLAST was used to align the amplified sequences with the genome sequence of serovar Typhi strain CT18 (accession number AL513382).

Table 1. Patterns of antibiotic resistance, plasmid profiles and characteristics of integrons of ciprofloxacin-susceptible and ciprofloxacin-resistant clinical isolates of *S*. Typhi and *S*. Paratyphi A and *E. coli* transconjugants

Isolates		R-type	Ciprofloxacin MIC (mg/L)	Plasmid	Transconjugant R pattern	*intI1* gene	VR size	Putative gene cassettes
ST 31/3	*S*. Typhi		0.003	–	–	none	–	–
ST 419/2	*S*. Typhi		0.025	–	–	none	–	–
ST 512/6	*S*. Typhi	NAL[I]	0.5	–	–	none	–	–
ST 437/2	*S*. Typhi	NAL	0.5	–	–	none	–	–
ST 169/5	*S*. Typhi	NAL-SXT-TET	0.75	50 kb	SXT-TET	*intI1*	1.6 kb	*dfrA15 aadA1*
STA 32/2	*S*. Paratyphi A	NAL	0.25	–	–	none	–	–
STA 74/4	*S*. Paratyphi A	NAL	0.125	–	–	none	–	–
ST 55/4	*S*. Typhi	NAL-CIP-SXT-TET	≥32	50 kb	SXT-TET	*intI1*	1.6 kb	*dfrA15 aadA1*
ST 764/5	*S*. Typhi	NAL-CIP-SXT-TET	≥32	50 kb	SXT-TET	*intI1*	1.6 kb	*dfrA15 aadA1*
ST 642/12	*S*. Typhi	NAL-CIP-SXT-TET	≥32	50 kb	SXT-TET	*intI1*	1.6 kb	*dfrA15 aadA1*
STA 92/5	*S*. Paratyphi A	NAL-CIP	8	–	–	none	–	–
STA 114/11	*S*. Paratyphi A	NAL-CIP	8			none	–	–

NAL, nalidixic acid; CIP, ciprofloxacin; SXT, co-trimoxazole; TET, tetracycline; NAL[I], nalidixic acid intermediate; VR, variable region of class 1 integron amplified with 5′-CS and 3′-CS primers.[19]

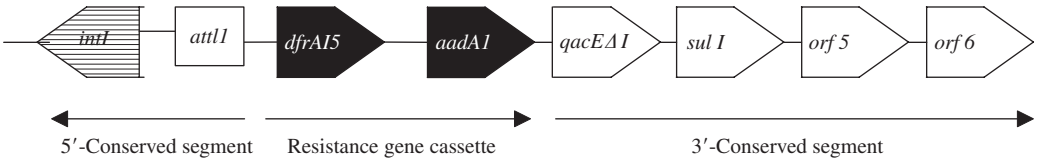

Figure 1. Schematic diagram of class 1 integron in *S*. Typhi ciprofloxacin-resistant isolates. Two resistance gene cassettes were detected—*dfrA15* conferring resistance to trimethoprim and *aadA1* conferring resistance to spectinomycin and streptomycin. The combination of the *dfrA15* gene with the *sul1* gene (sulfamethoxazole) results in resistance to co-trimoxazole.

PFGE

S. Typhi and *S*. Paratyphi A isolates were typed by PFGE according to a standardized protocol described previously.[22] Briefly, after cell lysis by proteinase K, genomic DNA plugs were digested with 50 U of *Xba*I and separated on a 1% agarose gel (Agarose LE, Roche) using Gene Navigator apparatus (Pharmacia-LKB). Electrophoresis conditions were run for 22 h at 180 V, with a pulse time of 2–64 s.

Results

Molecular characterization of antibiotic resistance determinants

The patterns of antibiotic resistance, plasmid profiles and characteristics of integrons of five ciprofloxacin-resistant *S*. Typhi and *S*. Paratyphi A isolates are summarized in Table 1, together with two ciprofloxacin-susceptible isolates (MICs < 0.125 mg/L) and five isolates with reduced susceptibility to (MICs 0.125–1 mg/L). Four of the five isolates with reduced susceptibility to ciprofloxacin were nalidixic-acid-resistant (*S*. Typhi ST 437/2 and ST 169/5, and *S*. Paratyphi A STA 32/2 and STA 74/4) while a fifth isolate (ST 512/6) was intermediately susceptible to nalidixic acid. In addition, isolate ST 169/5 was also resistant to co-trimoxazole and tetracycline (R-type NAL-SXT-TET). Of the five ciprofloxacin-resistant isolates three were *S*. Typhi (MICs ≥ 32 mg/L) and two were *S*. Paratyphi A (MICs 8 mg/L). *S*. Typhi isolates were additionally resistant to co-trimoxazole and tetracycline (R-type CIP-NAL-SXT-TET) and harboured a plasmid of about 50 kb plasmid carrying a class 1 integron. Sequencing of the 1.6 kb integron variable region revealed the presence of two gene cassettes: *dfrA15* and *aadA1* which confer resistance to trimethoprim and to spectinomycin and streptomycin, respectively (Figure 1). However, resistance to spectinomycin and streptomycin was not phenotypically expressed. The transfer capability of antibiotic resistance was tested by conjugation experiments. Only resistance to co-trimoxazole and tetracycline and not to ciprofloxacin was transferable. *E. coli* transconjugants were found positive for both the 50 kb plasmid and class 1 integron indicating that the integron (conferring resistance to co-trimoxazole) and tetracycline resistance gene were plasmid-borne.

Sequence analysis of QRDR genes

Correlation between the ciprofloxacin MIC and nucleotide changes within the QRDRs of DNA gyrase and topoisomerase IV subunit genes is shown in Table 2. No mutations were detected in QRDRs of *gyrA* and *parC* genes of nalidixic-acid-susceptible isolates (ciprofloxacin MICs < 0.125 mg/L). Different assortments of nucleotide substitutions were identified among isolates of *S*. Typhi and Paratyphi A. In particular, nalidixic-acid-resistant and -intermediately-susceptible *S*. Typhi isolates with ciprofloxacin MICs ≥ 0.5 mg/L, exhibited a single point mutation in *gyrA*

Table 2. MICs of ciprofloxacin and nucleotide changes in DNA gyrase and topoisomerase IV subunits in clinical isolates of *S*. Typhi and *S*. Paratyphi A

Isolates	Ciprofloxacin MIC (mg/L)	Nucleotide change in DNA gyrase			Nucleotide change in DNA topoisomerase IV			
		GyrA			ParC			
		83 [TCC (Ser)]	87 [GAC (Asp)]	GyrB	80 [AGC (Ser)]	69 [GAC (Asp)]	78 [GGC (Gly)]	ParE
NAL^S *S.* Typhi								
ST 31/3	0.003	nil	nil	nil	nil	nil	nil	nil
ST 419/2	0.025	nil	nil	nil	nil	nil	nil	nil
NAL^I *S.* Typhi								
ST 512/6	0.5	TAC (Tyr)	nil	nil	nil	nil	GAC (Asp)	nil
NAL^R *S.* Typhi								
ST 437/2	0.5	TTC (Phe)	nil	ND	Nil	GAA (Glu)	nil	ND
ST 169/5	0.75	TTC (Phe)	nil	ND	ATC (Ile)	nil	nil	ND
NAL^R *S.* Paratyphi A								
STA 32/2	0.25	TTC (Phe)	nil	ND	nil	nil	nil	ND
STA 74/4	0.125	TTC (Phe)	nil	ND	nil	nil	nil	ND
CIP^R *S.* Typhi								
ST 55/4	$\geq$32	TTC (Phe)	AAC (Asn)	nil	ATC (Ile)	nil	nil	nil
ST 764/5	$\geq$32	TTC (Phe)	AAC (Asn)	nil	ATC (Ile)	nil	nil	nil
ST 642/12	$\geq$32	TTC (Phe)	AAC (Asn)	nil	ATC (Ile)	nil	nil	nil
CIP^R *S.* Paratyphi A								
STA 92/5	8	TTC (Phe)	GGC (Gly)	nil	AGC (Arg)	nil	nil	nil
STA 114/11	8	TTC (Phe)	GGC (Gly)	nil	AGC (Arg)	nil	nil	nil

ND, not determined.

and *parC* genes. Interestingly, a single *gyrA* mutation was observed in nalidixic-resistant *S*. Paratyphi A with a comparatively lower ciprofloxacin MIC (0.125–0.25 mg/L). All ciprofloxacin-resistant isolates (MICs $\geq$ 8 mg/L) showed three mutations, two mutations within the QRDR of *gyrA*, at positions 83 and 87, and a single mutation in *parC*, at position 80. The first *gyrA* mutation leading to a phenylalanine substitution of the serine residue at codon 83 (Ser-83→Phe) was common to both serovars, while the second substitution at codon 87 was different in *S*. Typhi (Asp-87→Asn) and *S.* Paratyphi A (Asp-87→Gly). The Ser-83→Phe substitution was also shared by isolates with reduced susceptibility to ciprofloxacin, with the exception of the isolate with intermediate susceptibility to nalidixic acid (ST 512/6) where Tyr substituted Ser-83. Four types of mutations were observed within *parC* in all isolates with ciprofloxacin MICs $\geq$ 0.5 mg/L, regardless of the serovar (Table 2). None of the isolates carried mutations in the *gyrB* and *parE* genes.

PFGE

The PFGE patterns of the three ciprofloxacin-resistant *S.* Typhi and *S*. Typhi isolate ST 169/5 were indistinguishable as were those of the two ciprofloxacin-resistant *S.* Paratyphi A isolates (data not shown).

Discussion

The emergence of MDR *S*. Typhi and *S.* Paratyphi A strains in Asia in the late 1980s and early 1990s led to the widespread use of fluoroquinolones for treating enteric fever. However, during the last decade treatment failures with ciprofloxacin have been increasingly reported. These failures have been associated with infection with *S*. Typhi and Paratyphi A strains that are resistant to nalidixic acid and exhibiting decreased susceptibility to ciprofloxacin.[4,5,23] Strains that are already resistant to nalidixic acid may require fewer exposures to fluoroquinolones to develop high-level resistance to ciprofloxacin, than the strains that are fully ciprofloxacin susceptible.[24]

To our knowledge this is the first report of molecular characterization of clinical isolates of *S.* Typhi with full resistance to ciprofloxacin. In Enterobacteriaceae, high-level fluoroquinolone resistance has been associated with mutations within the different QRDRs of the DNA gyrase and topoisomerase IV genes. In this study we have investigated the presence of such mutations in our isolates (Table 2). Although the sample size is relatively small, our study highlighted that mutations conferring resistance to fluoroquinolones occur in a stepwise manner, demonstrated by gradual increases in MIC values. A single *gyrA* mutation at Ser-83 alone was associated with resistance to nalidixic acid or reduced susceptibility to ciprofloxacin (MICs 0.125–0.25 mg/L). Ser-83 is suggested to be an important site for determining fluoroquinolone resistance within *S*. Typhi and Paratyphi A isolates[4,10,12] and is supported by our results. Mutation in *parC* was the second step leading to high-level fluoroquinolone resistance, resulting only in a slight increase in resistance to ciprofloxacin in serovar Typhi (MICs 0.5–1 mg/L) when present together with *gyrA* mutation. *S*. Paratyphi A isolates with a lower extent of reduction of

ciprofloxacin resistance (MICs of 0.125 and 0.25 mg/L) exhibited no mutation in *parC*, suggesting a correlation between *parC* mutation and levels of ciprofloxacin MIC. A second mutation in *gyrA* was found to be essential to cause high-level resistance to ciprofloxacin (MICs > 4 mg/L). It can be concluded that ciprofloxacin-resistant *S.* Typhi isolates with R-type CIP-NAL-SXT-TET and MICs $\geq$ 32 mg/L were clonally related to isolate *S*. Typhi 169/5 (R-type NAL-SXT-TET and ciprofloxacin MIC 0.75 mg/L) as the PFGE (data not shown) and mutations in *gyrA* Ser-83 and *parC* Ser-80 (Table 2) were identical in these isolates. Sequential acquisition of a second mutation in *gyrA*, Asp-87 to Asn in *S.* Typhi 169/5 resulted in emergence of ciprofloxacin resistance in *S.* Typhi isolates. As ciprofloxacin-resistant isolates of *S*. Typhi and Paratyphi A had double mutations in *gyrA* and a single mutation in *parC*, it appears that increase of MIC above 4 mg/L was caused by a second mutation in *gyrA*, rather than the *parC* mutation. Although the substitution at Ser-83 to Phe was identical in ciprofloxacin-resistant isolates of *S.* Typhi and Paratyphi A, the differences in mutation at *gyrA* Asp-87 and *parC* Ser-80 resulted in different MICs between the two serovars. While mutations in both *gyrA* and *parC* have been identified in bacterial isolates highly resistant to fluoroquinolones, the role of *parC* mutation is however less clear.[11] In Gram-negative bacteria the primary target of fluoroquinolones is gyrase rather than topoisomerase IV, hence *gyrA* mutations precede those of *parC*. As single *parC* mutations provide no selective advantage they are generally accompanied by *gyrA* mutation. They are, however, required to achieve high-level fluoroquinolone resistance[10,11,24–26] with amino acid changes usually at codons 80 and 84 (Ser-80 to Ile, Arg; Glu-84 to Gly, Lys).[25] These mutations have not been reported in clinical isolates of *S.* Typhi. Ling *et al*.[26] reported *parC* mutations in *Salmonella* in the absence of *gyrA* mutation in isolates with ciprofloxacin MICs < 0.06 mg/L and were also the first to report *parC* Tyr-57→Ser in an isolate of *S.* Paratyphi A with reduced susceptibility to fluoroquinolones. However, Piddock *et al*.[11] did not detect any *parC* mutants among veterinary *Salmonella* isolates with MICs $\geq$ 0.5 mg/L. In our study, nucleotide changes in *parC* were identified at codons 69, 78 and 80 in isolates of *S*. Typhi with reduced susceptibility to ciprofloxacin (MICs 0.5–1 mg/L) and at codon 80 in ciprofloxacin-resistant isolates of *S.* Typhi and Paratyphi A. The Asp-69→Glu mutation identified in the *S*. Typhi 437/2 isolate was a novel one, not reported so far. This is also the first report of a *parC* mutation in serovar Typhi. Since each mutation in *gyrA* and *parC* was associated with different ciprofloxacin MICs, further studies on other resistance mechanisms, such as alterations in membrane permeability and changes in efflux and influx, are required to evaluate the contribution of *parC* mutations to fluoroquinolone resistance in *S.* Typhi and Paratyphi A and are presently under investigation.

Our study suggests that isolates with reduced susceptibility to fluoroquinolones might be important in clinical development of resistance as they could become highly resistant upon sequential acquisition of resistance.

To date only two clinical isolates of *S*. Paratyphi A showing ciprofloxacin resistance have been characterized. The isolate from Japan had an MIC of 128 mg/L, with a double mutation in *gyrA* (Ser-83→Phe and Asp-87→Asn) and a third in *parC* (Glu-84→Lys).[9] However, the isolate from Pondicherry, India,[8] showed an identical ciprofloxacin MIC and mutations found in ciprofloxacin-resistant *S.* Paratyphi A isolates of our series isolated from New Delhi, India, suggesting a clonal spread of this strain within India. Double mutations in *gyrA*, along with a single mutation in *parC*, have also been reported in *in vitro* selected ciprofloxacin-resistant mutants of *S*. Paratyphi A,[10] strongly suggesting that such triple mutation is important for the development of high-level fluoroquinolone resistance.

Lately there has been a report of a ciprofloxacin-resistant *S.* Typhi (MIC 16 mg/L), with a double mutation in *gyrA* (Ser-83→Phe and Asp-87→Asn); however, the authors have not looked into the role of *parC* mutations and other mechanisms of resistance.[27]

All the above reports describe single isolates of *S*. Typhi or Paratyphi A with high-level fluoroquinolone resistance. In our study all three ciprofloxacin-resistant *S.* Typhi isolates demonstrated an identical PFGE pattern and mutations in DNA gyrase and topoisomerase IV as did the two *S.* Paratyphi A isolates. The patients infected with these resistant isolates did not give a history of prior treatment with fluoroquinolones. This is the first report suggesting the spread and the infection by a circulating resistant strain rather than the emergence of resistance during treatment.

The presence of integrons in these ciprofloxacin-resistant *S.* Typhi isolates is also worrying since integrons represent the main vehicle of antibiotic resistance. Two reports have described multidrug-resistant *S*. Typhi strains harbouring integrons with up to six drug resistance genes.[19,28] This is the first report of ciprofloxacin-resistant strains of *S*. Typhi harbouring a plasmid-borne class 1 integron. Integrons could play a role in the development and dissemination of new MDR strains of *Salmonella* spp.[29] By retrospective investigation, Carattoli *et al*.[30] were able to demonstrate the plasmid-borne involvement of integrons in the development of MDR strains of *S*. Typhimurium. The strains of *S*. Typhi described here, with multiple resistance mechanisms, including a class 1 integron on a 50 kb plasmid, and associated chromosomally-mediated resistance to fluoroquinolones, thus have the possibility of becoming resistant to the third-generation cephalosporins which are currently the drugs of choice for treating enteric fever. This is possible by the acquisition of gene cassettes such as *veb-1* or $bla_{VIM,}$ by the plasmid-borne integron thus leading to untreatable enteric fever in the near future.

In many tropical countries, including the Indian subcontinent, there is widespread availability and uncontrolled use of antibiotics including quinolones. Therefore, there is selective pressure on a large bacterial population of endemic *Salmonella* spp. Reducing the exposure to fluoroquinolones would definitely lessen the likelihood of selecting mutants. As isolates with reduced susceptibility to fluoroquinolones could become highly resistant upon sequential accumulation of mutations in topoisomerase genes, the use of fluoroquinolones as first-line drugs for management of enteric fever in areas where these strains are endemic, therefore, requires urgent review.

Continuous surveillance of the plasmid and chromosome of *S*. Typhi and *S*. Paratyphi A is essential to alter treatment strategies aimed at maintaining the useful life of the few remaining antimicrobials available to treat enteric fever.

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Transparency declarations

None to declare.

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