Intermediate Susceptibility to Ciprofloxacin among *Salmonella enterica* Serovar Typhi Isolates in Lima, Peru

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Thirty-three *Salmonella enterica* serovar Typhi blood isolates from Lima, Peru (2008 to 2012), were fully susceptible to trimethoprim-sulfamethoxazole, chloramphenicol, ceftriaxone, and tetracycline; 8/33 (24.2%) showed intermediate susceptibility to ciprofloxacin carrying mutations in the quinolone resistance-determining region of the *gyrA* gene (Ser83-Phe and Asp87-Asn) and in the *gyrB* gene (Ser464-Phe).

Typhoid fever is a systemic disease caused mainly by *Salmonella enterica* serotype Typhi. Although there is a decreasing incidence, it is still endemic in many countries of Latin America (1). Since multidrug-resistant (MDR) isolates (i.e., resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole) have become widespread, fluoroquinolones (e.g., ciprofloxacin) have been the first-line therapy for typhoid fever in adult patients. However, reports of clinical failure in patients treated with ciprofloxacin were described in the southeast of Asia in the 1990s (2). These isolates were resistant to nalidixic acid (NA), showing an increased MIC value of ciprofloxacin (≥ 0.125 $\mu\text{g/ml}$) but still within the range for susceptibility as defined for *Enterobacteriaceae*; this phenomenon was named “decreased ciprofloxacin susceptibility.” The presence of point mutations in the genes encoding the fluoroquinolone targets, DNA gyrase (*gyrA* and *gyrB*), and/or topoisomerase IV (*parC* and *parE*), was detected among them (3). Several studies subsequently emphasized the need of changing the breakpoints for fluoroquinolones for *Salmonella* (4); as a consequence, the Clinical and Laboratory Standards Institute changed the breakpoints as follows: (i) susceptible, ≤ 0.06 $\mu\text{g/ml}$; (ii) intermediate susceptible, 0.12 to 0.5 $\mu\text{g/ml}$; (iii) resistant, ≥ 1 $\mu\text{g/ml}$ (5).

The emergence of *S. Typhi* showing intermediate susceptibility to ciprofloxacin (ISC) has been almost unstudied in Latin America. Our objective was to assess the susceptibility pattern of *S. Typhi* from Peruvian patients, to describe the molecular characteristics of resistance to fluoroquinolones, and to analyze their

clonal relationship. Blood culture isolates were collected as part of a microbiological surveillance study from 2008 to 2012 through a network of eight public hospitals in Lima, Peru. Blood cultures were drawn during routine patient care. Each hospital followed its own protocol for blood culturing. Of 2,897 Gram-negative isolates, 154 (5.3%) belonged to the genus *Salmonella*. Only one isolate per patient was considered. Lactose-negative colonies with typical biochemical characteristics of *S. Typhi* were further confirmed by agglutination with *Salmonella* O9, Vi-specific and H:d antisera.

Susceptibility testing was performed by disk diffusion for ampicillin, ceftriaxone, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole (6). MIC values for NA and ciprofloxacin were determined by agar dilution (5). *Escherichia coli* strain ATCC 25922 was used as a control for susceptibility testing.

Screening for mutations in the quinolone resistance-determining region (QRDR) was performed by amplification of a fragment of the

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TABLE 1 Cases of typhoid fever caused by *Salmonella* Typhi with intermediate susceptibility to ciprofloxacin

Case code	Age (yrs)	Origin	Sampling date (day/mo/yr)	MIC ($\mu\text{g/dl}$) ^a		Alignment with <i>Salmonella</i> Typhi CT18 and Ty2 ^b	
				CIP	NA	<i>gyrA</i>	<i>gyrB</i>
HAMA-081	5	Pediatric ward	13/05/2009	0.125	128	Ser83-Phe	
HAMA-136	12	Pediatric ward	15/02/2010	0.125	64	Asp87-Asn	
HSEB-073	13	Pediatric ward	10/08/2009	0.125	>256	Asp87-Asn	
HCH-804	21	Outpatient clinic	19/01/2012	0.125	16 ^c		Ser464-Phe
HGAI-470	14	Emergency room	13/02/2009	0.25	256	Asp87-Asn	
HAMA-205	28	Medicine ward	01/12/2011	0.25	16 ^c		Ser464-Phe
HCH-816	20	Medicine ward	04/01/2012	0.25	256	Ser83-Phe	
HAMA-077	13	Pediatric ward	28/04/2009	0.5	256	Ser83-Phe	

^a CIP, ciprofloxacin; NA, nalidixic acid.

^b All isolates tested carried a Glu133-Gly mutation in the *gyrA* gene.

^c Isolates susceptible to nalidixic acid.

Dice (Opt:0.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-100.0%]
pfge

FIG 1 Pulsed-field gel electrophoresis analysis of 33 *Salmonella* Typhi isolates from Lima, Peru. S, susceptible; I, intermediate susceptibility.

gyrA, *gyrB*, and *parC* genes containing the QRDR as previously described (6), and the fragments were sequenced on an ABI 3130 sequencer. The presence of the plasmid-mediated quinolone resistance *qnr* genes (*qnrA*, *qnrB*, and *qnrS*) was determined using PCR (7). The alignment was done using the following strains: *Salmonella enterica* serovar Typhimurium LT2, ST CT18, and ST Ty2.

Clonal relationships were assessed by pulsed-field gel electrophoresis (PFGE) according to the PulseNet Europe protocol (8). Genomic DNA was digested with the XbaI restriction enzymes; *Salmonella* Braenderup H9812 was used as a size marker. For cluster analysis, BioNumerics 5.1 was used. The cluster analysis settings applied were the Dice similarity coefficient and unweighted-pair group method using average linkages (UPMGA) dendrogram type with the following parameters: (i) optimization of 0.50% and (ii) position tolerance of 1.50%.

A total of 33 isolates were recovered, and 11 cases (33.3%) were children (<14 years). All were fully susceptible to trimethoprim-

sulfamethoxazole, chloramphenicol, ceftriaxone, ampicillin, and tetracycline, except two which showed intermediate susceptibility to ampicillin. Six isolates (18.2%) were resistant to NA and eight (24.2%) showed ISC (Table 1). Most previous studies in Latin American countries focused on *Salmonella* non-Typhi (9). Over the last 20 years, only two studies assessing *S. Typhi* were found, reporting full susceptibility of 151 *S. Typhi* isolates from Colombia and Argentina. Of note, disk diffusion was the method used in both studies. Since MIC values were not determined, it is possible that isolates with ISC were missed (10, 11).

A mutation in *gyrA*, Gly133-Glu, was detected in all ISC isolates when *S. Typhimurium* LT2 was used for alignment. However, it was not detected when *S. Typhi* CT18 and *S. Typhi* Ty2 were used. This is very likely a serotype-specific mutation. It is present in isolates susceptible to NA irrespective of their MIC values for ciprofloxacin. It has been rarely described in Asian countries but was recently found to be the most common muta-

tion in the Democratic Republic of the Congo (5, 12–14). Six isolates had a second mutation in the *gyrA* gene, Ser83-Phe ($n = 3$) and Asp87-Asn ($n = 3$). These mutations are widespread in *S. Typhi* from Asian countries, including India (4, 12). One study which included 12 NA-resistant *S. Typhi* isolates from travelers found that seven isolates carried the Ser83-Phe mutation; interestingly, six travelers came from India and one from Peru (13). In addition, the two remaining isolates had a mutation in the *gyrB* gene, Ser464-Phe (Table 1). This mutation has been associated with the nonclassical fluoroquinolone resistance phenotype, as both isolates were susceptible to NA; as a consequence ISC is not predicted by the detection of resistance to NA. Such isolates are typically non-MDR; they have been described from France, the United States, and England, but they were primarily acquired in India (15). No mutations were found in the *parC* and *qnr* genes.

PFGE revealed a total of 28 different pulsotypes among the 33 isolates, with a maximum of two isolates sharing the same pulsotype (Fig. 1). This high diversity has been reported by Salve and Pichel in Argentina and Colombia (11).

This study has several limitations. A small number of isolates were included, but they were obtained during a 5-year multicenter laboratory surveillance. Besides, we did not collect clinical information regarding treatment follow-up.

Among 33 *S. Typhi* isolates, none displayed MDR, but ISC was demonstrated in almost a quarter of them. All ISC isolates showed a mutation in *gyrA* (Gly133-Glu), combined with either a mutation in *gyrA* at codons 83 and 87 or in the *gyrB* gene at codon 464. A diverse clonal distribution was demonstrated. Since fluoroquinolones are still the cornerstone of the treatment of typhoid fever in Latin American countries, active surveillance is needed in order to monitor resistance trends to these antimicrobials.

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