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Multidrug-Resistant *Salmonella enterica* Serovar Typhi Isolates with High-Level Resistance to Ciprofloxacin in Dhaka, Bangladesh

Typhoid fever due to multidrug-resistant *Salmonella enterica* serovar Typhi is a global public health problem, with an estimated 30 million cases and 600,000 deaths annually (7). Since the beginning of the 1990s, there has been an increasing prevalence of multidrug resistance to the first-line antimicrobials, such as chloramphenicol, ampicillin, and co-trimoxazole, shifting the drug of choice for the treatment of typhoid fever to fluoroquinolones on the Indian subcontinent (4, 5). Several clinical failures due to infection with isolates of *S. enterica* serovar Typhi with decreased susceptibilities to fluoroquinolones but resistance to nalidixic acid, and their emergence, incidence, and spread have been reported from other developing countries (2, 3). The purpose of this report was to investigate whether such isolates were present in Bangladesh during the period between January and December 2005 by retrospective analysis of the data for blood cultures and antimicrobial susceptibility tests performed in the Clinical Microbiology Laboratory of the International Centre for Health and Population Research, Bangladesh (ICDDR,B). This is the first report to document the prevalence of isolates of *S. enterica* serovar Typhi expressing high-level resistance to ciprofloxacin in Bangladesh.

S. enterica serovar Typhi strains were isolated by blood culture by using the BactAlert 3D system (BioMerieux, Marcy l'Etoile, France). Antimicrobial susceptibility was determined by the standard disk diffusion method of the Clinical and Laboratory Standards Institute (CLSI), and the highly resistant isolates were subjected to MIC determination by Etest (AB Biodisk, Solna, Sweden). In 2005, 9,600 blood cultures were processed and *S. enterica* serovar Typhi was isolated from 428 patients (4.5%). Of these, 388 isolates (90.7%) of *S. enterica* serovar Typhi showed resistance to nalidixic acid. The ciprofloxacin susceptibilities of these isolates varied; 50 isolates (11.7%) had ciprofloxacin zone diameters of ≥21 mm (regarded as susceptible by CLSI), 368 (88.3%) had zone diameters of 16 to 20 mm (regarded as intermediate by CLSI), and 10 isolates (2.3%) had zone diameters of 15 mm or less (regarded as resistant by CLSI). On repeat testing, these 10 resistant isolates did not show any zone of inhibition by the disk diffusion method. The phenotypic pattern of resistance showed that 91.4% of the isolates were multidrug resistant (Table 1). The ciprofloxacin MICs of the 10 resistant isolates were also high and varied between 6.0 and 16.0 μg/ml, thus exceeding the CLSI resistance breakpoint of 4 μg/ml. The levels of resistance to nalidixic acid expressed by all 10 ciprofloxacin-resistant isolates were even higher (>256 μg/ml). All isolates, including the 10 highly resistant isolates, however, were susceptible to ceftriaxone, with zone diameters of ≥21 mm (regarded as susceptible by CLSI).

TABLE 1. Resistance phenotype and MICs of ciprofloxacin and nalidixic acid for *Salmonella enterica* serovar Typhi (n = 428), January to December 2005

Resistance phenotype(s)[a]	No. (%) of isolates	MIC (μg/ml)	
		Ciprofloxacin	Nalidixic acid
Amp^r Chl^r Sxt^r NA^r Cip^r	10 (2.33)	6.0–16.0	≥256
Amp^r Chl^r Sxt^r NA^r Cip^{rs}	234 (54.67)	0.19–0.25	≥256
Amp^r Chl^r NA^r Cip^{rs}	1 (0.23)	0.19–0.25	≥256
Chl^r Sxt^r NA^r Cip^{rs}	5 (1.16)	0.19–0.25	≥256
Amp^r Chl^r Sxt^r	3 (0.70)	ND[b]	ND
NA^r Cip^{rs}	138 (32.24)	0.19–0.25	≥256
Susceptible	37 (8.64)	0.012–0.016	2.0–3.0

[a] Amp, ampicillin; Chl, chloramphenicol; Sxt, trimethoprim-sulfamethoxazole; NA, nalidixic acid; Cip, ciprofloxacin; r, resistant; rs, reduced susceptibility.
[b] ND, not determined.

In the mid-1990s, the emergence of *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A strains with decreased susceptibilities to fluoroquinolones was reported from the Indian subcontinent and southeast and central Asia. Disk diffusion testing revealed that these isolates were resistant to nalidixic acid but sensitive to ciprofloxacin, according to the current CLSI interpretive criteria. However, the MICs of ciprofloxacin increased from 0.25 to 4 μg/ml, which are 10- to 100-fold higher than those for the usual nalidixic acid-susceptible isolates, but these isolates were still categorized as "susceptible" by current CLSI criteria. This "susceptible" reporting prompted clinicians to use ciprofloxacin, which resulted in delayed clinical responses or treatment failures in the ciprofloxacin-treated cases of typhoid fever caused by these isolates. Accordingly, the CLSI currently recommends the routine use of disk diffusion testing of nalidixic acid as a marker for the detection of reduced susceptibility of *Salmonella* spp. to fluoroquinolones. Resistance to nalidixic acid in *S. enterica* serovar Typhi has been reported to be mediated by a single point mutation at the quinolone resistance-determining region of the *gyrA* gene. Complete resistance to fluoroquinolones is usually associated with a double mutation in *gyrA*, but multiple mutations with the involvement of other genes have also been mentioned (1). The injudicious administration and rampant use of quinolones in Bangladesh probably contributed to the high prevalence of reduced susceptibility (>88%) and the emergence of very high level or complete resistance (>4 μg/ml) of isolates of *S. enterica* serovar Typhi to fluoroquinolones. The prevalence of multidrug resistance (resistance to two or more drugs) is also high (91.4%). Alternative effective drugs available for treatment of these resistant isolates are ceftriaxone and cefixime, but they are expensive and one ceftriaxone-resistant case of *S. enterica* serovar Typhi infection has been reported (6). We therefore face the imminent prospect of encountering untreatable typhoid fever in the near future. A national guideline on the proper usage of antibiotics is required for urgent implementation in Bangladesh. In addition, a reevaluation of the MIC breakpoint criteria by the CLSI is necessary, as the current reference standards are unable to differentiate between fully susceptible isolates and isolates showing reduce susceptibility.

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