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Emergence of multidrug-resistant *Salmonella enterica* serotype Typhi with decreased ciprofloxacin susceptibility in Bangladesh

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SUMMARY

During 1989–2002, we studied the antimicrobial resistance of 3928 blood culture isolates of *Salmonella enterica* serotype Typhi (*S.* Typhi) in Dhaka, Bangladesh. Overall 32% (1270) of the strains were multidrug-resistant (MDR, resistant to chloramphenicol, ampicillin and trimethoprim–sulphamethoxazole); first detected in 1990 (rate of 8%), increased in 1994 (44%), declined in 1996 (22%, $P<0{\cdot}01$ compared to 1994) and re-emerged in 2001 (36%) and 2002 (42%, $P<0{\cdot}01$ compared to 1996). An increased MIC of ciprofloxacin (0·25 μg/ml) indicating decreased susceptibility to ciprofloxacin was detected in 24 (18·2%) out of 132 randomly selected strains during 1990–2002; more frequently in MDR than susceptible strains (46·3% *vs.* 5·5%, $P<0{\cdot}001$), and the proportion of them rose to 47% in 2002 from 8% in 2000 ($P<0{\cdot}01$). Ciprofloxacin (5 μg) disk diffusion zone diameters of $\leqslant$24 mm as break-point had 98% sensitivity and 100% specificity when compared with a ciprofloxacin MIC of 0·25 μg/ml as break-point for decreased susceptibility; being a useful and easy screen test. All strains were susceptible to ceftriaxone. The emergence of MDR *S.* Typhi with decreased ciprofloxacin susceptibility will further complicate the therapy of typhoid fever because of the lack of optimum treatment guidelines.

INTRODUCTION

An estimated 16 million new cases of typhoid fever with 600 000 deaths caused by *Salmonella enterica* serotype Typhi (*S.* Typhi) occur globally each year [1] with the highest incidence (1000 cases per 100 000 people per year) in Southeast Asia [2]. Typhoid fever is highly endemic in Bangladesh, resulting in high morbidity, devastating economic loss and case fatalities [2–4]. In late 1987, outbreaks of typhoid fever caused by a multidrug-resistant (MDR) strain of *S.* Typhi, resistant to three first-line antimicrobial agents [ampicillin (Ap), chloramphenicol (Cm) and trimethoprim–sulphamethoxazole (SXT)], occurred in the suburbs of Shanghai, China [5] and subsequently in India [6], Pakistan [7], Bangladesh [8], the Arab Gulf [9], Vietnam [10] and Africa [11] resulting in severe illness and high incidence of complications and mortality [4–7, 10]. MDR strains were also detected in the United Kingdom and the United States among immigrants and travellers from epidemic-affected countries complicating the treatment of typhoid fever [7, 12]. Ciprofloxacin (Cp) and the allied fluoroquinolone ofloxacin have been the antimicrobial drugs of choice for treatment of MDR typhoid fever since 1990 in Bangladesh and other countries. However, a strain of *S.* Typhi with increased ciprofloxacin MIC (0·25–1 μg/ml) exhibiting

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decreased susceptibility to ciprofloxacin, and resistant to nalidixic acid (MIC $\geqslant 32\,\mu$g/ml) was detected in 1991 resulting in treatment failure in a patient who had recently returned to the United Kingdom from India. Such strains were subsequently isolated in many countries resulting in suboptimal clinical responses and therapeutic failures [13–16]. An epidemic caused by similar type of strain of *S*. Typhi (R type: ApCmSXTCp) has been reported in Tajikistan [17]. Nalidixic acid-resistant *S*. Typhi with decreased susceptibility to ciprofloxacin is now endemic in Vietnam [18], India [13, 19] and neighbouring countries complicating the treatment of typhoid fever. Recently, ciprofloxacin treatment failure in a patient with typhoid fever caused by a MDR strain of *S*. Typhi having decreased susceptibility to ciprofloxacin (MIC $\geqslant 0{\cdot}25\,\mu$g/ml), has been reported for the first time in the southern part of Bangladesh [16]. Thus, the detection of decreased susceptibility to ciprofloxacin in the laboratory is essential for treating typhoid fever. The commonly used disk diffusion technique is not useful for detecting decreased susceptibility to ciprofloxacin, and determination of the MIC of ciprofloxacin is the gold standard for detecting decreased susceptibility to ciprofloxacin [13, 14]. When compared with the disk diffusion technique, determination of MIC in the laboratory is expensive and needs trained personnel to perform the test. Moreover, the facility for determination of MICs is not available in many laboratories of developing countries where MDR typhoid fever is endemic. Thus, a disk diffusion technique for detecting decreased susceptibility to ciprofloxacin would be easy, less expensive and a user-friendly means for helping physicians to administer the proper treatment for typhoid fever. We, therefore, studied the present trends in antimicrobial resistance of *S*. Typhi in Dhaka, the capital of Bangladesh, to define optimal therapeutic strategies in this impoverished setting with particular reference to the emergence and detection of decreased ciprofloxacin susceptibility in prevalent MDR *S*. Typhi isolates.

MATERIALS AND METHODS

Clinical samples

The study was conducted in Dhaka Clinical Research and Service Centre (CRSC), of ICDDR,B:Centre for Health and Population Research, Bangladesh. It serves 100 000 diarrhoeal patients annually. The clinical microbiology laboratory cultures blood from in-patients with diarrhoea and fever as determined by the centre's physicians as well as from outpatients who are referred to the this laboratory by physicians in Dhaka city.

Bacterial strains

We studied all isolates of *S*. Typhi from blood cultures of sporadically occurring enteric fever cases reporting to the ICDDR,B hospital in Dhaka between 1989 and 2002. Blood was cultured by standard methods, as previously described [8]. All microbiological and epidemiological information was collected.

Antimicrobial susceptibility testing

In vitro susceptibilities to chloramphenicol, trimethoprim-sulphamethoxazole, ampicillin, ciprofloxacin and ceftriaxone were performed by the disk diffusion method [8, 20] during 1989–2002 using commercial antimicrobial disks (BBL, Baltimore, MD, USA) with *Escherichia coli* ATCC 25992 as the control strain following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS), USA. MIC of antimicrobial agents was determined for selected isolates. Since decreased ciprofloxacin susceptibility was first reported in 1990 [15], 132 *S*. Typhi strains isolated in years 1990 (25 isolates), 1995 (25 isolates), 2000 (25 isolates), 2001 (25 isolates) and 2002 (32 isolates) were randomly selected for determining MIC without pre-existing knowledge of their antimicrobial susceptibility patterns. The isolates were subcultured from glycerol stock ($-80\,^{\circ}$C) in 2002 and tested for susceptibility to the above-mentioned antimicrobial agents and nalidixic acid by disk diffusion method, and by *E* tests (AB-Biodisk, Solna, Sweden) or agar dilution technique (only for nalidixic acid) to obtain the MIC values of these antimicrobial agents.

The inhibition zone diameters obtained by disk diffusion method, and MIC values were compared by scattergram to determine zone diameter for detecting decreased susceptibility to ciprofloxacin by disk diffusion method.

Resistance simultaneously to three or more different groups of antimicrobial drugs was defined as MDR; decreased ciprofloxacin susceptibility was defined as an isolate with ciprofloxacin MIC within the range of 0·25–1 μg/ml and ciprofloxacin resistant if the MIC was $\geqslant 4\,\mu$g/ml.

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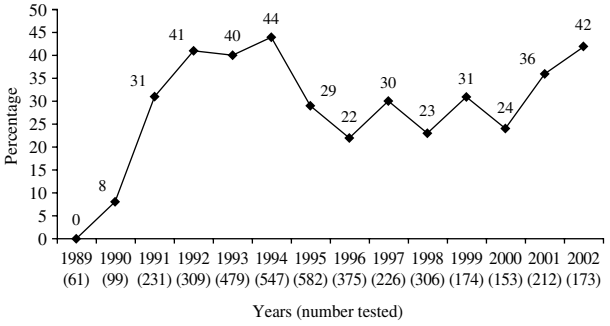

Fig. 1. Percentage of *Salmonella* Typhi isolates from blood cultures simultaneously resistant to ampicillin, chloramphenicol and trimethoprim–sulphamethoxazole (MDR *S.* Typhi), 1989–2002.

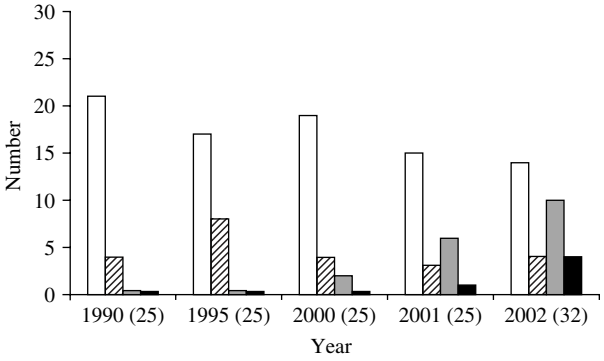

Fig. 2. Number of *Salmonella* Typhi strains resistant to ampicillin, chloramphenicol and trimethoprim–sulphamethoxazole (MDR) and strains with decreased ciprofloxacin susceptibility. □, Susceptible *S.* Typhi; ▨, MDR *S.* Typhi; ▩, MDR with decreased ciprofloxacin susceptibility; ■, decreased ciprofloxacin susceptibility (non-MDR).

RESULTS

The proportion of MDR *S.* Typhi isolates increased to a peak of 44% in 1994 from 8% in 1990, then decreased and ranged from 22 to 31% during 1995–2000, increased again to 36% in 2001, and to 42% in 2002 (Fig. 1), suggesting the emergence, decline and re-emergence of MDR *S.* Typhi in Bangladesh. Other resistance patterns such as resistance to one and two drugs were low (5–7%). All isolates were susceptible to ciprofloxacin (inhibition zone diameter of $\geqslant$21 mm) and ceftriaxone by the disk diffusion method by NCCLS criteria.

Of 132 isolates studied for MIC, overall 41 (31%) were MDR with MICs above break-points for ampicillin, chloramphenicol and trimethoprim–sulphamethoxazole; 24 (18·2%) isolates showed decreased ciprofloxacin susceptibility (MIC of 0·25 μg/ml); and 107 (8%) isolates had a MIC of $\leqslant$0·094 μg/ml (range 0·008–0·064 μg/ml, Table).

Decreased ciprofloxacin susceptibility was detected in 19 (46·3%) out of 41 MDR strains compared with 5 out of 91 (5·5%) susceptible strains (relative risk 31·7, $P<0·001$) showing a significant association with MDR traits. When the MIC of ciprofloxacin was compared with the inhibition zone diameter of ciprofloxacin by scattergram for determining decreased ciprofloxacin susceptibility by zone diameter, 108 (82%) isolates had inhibition zone diameters of $\geqslant$25 mm (range 25–41 mm) and MICs of $\leqslant$0·125 μg/ml (range 0·008–0·125 μg/ml) exhibiting no decreased susceptibility to ciprofloxacin. The isolates with decreased susceptibility to ciprofloxacin [ciprofloxacin MICs of 0·25 μg/ml (range 0·25 μg/ml)] had zone diameter of $\leqslant$24 mm (range 21–24 mm). When a MIC of 0·25 μg/ml was used as the break-point for determining decreased ciprofloxacin susceptibility, a ciprofloxacin inhibition zone of $\leqslant$24 mm resulted in 98% sensitivity and 100% specificity. None was completely resistant to ciprofloxacin or ceftriaxone by NCCLS criteria. Two out of 25 (8%) isolates (Fig. 2) exhibited decreased ciprofloxacin susceptibility in 2000, seven (28%) in 2001 and 15 out of 32 (47%) in 2002 ($P<0·01$, when 2002 data are compared with 2000 and 2001). Seventy-one percent (10/14) of MDR *S.* Typhi exhibited decreased ciprofloxacin susceptibility in 2002 compared to 25% (2/8) in 2000 ($P<0·01$). One isolate (4% of 25) in 2001 had a higher MIC to ciprofloxacin alone but was not MDR compared to four (12·5% of 32) in 2002. One isolate was categorized as intermediate to nalidixic acid by the disk diffusion method and had a ciprofloxacin MIC of 0·125 μg/ml showing no decreased susceptibility.

DISCUSSION

Inappropriate use of antimicrobial agents in humans and animals has led to widespread resistance among bacterial pathogens including *S.* Typhi, an invasive facultative intracellular pathogen responsible for septicaemic and prolonged febrile illness in humans [1, 11, 21]. MDR typhoid fever epidemic started in Bangladesh in 1990, peaked in 1994, subsequently declined and re-emerged in 2001 and 2002. The factors causing this phenomenon are not known except the role of conjugative R plasmid [8, 21]. A community-based surveillance in a slum area of Dhaka in 2001 detected MDR (R-type: CmApSXT)

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Table. *Ciprofloxacin and nalidixic acid disk diffusion results as indicators for ciprofloxacin MICs in* Salmonella *Typhi* (*n* = *132*) *for detecting decreased ciprofloxacin susceptibility*

No. of strains	Disk diffusion results of (NCCLS)†		Proposed zone diameter for detecting decreased ciprofloxacin susceptibility	Range of MIC (MIC_{90})* μg/ml of	
	Nalidixic acid	Ciprofloxacin		Nalidixic acid	Ciprofloxacin
107	S	S	S (⩾25 mm)	2–8 (8)	0·008–0·064 (0·016)
1	I	S	S (⩾25 mm)	16	0·125 (0·125)
24	R	S	R (⩽24 mm)	128 to >256 (>256)	0·25 (0·25)

* MIC_{90} was calculated separately for 108 nalidixic acid-susceptible and 23 nalidixic acid-resistant strains.
† S, Susceptible; I, intermediate; R, resistance.

phenotype in more than 50% of 49 *S*. Typhi isolates suggesting the dissemination of the MDR strains in the community [22]. Recently, a similar prevalence of MDR *S*. Typhi has been reported in Kolkata, India [23] confirming its re-emergence in the Indian subcontinent. A high (52–82%) prevalence of MDR *S*. Typhi has also been reported in Kenya and Ghana [24].

In 2000, typhoid fever caused by strains of *S*. Typhi exhibiting nalidixic acid resistance and decreased ciprofloxacin susceptibility was detected for the first time in Bangladesh. The isolation of such strains seemed to increase sharply in 2002. In addition, a large number of MDR *S*. Typhi isolates also exhibited decreased ciprofloxacin susceptibility in Bangladesh. Recently, MDR *S*. Typhi strains with decreased ciprofloxacin susceptibility, and strains exhibiting decreased ciprofloxacin susceptibility only have been reported in India [13], Bangladesh [16] and Kenya [24]. In Bangladesh, 74% of *S*. Typhi strains isolated with decreased ciprofloxacin susceptibility were MDR compared to 50% in the United Kingdom [14]. On the contrary, no association between decreased ciprofloxacin susceptibility and MDR phenotype was observed among *S*. Typhi in a recent study in Kenya [24].

With the decline of the MDR typhoid epidemic in the mid-1990s in Bangladesh, it was expected that the first-line conventional antimicrobial agents might once again become drugs of choice for the treatment of typhoid fever [8]. With the recent emergence of MDR strains that have decreased susceptibility to ciprofloxacin, however, therapy of typhoid fever in Bangladesh has become even more complicated. In such cases, ceftriaxone or cefixime or azithromycin could be considered as possible alternatives although the optimum treatment of these infections is still unclear [25].

With the increasing prevalence of MDR strains that have decreased ciprofloxacin susceptibility and resistance to nalidixic acid, there is a need for careful observation of outcome of therapy for typhoid fever. Failure of ciprofloxacin treatment of typhoid fever occurred due to infection with such *S*. Typhi strains in Bangladesh, as in many other countries [13, 14, 16, 21]. These strains appear susceptible when subjected to ciprofloxacin susceptibility testing by disk diffusion method, or by current MIC break-points by NCCLS criteria but treatment failure still occurs [13, 14]. To address this problem, many studies have shown nalidixic acid resistance as a surrogate marker for decreased ciprofloxacin susceptibility among *S*. Typhi [14, 21, 24]. But, recently, strains of *S*. Typhi with decreased ciprofloxacin susceptibility but susceptible to nalidixic acid were reported [24] questioning the utility of nalidixic acid resistance for detecting such strains. However, in our study the ciprofloxacin (5 μg) disk diffusion test with inhibition zone diameters of ⩽24 mm as break-point had very good sensitivity and specificity for detecting decreased ciprofloxacin susceptibility of *S*. Typhi unlike ofloxacin disk that had low specificity [26]. Thus, ciprofloxacin disk could be used to detect decreased ciprofloxacin susceptibility in *S*. Typhi, which appears to be a useful and easy screen for its detection and future studies should evaluate clinical outcome of treatment of typhoid fever caused by strains that show decreased susceptibility to ciprofloxacin. Moreover, the method is easily available in the laboratory, less expensive and user-friendly for guiding physicians to the proper treatment of typhoid fever.

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Ciprofloxacin is widely used in Bangladesh to treat many infections without prescription and is likely to result in high prevalence of resistance, limiting its utility [23, 27, 28]. A recent observation of R plasmid-mediated quinolone resistance in Enterobacteriaceae [29] is of great concern since this resistance gene could be disseminated rapidly across bacterial populations by conjugation. Hence, further studies are needed to detect mechanisms for decreased ciprofloxacin susceptibility of strains from Bangladesh.

Finally, the development of resistance to ciprofloxacin has been suggested as being due to exposures of these organisms to ciprofloxacin concentrations near their MICs [30]. With an increase in MIC of ciprofloxacin in Bangladesh and other countries, effective use may require parenteral or higher dosages to achieve serum levels required for effective therapy; however, the latter could have unwanted health consequences. Studies are necessary to address these important issues. Continuous surveillance for the susceptibility patterns of *S*. Typhi isolates by disk method is useful and easy if one uses a new break-point zone diameter of ciprofloxacin in evaluating the role of ciprofloxacin in the treatment of MDR typhoid fever. An effective programme to promote rational use of antimicrobial agents is essential to avoid a realistic threat of untreatable MDR typhoid fever.

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