Decline in epidemic of multidrug resistant *Salmonella* Typhi is not associated with increased incidence of antibiotic-susceptible strain in Bangladesh

M. RAHMAN*, A. AHMAD AND S. SHOMA

Laboratory Sciences Division, ICDDRB: Centre for Health and Population Research, GPO Box 128, Dhaka 1000, Bangladesh

SUMMARY

Since 1987, multidrug resistant (MDR) strains of *Salmonella* Typhi, resistant simultaneously to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole, have caused epidemics of severe typhoid fever in Asia and Africa. A retrospective analysis of blood culture results (1989–96) in a Diarrhoea Treatment Centre in Dhaka, Bangladesh detected MDR strains in 0·3 % (8 of 2793) of samples in 1990. The isolation rate peaked to 3·2 % (240 of 7501) in 1994 ($P < 0.01$) and decreased to 1·8 % (165 of 9348) in 1995 and further to 1·0 % (82 of 8587) in 1996 ($P < 0.01$ compared to 1994) indicating the emergence and decline of MDR typhoid epidemic. Ten of 15 MDR strains tested had a 176 kb conjugative R plasmid that mediates resistance to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole to *Escherichia coli* K12. Unlike MDR strains, the isolation rate (~ 3.3 %) of susceptible *S*. Typhi remained remarkably unchanged during the study. The significant decrease in isolation of MDR strains suggests that cheaper and effective first-line antibiotics may re-emerge as drugs of choice for the treatment of typhoid fever in Bangladesh.

INTRODUCTION

Typhoid fever caused by *Salmonella enterica* serotype Typhi (*Salmonella* Typhi), continues to be a major public health problem with an estimated 16·6 million cases and 600 000 deaths annually with 440 000 deaths in Asia alone [1]. It occurs predominantly in the developing world particularly in Southeast Asia with highest mean incidence (1000 cases per 100 000 people per annum) [2]. It is highly endemic in Bangladesh and is an important cause of health problems involving huge health care costs, high morbidity and economic loss [2–4]. Since 1948 chloramphenicol (Cm) has effectively reduced the morbidity and mortality associated with typhoid fever [5]. Cm resistance in *S*. Typhi isolates was reported in 1950 in England, but there had been no accounts of an epidemic caused by resistant strains until 1972 when it occurred in Mexico,

India and Vietnam [6–8]. Ampicillin (Ap) and trimethoprim-sulfamethoxazole (Ts) were considered suitable alternatives for the treatment of typhoid fever caused by chloramphenicol-resistant strains.

Until 1990, Bangladesh was remarkably free of drug-resistant strains of *S*. Typhi although multiresistant strains were isolated from sporadic cases on two occasions. In 1982, there was a report of a single isolate of *S*. Typhi that was resistant to Cm, streptomycin (Sm), sulfonamide (Su) and tetracycline (Te) followed by isolation of another strain in 1986 which was resistant to Cm, Ap, Ts, cloxacillin and cephalexin [9, 10].

In late 1987 an outbreak of typhoid fever caused by multidrug resistant (MDR) *S*. Typhi strain, resistant simultaneously to three first-line antibiotics Ap, Cm and Ts, occurred in suburbs of Shanghai, China [11]. During 1989–90, there were reports of emergence of a similar MDR strain of *S*. Typhi with incompatibility

* Author for correspondence.

group H1 R plasmid that caused epidemic typhoid fever in India [12], Pakistan [13] Arab Gulf [14] and Vietnam [15]. The MDR epidemic strain was unusually associated with severe illness and high incidence of complications and mortality in many countries [4, 11–13, 15]. In early 1990, MDR S. Typhi (R type – ApCmTs) was detected in Bangladesh as an extension of typhoid epidemic in Southeast Asia [16]. Subsequently, it spread to many countries of Asia and Africa [15]. MDR epidemic strains were also detected in the United Kingdom and the United States among immigrants from the epidemic-affected countries raising problems in the detection and treatment of MDR typhoid fever [13, 17]. MDR strains subsequently developed resistance to fluoroquinolones such as ciprofloxacin (Cp) and ofloxacin [18, 19]. Recently, an epidemic caused by quinolone-resistant S. Typhi (R type – ApCmTsCp) has been reported in Tajikistan [20].

On the contrary, a declining trend in epidemic MDR typhoid has been reported from the Indian subcontinent at the same time [21]. Therefore, we studied the emergence, duration and the status of this prolonged MDR typhoid epidemic in Bangladesh so that a rational approach to therapy might be adopted with a probability of reintroducing cheaper first-line conventional antibiotics with proven efficacy.

METHODS

Clinical samples

The study was conducted in Dhaka Clinical Research and Service Centre (CRSC), of ICDDRB: Centre for Health and Population Research, Bangladesh (ICDDR, B), Bangladesh. It serves 100 000 diarrhoeal patients annually. The clinical microbiology laboratory cultures blood from inpatients with diarrhoea and fever as determined by the centre's physicians as well as from outpatients who are referred to this laboratory by physicians of Dhaka city. All salmonella strains were isolated from blood cultures in Dhaka clinical microbiology laboratory, ICDDR, B between 1989 and 1996, were included in the study.

Microbiological techniques

Blood (1–2 ml from children, 4–5 ml from adults) for culture, was drawn by puncture of the femoral vein in children and anterior cubital vein in adults with sterile disposable needle and plastic syringe after cleansing the puncture site with tincture iodine (1%) and ethanol (70%). For children, DuPont's isolator 1.5 (DuPont Company, Wilmington, USA) was used for isolating salmonella according to instructions of the company. A bottle containing 50 ml of Trypticase Soya broth was used for adults. The bottle was incubated aerobically at 37 °C for 7 days and broth was subcultured on appropriate agar plates when growth was suspected and blindly on the seventh day of incubation. Suspected bacterial colonies were identified by standard biochemical tests, serogrouped and serotyped by slide agglutination using salmonella O and H group antisera (Difco Laboratories, Detroit, MI, USA) by standard method. Antimicrobial susceptibility was determined by Kirby-Bauer disk diffusion technique using NCCLS standards, and E. coli (ATCC 25992) and Staphylococcus aureus (ATCC 25923) (22) as control strains, Mueller-Hinton agar (MHA) and commercial antibiotic disks (Oxoid, Basingstoke, UK). The following commercial disks were used: ampicillin 10 (μg), chloramphenicol (30 μg), trimethoprim-sulfamethoxazole (1·25 μg/ 23·75 μg), ceftriaxone (30 μg), and ciprofloxacin (5 μg). MICs of antibiotics were determined by agar dilution method by inoculating 10^4 CFU per spot on MHA containing antibiotic in appropriate concentrations.

Plasmid DNA was extracted from 15 MDR (R type – ApCmTs) and five susceptible S. Typhi isolates according to the method of Portnoy et al. [23] and separated by electrophoresis in 0·7 % agarose gel. Gels were stained with ethidium bromide and visualized by UV transilluminator for plasmid DNA. The transfer of R plasmid was determined by conjugation between MDR S. Typhi and rifampicin-resistant Escherichia coli K12 (F-, lac-, Rif^R) according to the method of Neu et al. [24]. Transconjugants were selected on brain-heart infusion agar containing rifampicin (200 μg/ml) and chloramphenicol (125 μg/ml). To obtain transconjugants, all putative transconjugants were tested for antimicrobial susceptibility, plasmid profiles and lactose fermenting property to differentiate from spontaneous rifampicin-resistant-mutant of donor.

RESULTS

Between 1989 and 1996, a total of 2683 (5·3%) S. Typhi were isolated from 50677 blood cultures processed in the clinical microbiology laboratory. The

Table 1. *Changes in isolation rates of multidrug resistant (MDR) and susceptible strains of* Salmonella *Typhi from blood cultures, 1989–96*

Year	No. of blood cultures	No. (isolation rate) of S. Typhi			No. (isolation rate) of S. Paratyphi
		All	MDR strains*	Susceptible strains	
1989	4385	61 (1·4)	0 (0)	61 (1·4)	9 (0·2)
1990	2793	99 (3·5)	8 (0·29)	91 (3·3)	14 (0·5)
1991	5163	231 (4·5)	60 (1·2)	169 (3·3)	35 (0·7)
1992	5456	309 (5·7)	126 (2·3)	182 (3·3)	27 (0·5)
1993	7444	479 (6·4)	191 (2·6)	268 (3·6)	75 (1·0)
1994	7501	547 (7·3)	240 (3·2)	299 (4·0)	56 (0·7)
1995	9348	582 (6·2)	165 (1·8)	364 (3·9)	81 (0·9)
1996	8587	375 (4·4)	82 (0·95)	283 (3·3)	79 (0·9)
Total	50 677	2683 (5·3)	872 (1·7)	1717 (3·4)	376 (0·74)

* MDR strains = strains simultaneously resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole.
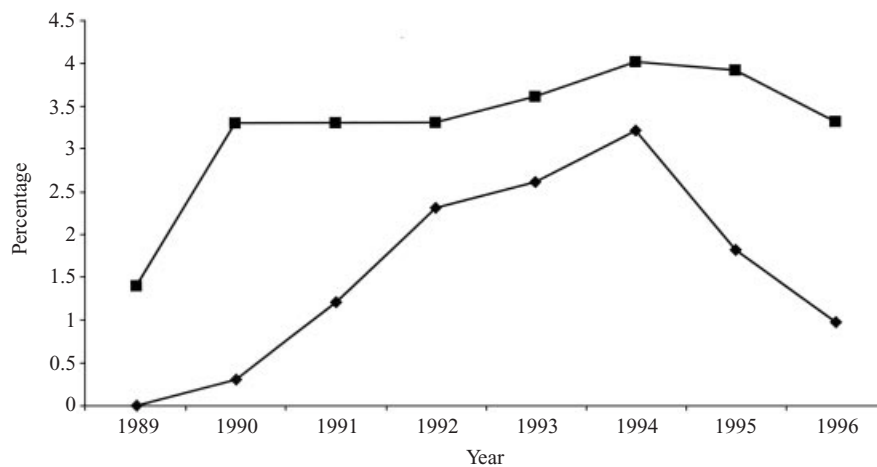

Fig 1. Increase and decrease of multidrug resistant (MDR) *Salmonella* Typhi isolation rate from blood cultures; ◆ MDR *S*. Typhi, ■ Susceptible *S*. Typhi.

isolation rate of *S*. Typhi increased significantly from 1·6% in 1989 to 3·5% in 1990 ($P < 0·01$) and 7·3% in 1994 ($P < 0·01$) in our laboratory (Table 1). However, the isolation rate decreased to 6·2% in 1995 and further to 4·4% in 1996 ($P < 0·01$ compared to 1994). In contrast, isolation rates of susceptible *S*. Typhi and *S*. Paratyphi from blood cultures (Table 1) remained more or less the same during the epidemic period of typhoid fever (1990–6).

In 1989, all *S*. Typhi isolates were susceptible to all antibiotics tested. MDR *S*. Typhi strain (strain simultaneously resistant to Ap, Cm and Ts) was first isolated in 1990. The isolation rate of MDR strain was 0·3% (8 of 2793 samples) from blood cultures in that year and increased by more than fourfold to 1·2% (60 of 5163 samples) in 1991 and peaked to 3·2% (240 of 7501 samples) in 1994 ($P < 0·01$ compared to 1990).

The rate decreased to 1·8% (165 of 9348 samples) in 1995 and further to 1% (82 of 8587 samples) in 1996 ($P < 0·01$ compared to 1994) indicating a decline in the epidemic of MDR typhoid fever.

In 1990, 8% (8 of 99 isolates) of *S*. Typhi isolates were simultaneously resistant to Ap, Cm and Ts. The frequencies of resistance to these drugs increased to 26% in the next year and peaked up to 51% in 1994 ($P < 0·01$ compared to 1990) (Table 2). In 1995, a lower percentage (range = 34–39%) of *S*. Typhi isolates were resistant to these antibiotics. They decreased further to a range of 24–31% in 1996 ($P < 0·01$ compared to 1994). Ceftriaxone (Cr) resistance was negligible and Cp resistance was not detected by disk diffusion method among *S*. Typhi isolates.

Almost all of the resistant strains of *S*. Typhi were MDR strains (Table 3). In 1989, 8% of 61 *S*. Typhi

Table 2. *Frequency of antibiotic resistance among* Salmonella *Typhi isolates from blood cultures, 1989–96*

Antimicrobial agents	Percentage of isolates resistant to antibiotics (no. of isolates tested)							
	1989 (61)	1990 (99)	1991 (231)	1992 (309)	1993 (479)	1994 (547)	1995 (582)	1996 (375)
Ampicillin	0	8	26	41	42	48	34	25
Trimethoprim-sulfamethoxazole	0	8	26	41	44	51	39	24
Chloramphenicol	0	8	26	41	42	46	34	31
Ceftriaxone	NT*	0	0	2·5	0	0	0·2	0
Ciprofloxacin	NT	NT	NT	0	0	0	0	0

* NT, Not tested.

Table 3. *Patterns of resistance to antibiotics among* Salmonella *Typhi isolates from blood samples, 1989–96*

Resistance phenotypes*	Percentange of isolates with resistance patterns (no. of isolates tested)							
	1989 (61)	1990 (99)	1991 (231)	1992 (309)	1993 (479)	1994 (547)	1995 (582)	1996 (375)
Ap, Cm, Ts	0	8	31	41	40	44	29	22
Ap, Cm	0	0	0	0	0·3	0	4	0
Ap, Ts	0	0	0	0·3	2	4	0	3
Cm, Ts	0	0	0·4	0	1	2	4	1
Cm	0	0	0	0·3	0	0	0	0·4
Ts	0	0	0·4	0	0·3	2·5	2	4

* Ap, ampicillin; Cm, chloramphenicol; Ts, trimethoprim-sulfamethoxazole.

were MDR strains. The percentage of MDR strains increased to 44% of 547 in 1994 ($P < 0.01$) indicating the peak of epidemic and then subsequently decreased to 22% of 375 in 1996 ($P < 0.01$ compared to 1994) showing the decline of epidemic. Resistance to one or two antibiotics was rarely detected among *S*. Typhi strains.

Ten (66·7%) of 15 MDR *S*. Typhi strains tested had one 176 kb plasmid. No plasmid was detected in five susceptible isolates. Resistance to Ap, Cm and Ts in MDR strains having plasmid, was transferred to *E. coli* K12 by conjugation and mediated by 176 kb conjugative R plasmid.

DISCUSSION

Until 1989, the treatment of typhoid fever was relatively simple in Bangladesh like many other countries since all *S*. Typhi isolates were uniformly susceptible to first-line antibiotics, including chloramphenicol. Although, a few sporadic cases of typhoid fever caused by antibiotic-resistant *S*. Typhi were reported, there was no evidence of a clinically important resistance among strains of *S*. Typhi in Bangladesh before 1990. In early 1990, MDR *S*. Typhi strain was detected in our clinical microbiology laboratory (16). Since then it was isolated at an increasing frequency and in 1996, nearly half of isolates were MDR strains. During the same period, epidemics caused by MDR *S*. Typhi were reported from Pakistan, India and other Southeast Asian and West Pacific countries, the Middle East and Africa [15]. MDR strains from all countries had similar resistance patterns being resistant to three first-line antibiotics Ap, Cm and Ts. Thus, MDR strain that emerged in 1987 appeared to have caused the largest and prolonged pandemic of typhoid fever for the first time infecting large number of peoples across Asia and Africa [2, 15, 17–20].

At the beginning of pandemic in China, MDR *S.* Typhi was resistant to three first-line antibiotics similar to that observed in Bangladesh. Fluroquinolones (Cp, ofloxacin etc.) and third generation cephalosporins (Cr, cefotaxime) were used to treat MDR typhoid fever in Bangladesh and other countries. The strain subsequently developed resistance to Cp resulting in treatment failures in patients from Indian subcontinent and Vietnam [18, 19]. In 1997, Cp-resistant *S.* Typhi caused an epidemic typhoid fever in Tajikistan (20) signaling the impending difficulties in the treatment of typhoid fever. Although, resistance to third generation cephalosporins is still rare among MDR isolates, Cr resistance is present in non-typhi salmonella strains which could serve as donors of an extended-*β*-lactamase gene to *S.* Typhi.

MDR *S.* Typhi was reported to cause severe illness resulting in serious complications and high mortality [2, 4]. It was associated with high and prolonged fever, marked toxaemia, prolonged bacteraemia and high incidence of complications such as intestinal haemorrhage, jaundice, hepatomegaly and disseminated intravascular coagulation in Bangladesh and many other countries [4, 11, 15]. These severe clinical features were not observed in the first multiresistant typhoid fever epidemic in 1972 in Mexico, Latin American countries, India and Vietnam [6–8]. It appears that MDR *S.* Typhi strain may be more virulent, perhaps due to presence of other virulence genes on its R plasmid.

The recent MDR strain appears likely to have spread from China to Bangladesh via neighbouring countries. Interestingly, the MDR strain did not replace or gain a survival advantage over susceptible *S.* Typhi strains in Bangladesh. Both MDR and susceptible strains were isolated during the epidemic period. However, a decline in isolation rate of MDR strain was observed after 4 years of the MDR epidemic period and continued to do so for the next few years indicating the waning of the MDR epidemic typhoid fever in Bangladesh. A similar observation was reported in India [25]. The isolation rate of susceptible strains in Bangladesh remained unchanged during the epidemic period without being influenced by the emergence and decline of epidemic MDR *S.* Typhi. The findings of our study in association with others who reported the concurrent prevalence of MDR and susceptible *S.* Typhi [25], support the views that there was no emergence of susceptible strains of *S.* Typhi in the Indian subcontinent during the post-epidemic period, contradicting the views that there was an reemergence of susceptible strains of *S.* Typhi [21].

The majority of MDR *S.* Typhi strains in Bangladesh had a 176 kb conjugative R plasmid that mediates resistance of Ap, Cm and Ts and was similar in size to MDR isolates from all countries affected by the epidemic [13, 15, 16]. Thus, it is reasonable to define this epidemic as a R plasmid epidemic, hosted and disseminated by *S.* Typhi. The decline in epidemic of MDR typhoid fever in the Indian subcontinent is perhaps related to the loss of this R plasmid.

Integration of the R plasmid into the chromosome of MDR *S.* Typhi strains explains the absence of R plasmid in some MDR strains in Bangladesh as described earlier among strains from other countries [13].

Like many bacterial pathogens, we continue to encounter the emergence of resistance to commonly used antibiotics among *S.* Typhi isolates at an increasing frequency resulting in difficulties in the treatment of typhoid fever in Bangladesh like other countries in Asia, Africa and Latin America. The incidence of typhoid fever in Southeast Asian countries is still alarmingly high. Thus, the use of an effective vaccine appears to be the key factor in containing typhoid fever in this part of the world in association with appropriate antimicrobial therapy for the treatment of typhoid cases. Our study suggests that cheaper and effective first-line antibiotics may reemerge as drugs of choice for the treatment typhoid fever in Bangladesh as indicated by a marked decline in isolation rate of MDR *S.* Typhi strain.

ACKNOWLEDGEMENTS

REFERENCES

1. Thong KL, Puthucheary S, Yassin RM, et al. Analysis of *Salmonella typhi* isolates from Southeast Asia by pulsed-field gel electrophoresis. J Clin Microbiol 1995; **33**: 1938–41.

2. Pang T, Buttha ZA, Finlay BB, Altwegg M. Typhoid fever and other salmonellosis: a continuing challenge. Trends Microbiol 1995; **3**: 253–5.

3. Akbar MS. Evaluation of chloramphenicol and cotrimoxazole in the treatment of enteric fever. Bangladesh J Child Health 1986; **10**: 11–4.

4. Ahasan HN, Rafiqueuddin AKM, Chowdhury MAJ, Azhar MA, Ara M, Farazi MA. Complications of enteric fever encountered in medical wards. J Dhaka Med Coll 1993; **2**: 32–3.

5. Woodward TE, Smadel JE, Ley HL, et al. Preliminary report on the beneficial effect of chloromycetin on the treatment of typhoid fever. Ann Intern Med 1948; **29**: 131–3.

6. Olarte J, Galindo E. *Salmonella typhi* resistant to chloramphenicol, ampicillin, and other antimicrobial agents: strains isolated during an extensive typhoid fever epidemic in Mexico. Antimicrob Agents Chemother 1973; **4**: 597–601.

7. Paniker CK, Vimala KM. Transferable chloramphenicol resistance in *Salmonella typhi*. Nature 1972; **239**: 109–10.

8. Butler T, Ling NN, Arnold K, Pollack M. Chloramphenicol-resistant typhoid fever in Vietnam associated with R factor. Lancet 1973; **ii**: 983–5.

9. Huq MI, Samadi AR. Chloramphenicol-resistant *Salmonella typhi* vi phage A isolated from patient in Bangladesh. Lancet 1982; **i**: 1125.

10. Morshed MG, Khan NZ, Khan WA, Akbar MS. Multiple drug resistant *Salmonella typhi* in Bangladesh. J Diarrhoeal Dis Res 1986; **4**: 241.

11. Wang F, Gu X, Zhang M, Tai T. Treatment of typhoid fever with ofloxacin. J Antimicrob Chemother 1989; **23**: 785–8.

12. Jesudasan M, John TJ. Multiresistant *Salmonella typhi* in India. Lancet 1990; **ii**: 252.

13. Rowe B, Ward LR, Threlfall EJ. Spread of multi-resistant *Salmonella typhi*. Lancet 1990; **336**: 1065.

14. Wallace M, Yousif AA. Spread of multiresistant *Salmonella typhi*. Lancet 1990; **336**: 1065.

15. Rowe B, Ward LR, Threlfall EJ. Multidrug-resistant *Salmonella typhi*: a worldwide epidemic. Clin Infect Dis 1997; **24** (Suppl 1): S106–9.

16. Albert MJ, Haider K, Nahar S, et al. Spread of multiresistant *Salmonella typhi* in Bangladesh. J Antimicrob Chemother 1990; **24**: 555–6.

17. Zenilman MJ. Typhoid fever. JAMA 1997; **278**: 847–50.

18. Rowe B, Ward LR, Threlfall EJ. Ciprofloxacin-resistant *Salmonella typhi* in the UK. Lancet 1995; **346**: 1302.

19. Pary C, Wain J, Chinh NT, Vinh H, Farrar JJ. Quinolone-resistant *Salmonella typhi* in Vietnam. Lancet 1998; **351**: 1289.

20. Murdoc DA, Banatvala NA, Bone A, Shoismatulloev BI, Ward LR, Threlfall EJ. Epidemic ciprofloxacin-resistant *Salmonella typhi* in Tajikistan. Lancet 1998; **351**: 339.

21. Sood S, Kapil A, Das B, Jain Y, Kabra SK. Re-emergence of chloramphenicol-sensitive *Salmonella typhi*. Lancet 1999; **353**: 1241–2.

22. Baur AW, Kirby WMM, Sherris JC, Tsuck M. Antibiotic susceptibility testing by a standard single disc method. Am J Clin Path 1966; **45**: 593–6.

23. Portnoy DA, Mosley SL, Falkow S. Characterization of plasmids and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis. Infect Immun 1981; **31**: 775–82.

24. Neu HC, Cherubin CE, Longo ED, Flouton B, Winter J. Antimicrobial resistance and R-factor transfer among isolates of *Salmonella* in the Northeastern United States: a comparison of human and animal isolates. J Infect Dis 1975; **132**: 617–22.

25. Jesudasan M, John R, John TJ. The concurrent prevalence of chloramphenicol-sensitive and multidrug-resistant *Salmonella typhi* in Velore, *S*. India. Epidemiol Infect 1996; **116**: 225–7.