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Rising prevalence of enteric fever due to multidrug-resistant *Salmonella*: an epidemiological study

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A prospective study of the prevalent aetiology of enteric fever was undertaken at a tertiary care hospital in North India at intervals of every 3 years. *Salmonella* spp. were isolated from 174 (7 %) patients. Amongst these, 140 (80 %) patients were infected by *Salmonella enterica* subspecies *enterica* serovar *Typhi* (*S.* Typhi) and 16 (9 %) by *S. enterica* serovar Paratyphi A; the remaining 11 % were infected by other *S. enterica* serogroups, Typhimurium, Paratyphi C and Senftenberg, and other group E salmonella. A significantly greater number of *S.* Typhi were isolated in the summer and monsoon months. Multidrug resistance (resistance to chloramphenicol, ampicillin and co-trimoxazole) sequentially increased from 34 % in 1999 to 66 % in 2005. Increasing resistance was also noticed to the other antibiotics, especially to the cephalosporins. Moreover 8 % of the *S.* Typhi isolates were found to be presumptive extended spectrum β-lactamase producers. There was a gradual development of resistance to fluoroquinolones over the 7 years. No resistance was observed to fluoroquinolones in 1999, while in 2005 4.4 % resistance was observed to sparfloxacin, 8.8 % resistance to ofloxacin and a high resistance, 13 %, to ciprofloxacin. This is an alarming development and it is of paramount importance to limit unnecessary use of fluoroquinolones and third generation cephalosporins so that their efficacy against salmonella is not jeopardized further.

INTRODUCTION

Typhoid fever is an important cause of morbidity and mortality in many developing countries, with an estimated 33 million cases worldwide (Edelman & Levine, 1986). In Asia, the mean incidence of enteric fever is estimated to be 900 per 100 000 people per annum (Ivanoff, 1994). Enteric fever is endemic in all parts of India and still constitutes a significant health hazard. The resistance of *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi) to chloramphenicol was first reported in India from Kerala, where a substantial outbreak took place in 1972 (Paniker & Vimla, 1972). Since then multidrug-resistant strains of *S.* Typhi have escalated into a worldwide problem (Ackers *et al.*, 2000; Jesudasan & John, 1990; Kamili *et al.*, 1993; Madhulika *et al.*, 2004). The steadily increasing multidrug resistance in *S.* Typhi strains is a cause of grave concern in India, where such strains are endemic in many parts. A prospective study was planned to assess the antimicrobial susceptibilities of all salmonella species isolated from blood. The study was conducted over 3 non-consecutive years (1999, 2002 and 2005) spanning a 7 year period.

METHODS

The study was carried out from January to December in the years 1999, 2002 and 2005, in the 1500 bed Lok Nayak Hospital, New Delhi (India). Blood samples were collected from febrile patients prior to initiation of antibiotic therapy. These samples, 5–10 ml from adults and 2–3 ml from children, were collected by venepuncture using aseptic technique, and inoculated directly into blood culture bottles containing 50 ml brain heart infusion broth. The samples were processed according to standard recommended techniques (Koneman *et al.*, 1997). A detailed clinical and treatment history was elicited from all of the patients. The bottles were incubated at 37 °C for 7 days and examined daily for bacterial growth. Subcultures were performed on the first, second, third, fifth and seventh day of incubation on 5 % sheep blood agar and MacConkey agar. Suspected non-lactose fermenting colonies were screened biochemically and their identity confirmed serologically.

Antibiotic sensitivity was tested by the Kirby–Bauer technique according to Clinical and Laboratory Standards Institute guidelines (Bauer *et al.*, 1966). The following antibiotics (Himedia) were used: amoxicillin (10 μg), ampicillin (10 μg), chloramphenicol (30 μg), nalidixic acid (30 μg), cefotaxime (10 μg), cefuroxime (30 μg), ceftriaxone (10 μg), cefpodoxime (30 μg), co-trimoxazole (25 μg), gentamicin (10 μg), netilmicin (10 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), sparfloxacin (5 μg), azithromycin (15 μg) and amoxicillin/clavulanic acid (20/10 μg). Presumptive extended spectrum β-lactamase (ESBL) producers were screened by combined disc test, as well as by double disc synergy test, using discs of amoxicillin/clavulanic acid, amoxicillin, cefoperazone sulbactam, cefoperazone, ceftriaxine and cefotaxime. The discs of substrates were kept at a distance of 15 mm from the inducers (amoxicillin/clavulanic acid and

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Abbreviation: ESBL, extended spectrum β-lactamase.

cefoperazone sulbactam). A difference of >5 mm in the zone of inhibition of the cephalosporin versus the inducer was a marker of a presumptive ESBL producer. Statistical analysis was performed using the chi-square test and Student's paired *t*-test.

RESULTS AND DISCUSSION

A total of 5565 patients entered the study, of which 174 (3.1 %) in all had bacteraemia due to *Salmonella* spp. The majority of isolates, 157 (90.2 %), were from children and 17 (9.8 %) were isolated from adults (Table 1). The cases were predominantly in the 6–15 year age group, representing 40.2 % of the total. An unusual feature was that there were as many as 7 (4 %) neonates from whom *S.* Typhi was isolated, the youngest being merely 8 days old. This is believed to be the first ever report of enteric fever in an 8-day-old neonate. More than half (55.7 %) of the patients were males.

S. Typhi, totalling 140 (80.5 %) isolates, predominated. There were 16 isolates (9.2 %) that were *S. enterica* serovar Paratyphi (*S.* Paratyphi) A, and 18 (10.3 %) isolates that were other *Salmonella* serogroups: 5 (2.9 %) *S. enterica* serovar Typhimurium (*S.* Typhimurium), 2 (1.1 %) *S.* Paratyphi C, 3 (1.7 %) *S. enterica* serovar Senftenberg, 3 (1.7 %) other group E salmonella and 5 (2.9 %) *Salmonella* serogroups other than groups A, B, C, D and E .

It was observed that a large number, 120 (69.0 %), of the cases of enteric fever clustered in the hot months of April, May and June, and in the monsoon season, with a larger peak in the summer and a smaller peak in the monsoon season (Fig. 1). The number of cases decreased during autumn and winter. During the summer and monsoon months the water supply and sanitation systems are under a great strain in Delhi, which could account for the higher incidence in these months.

Multidrug resistance in *S.* Typhi (resistance to ampicillin, chloramphenicol and co-trimoxazole) was observed in 74 (52.9 %) of the isolates. On studying the trend over the 7 year period, multidrug resistance increased from 16 (34 %) isolates in 1999 to 28 (58 %) isolates in 2002, and rose to 30 (66 %) isolates in 2005, along with a rising resistance to all the other drugs (Table 2). *S.* Paratyphi A did not exhibit any resistance to chloramphenicol (Table 3). Only one

Table 1. Age and sex distribution of patients suffering from enteric fever

Age	Female *n*=77 (44.3 %)	Male *n*=97 (55.7 %)	Total *n*=174 (%)
Neonates	3	4	7 (4.0)
<1 year	4	12	16 (9.2)
1–5 years	41	23	64 (36.8)
6–15 years	24	46	70 (40.2)
>15 years	5	12	17 (9.8)

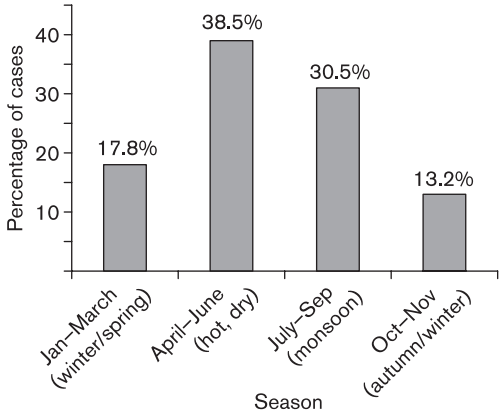

Fig. 1. Seasonal distribution of cases of enteric fever.

(6.3 %) isolate was resistant to co-trimoxazole. However, 9 (56.3 %) isolates were resistant to ampicillin. *S.* Typhimurium expressed 100 % resistance to ampicillin. *S.* Senftenberg and other group E serotypes shared similar resistance patterns: 33.3 % resistance to chloramphenicol and 66.7 % to ampicillin. *S.* Typhi exhibited extremely high, 59 (42.1 %) isolates, resistance to amoxicillin/clavulanic acid. Amongst the cephalosporins, the least, 8 (5.7 %) isolates, resistance was demonstrated against cefotaxime followed by 17 (12.1 %) isolates with resistance to ceftriaxone and cephalexin, and a high resistance, 20 (14.3 %) isolates, to cefpodoxime, and 22 (15.7 %) isolates with resistance to cefuroxime. Resistance to amoxicillin/clavulanic acid has increased significantly over the years studied. This high level of resistance to β-lactam β-lactamase inhibitor could be due to hyperproduction of class A β-lactamases such as TEM-1 or SHV-1. The most common inhibitor-resistant enzymes are derivatives of TEM-1. It is unlikely that all these organisms are ESBL producers, as most of the isolates are still sensitive to the third generation cephalosporins. This has also been suggested by Jesudasan & John (1990).

We feel that in the Indian scenario, with the high level of resistance to amoxicillin/clavulanic acid, this β-lactam β-lactamase combination is a poor inducer for the detection of ESBL producers. We recommend cefoperazone/sulbactam (1 : 1) as a better inducer for screening purposes. A total of 8 % isolates were identified as presumptive ESBL producers. Ceftriaxone proved to be a better substrate than cefpodoxime, cefotaxime and cefoperazone. Presumptive ESBL production increased from none in 1999 to 8 % in 2005 in *S.* Typhi. We also observed a gradual increase in resistance among the fluoroquinolones, with least resistance, 4 (2.9 %), to sparfloxacin, and a higher resistance to ofloxacin, 6 (4.3 %), and the highest resistance, 14 (10 %), to ciprofloxacin. A total of 9 (6.4 %) isolates were resistant to azithromycin. Nalidixic acid was used for the screening of fluoroquinolone resistance. Its resistance closely mirrors resistance to ciprofloxacin (Table 2). We feel that individual fluoroquinolones should be tested separately as

Table 2. Resistance pattern of *S.* Typhi to selected drugs over a 7 year period

Drug	No. of resistant isolates (%)			*P* value 1999 vs 2005
	1999 (*n*=47)	2002 (*n*=48)	2005 (*n*=45)	
Chloramphenicol	19 (40.4)	29 (60.4)	33 (73.3)	<0.003
Co-trimoxazole	17 (36.2)	29 (60.4)	36 (80)	<0.003
Amoxicillin	19 (40.4)	30 (62.5)	31 (68.9)	0.003
Amoxicillin/clavulanic acid	11 (23.4)	17 (35.4)	31 (68.9)	<0.001
Cephalexin	2 (4.3)	3 (6.3)	7 (15.6)	NS
Cefuroxime	5 (10.6)	8 (16.7)	9 (2.0)	NS
Cefotaxime	1 (2.1)	2 (4.2)	5 (11.1)	NS
Ceftriaxone	1 (2.1)	5 (10.4)	7 (15.6)	NS
Netilmicin	2 (4.3)	3 (6.3)	4 (8.9)	NS
Nalidixic acid	1 (2.1)	4 (8.3)	10 (22.2)	<0.01
Ciprofloxacin	0	4 (8.3)	10 (22.2)	<0.01
Ofloxacin	0	1 (2.1)	5 (11.1)	<0.05
Sparfloxacin	0	1 (2.1)	3 (6.7)	NS

NS, Not significant.

this study shows different sensitivity patterns among the three quinolones tested, which was confirmed by the patients recovering from the infection despite some being resistant to nalidixic acid. Incremental drug resistance was observed over the years. In 2005, multidrug resistance had increased to 66 % from 34 % in 1999, which is alarming. In our study emergence of resistance to fluoroquinolones was noticed. There have been varying reports from other parts of India. Some studies from India have reported no resistance to ciprofloxacin (Kabra *et al.*, 2000; Ciraj *et al.*, 1999), while others have reported development of resistance to ciprofloxacin and norfloxacin (Rathish *et al.*, 1995; Gaind *et al.*, 2006). Our study with the newer quinolones yielded lower resistance to them than to ciprofloxacin. Thus newer fluoroquinolones appear to be a better option than ciprofloxacin. There was a statistically significant rise in resistance from 1999 to 2005 against chloramphenicol, co-trimoxazole, ampicillin, ofloxacin and ciprofloxacin ($P<0.003$, $P<0.001$, $P<0.05$, $P<0.05$ and $P<0.01$, respectively).

Ciprofloxacin was significantly more effective in treating multiresistant typhoid fever than ceftriaxone. Although the

Table 3. Antimicrobial resistance patterns of *S.* typhi. *S.* Paratyphi and other *Salmonella* serogroups

	S. Typhi (group D) *n*=140 (%)	*S.* Para A (group B) *n*=16 (%)	STM (group B) *n*=5 (%)	*S.* Para C (group C) *n*=2 (%)	*S.* Senftenberg (group E) *n*=3 (%)	Group E other than *S.* Senftenberg *n*=3 (%)	Other *Salmonella* groups *n*=5 (%)
Chloramphenicol	79 (56.4)	0	1 (20)	0	1 (33.3)	1 (33.3)	0
Co-trimoxazole	82 (58.6)	1 (6.3)	0	1 (50)	0	0	2 (40)
Ampicillin	81 (57.9)	9 (56.3)	5 (100)	0	2 (66.7)	2 (66.7)	1 (20)
Amoxicillin	80 (57.1)	1 (6.3)	0	0	0	3 (100)	2 (40)
Ampicillin sulbactam	59 (42.1)	1 (6.3)	2 (40)	1 (50)	0	3 (100)	2 (40)
Cephalexin	10 (7.1)	1 (6.3)	0	0	2 (66.7)	1 (33.3)	0
Cefotaxime	7 (5)	0	0	0	2 (66.7)	1 (33.3)	0
Cefuroxime	22 (15.7)	1 (6.3)	2 (40)	0	0	3 (100)	1 (20)
Ceftriaxone	11 (7.9)	0	0	0	1 (33.3)	1 (33.3)	1 (20)
Cefpodoxime	20 (14.3)	1 (6.3)	0	0	1 (33.3)	0	0
Gentamicin	11 (7.9)	2 (12.5)	0	0	2 (66.7)	1 (33.3)	0
Netilmicin	7 (5)	0	0	0	1 (33.3)	1 (33.3)	0
Ciprofloxacin	10 (7.1)	0	0	0	2 (66.7)	0	0
Ofloxacin	5 (3.6)	0	0	0	0	0	0
Sparfloxacin	3 (2.1)	0	0	0	0	0	1 (20)
Azithromycin	9 (6.4)	0	0	0	0	0	0

S. Para A, *S.* Paratyphi A; S. Para C, *S.* Paratyphi C; STM, *S.* Typhimurium.

sensitivity profile does not show much difference between the two (Table 3), in 27 patients in whom ceftriaxone was used as the drug of first choice, fever did not subside. Ciprofloxacin was successfully resorted to in these cases. Failure of ceftriaxone in these cases could be attributed to ESBL production, and hyperproduction of TEM and SHV type of β-lactamases. No treatment failure occurred in the 73 cases where ciprofloxacin was used as a drug of first choice. Similar results have been reported in other studies (Mirza et al., 1995; Wallace *et al.*, 1993). Although fluoroquinolones are not advised for use in children in many countries, they have been used for treating typhoid with few side effects (Gupta, 1994). It was noticed that none of the other *Salmonella* serogroups exhibited triple drug resistance. Among them maximum resistance was observed against ampicillin. These point to divergent lines of antibiotic resistance.

Although in our study *S.* Paratyphi A was not the leading cause of enteric fever, there are reports of increasing isolation rates of *S.* Paratyphi A from India as well as Asia (Sood *et al.*, 1999; Ochiai *et al.*, 2005). The emergence of *S.* Paratyphi A has an important implication – licensed typhoid fever vaccines (Vi polysaccharide and live oral Ty21a) are not protective against *S.* Paratyphi A infections. The emergence of this serotype may portend a decline in the usefulness of these vaccines in controlling enteric fever in the future. New vaccination strategies should include bivalent vaccines that protect against both *S.* Typhi and *S.* Paratyphi A (Ochiai *et al.*, 2005).

At present, similar treatment strategies may work for both organisms but with the recent escalation of drug resistance in *S.* Paratyphi A future enteric fever prevention strategies in Asia must focus on it too (Harish *et al.*, 2004). It is clear that multidrug-resistant strains of *S.* Typhi have become firmly entrenched in various parts of the Indian subcontinent. In our opinion ofloxacin and sparfloxacin are better options then ciprofloxacin and cephalosporins. Urgent consideration should be given to what should be the most appropriate first line therapy for enteric fever in the subcontinent.

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