Sensitivity Pattern of *Salmonella* serotypes in Northern India

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Background. Enteric fever continues to be a major public health problem, especially in the developing countries of the tropics. We determined the incidence of *Salmonella* bloodstream infections and their antimicrobial resistance patterns from May to August in the years 1997-2001 in Haryana, a large state of India. The minimum inhibitory concentration (MIC) was also determined for 60 isolates of *S. typhi* to various commonly used antimicrobial agents. Material and Methods. Blood cultures of 6,956 patients (PUO/septicemia) were processed by standard procedures and the *Salmonella* spp. isolates were identified with specific antisera and with standard biochemical tests. Antimicrobial susceptibilities were determined by Stokes disc diffusion method. The MIC of 60 randomly isolated strains of *S. typhi* was determined by agar dilution method using Mueller Hinton Agar medium. Results. Isolation rates of *Salmonella* spp. increased in 2000 and 2001. Multidrug resistance (MDR) in *S. typhi* had increased while in *S. paratyphi* it had decreased markedly. Ninety per cent chloramphenicol sensitivity was seen in *S. typhi* by MIC method. There was a decrease in the susceptibility to ciprofloxacin of *S. typhi* with MIC showing an upward trend. All *S. typhi* tested were sensitive to third generation cephalosporins and aminoglycosides with MIC well below the breakpoint. Discussion. Our study indicates that MDR in *S. typhi* is on the rise in our area. There is also re-emergence of chloramphenicol sensitivity. Rising MIC values of ciprofloxacin may lead to prolonged treatment, delayed recovery or pose treatment failure. Thus, sensitivity pattern of causative organism must be sought before instituting appropriate therapy to prevent further emergence of drug resistance.

Key Words: *Salmonella*, multidrug resistance.

Enteric fever is a worldwide problem and widely prevalent in the developing countries of the tropics. An estimated 600,000 deaths from enteric fever occur annually throughout the world [1]. *Salmonella typhi* and *S. paratyphi* A are the predominant types of *Salmonella* responsible for enteric fever in India, particularly in the summer [2]. In India, *S. typhi* drug resistance has been reported since 1960; followed by the first outbreak of multidrug resistant *S. typhi* (MDRST) in Calicut [3,4]. Since then MDRST has appeared throughout the world, especially in South America, the Indian subcontinent, Africa and Southeast Asia [5-7]. Drug resistance is of considerable importance to microbiologists and is posing a major therapeutic problem for the public and for public health authorities. Resistance to commonly used antibiotics, such as chloramphenicol, ampicillin, and co-trimoxazole has been reported from different parts of India in the last two decades [8]. In the recent past, fluoroquinolones and cephalosporins have gained importance for the treatment of enteric infections. Considerable variation in antibiotic resistance patterns among salmonellae to various antibiotics has been reported from different parts of India. Hence we studied the incidence of *Salmonella* bloodstream infections and their antimicrobial resistance patterns from May to August, 1997 to 2001. Minimum inhibitory concentrations (MIC) were also determined by agar dilution method for 60 randomly isolated (May to August, 2001) strains of *S. typhi*.

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Material and Methods

Blood cultures of 6,956 patients (PUO/septicemia) attended at the Postgraduate Institute of Medical Sciences, Rohtak, Haryana and outpatient departments from May to August, 1997 to 2001 were processed by standard procedures [9]. Duplicate isolates from the same patient were excluded from the study.

Microbiological methods

All isolates of *Salmonella* spp. from blood were identified with specific antisera (Central Research Institute, Kasauli, India) and with standard biochemical tests [9]. Antimicrobial susceptibilities were determined at the time of isolation by the Stokes disc diffusion method, with discs containing chloramphenicol (30µg), ampicillin (10µg), co-trimoxazole (25µg), gentamicin (10 µg), amikacin (10µg), ciprofloxacin (5µg), norfloxacin (10µg), cefotaxime (30µg), ceftizoxime (30µg) and ceftriaxone (30µg). The results were interpreted using standard methods [10]. MIC of 60 isolates of *S. typhi* was determined by the agar dilution method using Mueller Hinton Agar medium (Hi Media, India) with an inoculum of 10^5 organisms/ml, followed by 24 hours incubation and interpreted using a standard protocol [10]. The control strain was NCTC 10418 *Escherichia coli*. Each isolated *Salmonella* was tested against 12 dilutions of 8 antibiotics viz. ampicillin, chloramphenicol, gentamicin, amikacin, ciprofloxacin, cefotaxime, ceftizoxime and ceftriaxone.

Results

A total of 533 isolates of *Salmonella* spp. were obtained from 6956 blood cultures. *S. typhi* was the predominant serotype, followed by *S. paratyphi* A and *S. typhimurium*. There were recent increases in *Salmonella* bloodstream infections in 2000 (7.7%) and 2001 (9.3%), when compared to previous years (Table 1).

The antimicrobial susceptibility pattern of *S. typhi* against various antibiotics tested was ampicillin (21% to 45%), chloramphenicol (41% to 52%), co-trimoxazole (15% to 74%), gentamicin (57% to 76%), norfloxacin (34% to 83%), amikacin (76% to 87%), ciprofloxacin (62% to 89%), cefotaxime (74% to 88%), ceftizoxime (89% to 95%), ceftriaxone (87% to 90%) (Table 2). While ampicillin, cotrimoxazole and norfloxacin showed a downward trend, chloramphenicol showed an upward trend in the sensitivity pattern. All the newer drugs, eg. fluoroqinolones and cephalosporins were more effective, however some resistance problems, ranging from 5% to 25% have been encountered recently.

The antimicrobial sensitivity pattern of *S. paratyphi* against the various antibiotics (from 1997 to 2001) is shown in Table 3. There was decreased sensitivity pattern against chloramphenicol, ampicillin, cotrimoxazole and norfloxacin (12% to 38%) while the better sensitivity pattern was shown by aminoglycosides, fluoroquinolones and cephalosporins (70% to 87%) in the year 2001.

Multidrug resistance (MDR) i.e. resistance to three or more antibiotics in *S. typhi* and *S. paratyphi* A is as shown in Table 4. Overall MDR (from 1997 to 2001) in *S. typhi* was found in 242 (55.5%) out of 436 isolates and in 34 (36.2%) out of 94 isolates in *S. paratyphi* A. MDR in *S. typhi* showed an upward trend while *S. paratyphi* A showed a downward trend. According to the antibiotic resistance (ACCo) 38 of 436 isolates of *S. typhi* and 10 of 94 isolates of *S. paratyphi* were multidrug resistant. Out of these 48 MDR isolates, 36 were even resistant to amikacin, cefotaxime, ciprofloxacin, ceftizoxime and ceftriaxone by disc diffusion method.

The MIC of 60 isolates of *S. typhi* was determined; 100% of these salmonellae were found to be resistant to ampicillin (MIC=128µg/ml), 90% of the strains were sensitive to chloramphenicol (breakpoint MIC=32µg/ml) while only 63% of the strains were sensitive to ciprofloxacin (break point MIC=4µg/ml). *S. typhi* strains were found to be sensitive to gentamicin (0.015µg to 2µg/ml), amikacin (0.06µg to 2µg/ml), cefotaxime (0.03µg to 0.5µg/ml), ceftizoxime (0.06µg to 1µg/ml), ceftriaxone (0.06µg to 0.5µg/ml) below their MIC break point (Table 5).

Discussion

Enteric fever is a major public health problem in our country. Isolation of *Salmonella* spp. occurs throughout the year in Haryana. This means that

Table 1. Prevalence of *Salmonell*a spp. from May to August in 1997 to 2001

	1997(%)	1998(%)	1999(%)	2000 (%)	2001(%)	Total(%)
Total no.of blood samples received	1167	1101	1162	1743	1783	6956
S. typhi	56 (4.7)	38 (3.5)	42 (3.6)	134 (7.7)	166 (9.3)	436 (6.3)
S. paratyphi A	16 (1.4)	14 (1.3)	12 (1.0)	5 (0.3)	47 (2.6)	94 (1.4)
S. typhimurium	03 (0.3)	0	0	0	0 0	3 (0.04)
Total no. of salmonellae	65 (5.6)	52 (47)	54 (4.6)	139 (8.0)	213 (12)	533 (7.7)

Table 2. Sensitivity (in %) of *Salmonella typhi* to various antibiotics by the disc diffusion method

Antibiotics/Year	1997	1998	1999	2000	2001	Overall
Chloramphenicol	41	41	43	48	52	45
Ampicillin	27	37	31	45	21	32
Gentamicin	65	68	57	76	68	67
Co-trimoxazole	74	47	40	13	15	38
Norfloxacin	83	79	60	65	34	64
Amikacin	85	85	76	87	87	84
Ciprofloxacin	89	76	62	87	81	79
Cefotaxime	74	76	74	82	88	79
Ceftizoxime	-	-	-	89	95	92
Ceftriaxone	-	-	-	87	90	88

Table 3. Sensitivity (in %) of *Salmonella paratyphi* to various antibiotics by the disc diffusion method

Antibiotics/Year	1997	1998	1999	2000	2001
Chloramphenicol	25.0	35.7	50.0	80.0	72.8
Ampicillin	50.0	57.1	33.3	40.0	14.9
Gentamicin	62.5	57.1	75.0	80.0	70.2
Co-trimoxazole	50.0	50.0	33.3	40.0	38.3
Norfloxacin	87.5	78.6	41.7	60.0	14.9
Amikacin	75.0	92.9	91.7	100.0	83.0
Ciprofloxacin	93.8	64.2	83.3	100.0	83.0
Cefotaxime	62.5	100.0	83.3	100.0	85.1
Ceftizoxime	-	-	-	100.0	87.2
Ceftriaxone	-	-	-	100.0	85.1

Table 4. Multidrug resistance among *Salmonella* spp.

Years	*S. typhi*			*S. paratyphi*		
	Total	**MDR (%)**	**ACCo (%)**	**Total**	**MDR (%)**	**ACCo (%)**
1997	56	30 (53.6)	2 (3.6)	16	11 (68.8)	0 (0)
1998	38	19 (50.0)	6 (15.8)	14	05 (35.7)	2 (14.3)
1999	42	24 (57.0)	7 (16.7)	12	05 (41.7)	2 (16.7)
2000	134	63 (47.0)	10 (7.5)	5	00 (20.0)	0 (0)
2001	166	106 (63.9)	13 (7.8)	47	13 (27.7)	5 (10.6)
Total	436	242 (55.5)	38 (8.7)	94	34 (36.2)	10 (10.6)

MDR = Multidrug resistance.
ACCo = Antibiotic resistance (ampicillin, chloramphenicol, co-trimoxazole).

Table 5. MIC values of *S. typhi* to various antimicrobial agents

Drug (µg /ml)	Ampi (≥32)*	Chlor (≥32)	Genta (≥8)	Amika (≥32)	Cipro (≥4)	Cefotax (≥64)	Ceftiz (≥32)	Ceftriax (≥64)
≥128	60	04	- **	-	-	-	-	-
64	0	0	-	-	-	0	0	0
32	0	02	-	-	-	0	0	0
16	0	12	0	0	0	0	0	0
08	0	02	0	0	12	0	0	0
04	0	16	0	0	10	0	0	0
02	0	14	14	02	04	0	0	0
01	0	0	42	56	0	0	02	0
0.5	0	04	0	0	0	02	0	22
0.25	0	0	0	0	20	10	0	34
0.12	0	0	0	0	0	02	0	0
0.06	0	06	02	02	06	12	58	04
0.03	-	-	0	0	0	34	0	0
0.015	-	-	02	0	0	-	-	-
0.007	-	-	0	0	08	-	-	-

Numbers in each row indicate the number of strains with that MIC value against each antimicrobial agent.
Ampi, ampicillin; Chlor, chloramphenicol; Genta, gentamicin; Amika, amikacin; Cipro, ciprofloxacin; Cefotax, cefotaxime; Ceftizox, ceftizoxime; Ceftriax, ceftriaxone.
* = breakpoint MIC.
** = dilution not made of that particular antibiotic.

drinking water conditions and sanitation have not improved or a large number of carriers are present in the society. Isolation rates of *Salmonella* spp. have increased in recent years (2000-2001), particularly in the summer months. Proper sanitation, public health education and vaccination are long term preventive measures that would improve this situation.

Chloramphenicol has been the mainstay of treatment for enteric fever, while ampicillin/co-trimoxazole are other cost-effective and well-tried primary drugs of choice. Drug resistance to chloramphenicol in *S. typhi* first emerged in the United Kingdom (UK) in the 1950s and subsequently in Greece and Israel followed by the epidemics of MDR *Salmonella* in Mexico, India and

other regions [11]. Though the resistance to chloramphenicol increased steadily in India from 1960 onwards the ACCo showed a downwards trend from 15.8% to 7.8% from 1998 to 2001 (Table 1). This indicates a reemergence of chloramphenicol sensitivity in *S. typhi* (Table 2), as reported previously [12,13]. MIC determination by the tube dilution method also showed that 90% of *S. typhi* strains were chloramphenicol sensitive in year 2001 while ampicillin resistance was 100% (MIC=128µg/ml).

An interesting feature that was observed in various other studies conducted worldwide as well as in our study was the lack of correlation between the results of disc diffusion and MIC methods [14-18]. While MIC was determined for only 60 isolates in 2001, we tested a large number of isolates with the disc diffusion method. Similar to our chloramphenicol sensitivity results, Jevanand et al. [14] found that 29.6% of the strains were sensitive to chloramphenicol by the disc diffusion method while 100% sensitivity was observed with MIC methodology. All the strains were found to be resistant to ampicillin by MIC methodology by Jevanand et al as determined in our study [14]. We observed an increased resistance to ampicillin with MIC methodology (= 128µg/ml in all the strains) when compared to the disc diffusion method (79% of the strains resistant in 2001). In a similar manner, more strains were considered resistant to drugs by MIC than with disc diffusion in another study [15]. These variations could also be due to fewer isolates being tested with MIC and through random selection of the isolates. This latter hypothesis is supported by the fact that when we used both methods on the 60 strains isolated in 2001 there was almost 90% correlation between the two methods. A study was conducted in Britain where each of 12 laboratories in different parts of Britain supplied approximately 80 consecutive bacterial isolates to Department of Bacteriology, St. Mary's Hospital, London. Not all laboratories provided sensitivity reports for every antimicrobial substance for which MIC determinations were made. There were sometimes considerable discrepancies between the two sets of data. Consequently, as stated by Barrett et al., Stokes results may not always correlate well with those determined by MIC or breakpoint [16].

MDRST is a major therapeutic concern for physicians in developing countries. Contributory factors may be drug overuse, misuse and inappropriate prescribing practices by physicians along with intrinsic microbiological plasmid-mediated factors. In the years 1982-89 the rate of MDRST in India was below 15%, but it increased to 50% in 1990, to more than 70% in 1992 [19] and then to 90% in Bangalore in 1994 [20]. In our region, MDRST varied from 38.5% in 1990 [21] to 27.11% in 1995 [22]. We found MDR in *S. typhi*to increase from 53.6% to 63.9%, while in *S. paratyphi* A it dropped from 68.8% to 27.7% from 1997 to 2001 (Table 4).

In a search for improved treatments for enteric fever and MDRST in particular, attention has been focused on fluoroquinolone compounds and broad-spectrum cephalosporins because of their excellent properties. These highly active drugs reduce the duration of treatment from the traditional 14 days that is necessary with chloramphenicol. Short treatment regimens and reduced periods of hospitalization have obvious financial benefits, particularly in developing countries. They are also more likely to ensure compliance [23]. In our study there was 88% sensitivity to ciprofloxacin while sensitivities to third generation cephalosporins – cefotaxime, ceftizoxime and ceftriaxone were 81%, 90% and 95%, respectively, by the disc diffusion method in 2001. However, the MIC of *S. typhi* to ciprofloxacin has shown an upward trend with 20% strains with MIC = 8µg/ml and 16.6% strains with MIC = 4µg/ml (breakpoint MIC inoculum =4µg/ml). It is important to remember that the ability of bacteria to develop resistance is partly related to the concentrations of the drugs to which they are exposed. It is therefore recommended that therapeutic blood levels of antibiotics should be four times higher than MIC. This has been observed for quinolones by Cullmann et al., but this aspect of pharmacodynamics needs to be investigated [24,25]. However, all *S. typhi* tested were sensitive to third generation cephalosporins (MIC range 0.03 to 1µg/ml) and aminoglycosides (gentamicin and amikacin), with MIC well below the breakpoint. However, parenterally applied broad spectrum cephalosporins are inferior to fluoroquinolones and it

is unlikely that oral drugs of the same class would be any better [26].

The use of fluoroquinolone antibiotics in children is contraindicated because of dose-dependent drug-induced damage to the articular cartilage of weight bearing joints. However, fluoroquinolones have now been safely used in typhoid and other types of systemic salmonellosis, as well as for other childhood life-threatening infections such as MDR pseudomonas and drug resistant shigellosis [27,28]. In our study, 5-10% drug resistance was observed to third generation cephalosporins by *S. typhi* with the disc diffusion method, though all the strains tested had MIC values considerably below the breakpoint MIC. Similar differences have been reported by other researchers [17].

Thus our study indicates that MDR in *S. typhi* is on the rise in our area. On the other hand there has been a reemergence of chloramphenicol sensitivity. Rising MIC values of ciprofloxacin may lead to prolonged treatment, delayed recovery or post treatment failure [29]. Thus the sensitivity pattern of causative organism must be studied before instituting appropriate therapy to prevent further emergence of drug resistance.

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