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Full Length Research Paper

A changing pattern in antimicrobial susceptibility of *Salmonella enterica* serotype isolated In North India

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Typhoid fever continues to remain a health problem as the causative organism *Salmonella typhi* has developed resistance to many of the antibiotics used. This study was undertaken to determine the current pattern of resistance to antimicrobial agents of *S. typhi* isolates obtained in a tertiary Health care Hospital. 309 samples were taken. Sensitivity to ampicillin, chloramphenicol, cotrimoxazole, ciprofloxacin and ceftriaxone was determined by disc diffusion, and the minimum inhibitory concentration (MIC) was determined. Antibiotic sensitivity was carried out by Kirby-Bauer method. ESBL screening and phenotype confirmation were done following National Committee for Clinical Laboratory Standards (NCCLS) recommendations for Escherichia coli. Isolation rates of Salmonella typhi was prominent in 2002 - 2003 and have remained stable. *S. paratyphi-A* is showing an increasing trend. The 3 common antibiotics which were showing resistance earlier are now showing sensitivity. The study indicates that MDR S. typhi is on the rise. There is also re-emergence of chloramphenicol sensitivity. Sensitivity pattern of causative organism must be sought before instituting appropriate therapy to prevent further emergence of drug resistance. Indiscriminate use of ciprofloxacin or ceftriaxone should be strongly discouraged. They should be used as second line agents. Also, the treatment must not be completely dependent on the Widal test. Blood culture and clinical history must be taken into consideration for treatment.

Key words: Ciprofloxacin, ceftriaxone, Enteric fever, multi-drug resistant (MDR) *Salmonella,* Minimum inhibitory concentration (MIC).

INTRODUCTION

Enteric fever (EF) is one of the most common causes of pyrexia of unknown origin (PUO) in most parts of the world. Enteric fever includes Typhoid fever caused by *Salmonella Typhi* and Paratyphoid fever caused by *S. Paratyphi* A, B and C. Enteric fever caused high morbidity and mortality in the earlier years and still causing a lot of trouble in most of the developing countries. It continues to be a major health problem despite the use of antibiotics and the development of newer antimicrobial agents. The causative organism, *S. typhi* has rapidly gained resistance to antibiotics like ampicillin, chloram-phenicol and cotrimoxazole, and also to previously efficacious agents like ciprofloxacin (Butt et al., 2003; Jesudason and John, 1992). The incidence of multi drug resistant (MDR*) S. typhi* was reported to be as high as 60% while there are reports noting a decline (Chande et al., 2002; Saha et al., 1990 - 2000; Sanghavi et al., 1999). The present study was undertaken to know the frequency of isolation of salmonella serotypes and their antibiotic susceptibility to the more commonly used agents like ampicillin, chloramphenicol, cotrimoxazole, ciprofloxacin and ceftriaxone.

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MATERIALS AND METHODS

Blood and Bone Marrow samples were collected from suspected cases of Enteric fever admitted to our hospital. The blood culture bottles were placed in BACTEC and the bottles were subcultured onto the culture plates. The culture plates were examined for

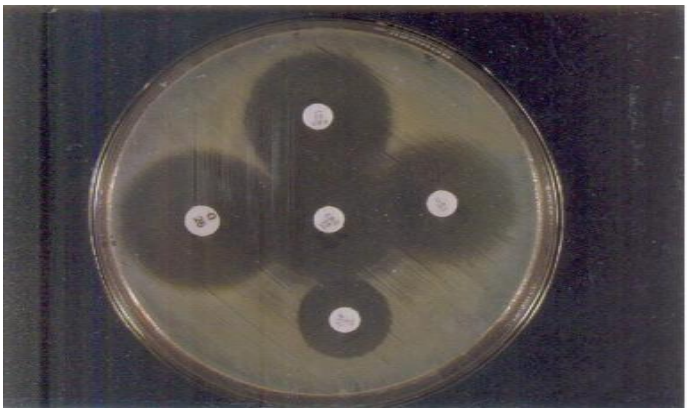

Figure 1. Kirby- Bauer method of sensitivity.

Figure 2. E-test for MIC calculation.

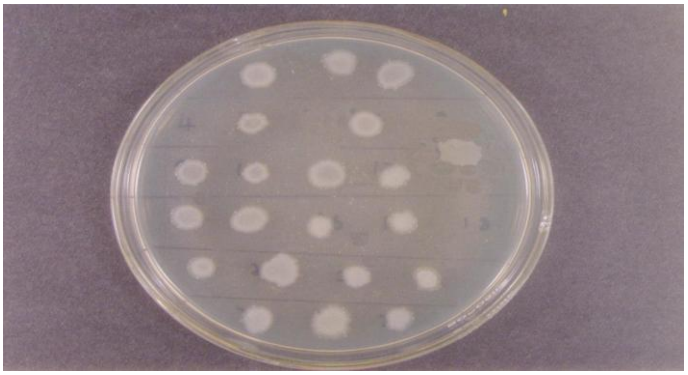

Figure 3. MIC of ciprofloxacin <0.125 µg/ml.

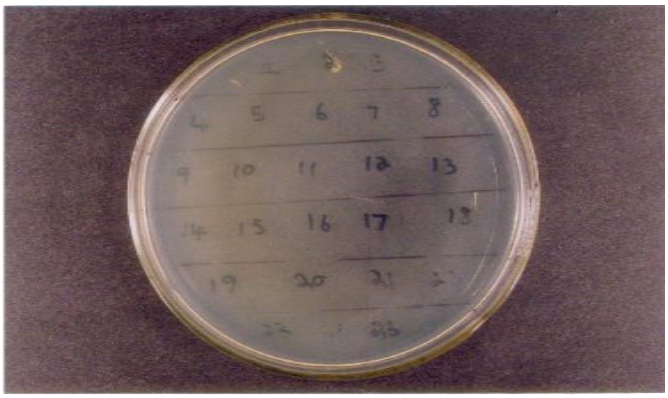

Figure 4. MIC of ciprofloxacin 0.5 µg/ml.

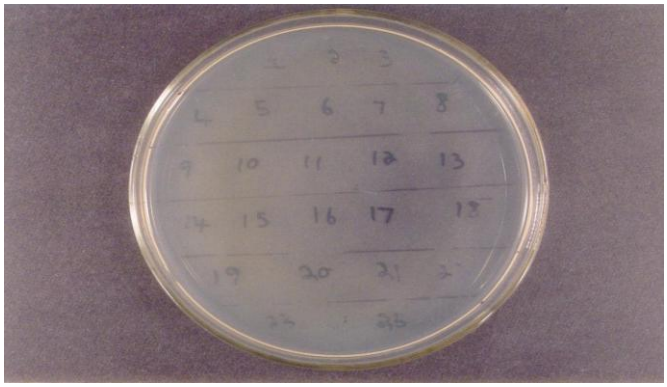

Figure 5. MIC of ceftriaxone <0.125 µg/ml.

Salmonella like colonies and Salmonella species was identified by Biochemical Reactions and Antibiotic sensitivity. Further confirmation was done by serological reactions which were performed by slide Agglutination with anti-sera. After identification, Antibiotic sensitivity testing was done by Kirby-Bauer Method as seen in Figure 1. The tests were interpreted by comparing with the Kirby-Bauer table. The control strain used was E. coli (ATCC 25922). MICs of isolates were determined by the standard plate agar dilution method according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (14). The Minimum inhibitory concentration (MIC) calculation was done by using E test & Agar dilution method using Ciprofloxacin and Ceftriaxone with dilution ranging from 0.125µg/ml to 512 µg/ml (doubling dilutions) as is shown in the (Figures 2 - 5). The quinolone evaluated was ciprofloxacin; the other antimicrobials were ampicillin, ceftriaxone, chloramphenicol, trimethoprim, and sulfamethoxazole (cotrimoxazole). Mueller-Hinton II agar (BBL, Becton Dickinson and Co., Cockeysville, MD) was used as the culture media. *Staphylococcus aureus* American Type Culture Collection (ATCC) 29213, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 were used as controls in testing for susceptibility. The breakpoint value for reduced ciprofloxacin susceptibility was chosen as >0.125 g/ml on the basis of earlier publications (12). For other antimicrobials, MIC breakpoints for resistance used were those recommended by NCCLS (14) as shown in Table 7. Susceptibility data were analyzed by using the WHONET5 computer program (O'Brien, Stelling).

RESULTS

Three hundred and nine strains of *salmonella* serotypes were isolated over a period of 6 years and 9 months that is from January, 2002 - October, 2008. Out of 309 cases, 240 were due to S. Typhi and 69 were due to Paratyphi -A. Enteric fever caused by *S. Typhi* was prominent in 2002 - 2003. After that S. Paratyphi A showed an increasing trend from 2004 onwards and was the most common

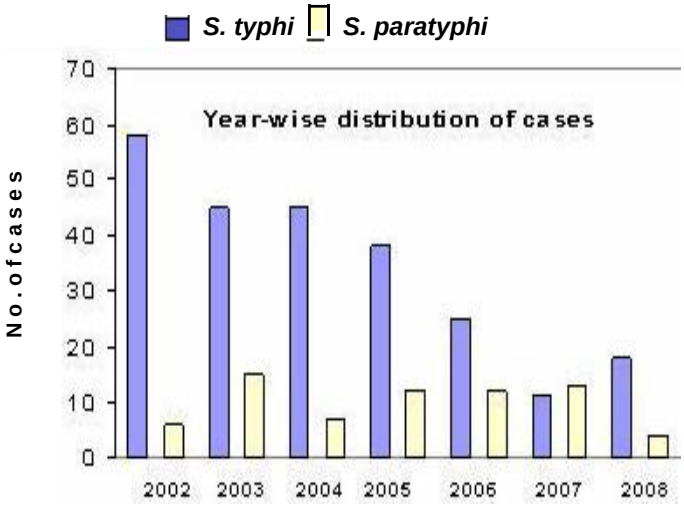

Figure 6. Year wise distribution of S. Typhi and S. Paratyphi cases

Table 1. Year-wise distribution of cases.

Year	*S. typhi* No. (%)	*S. paratyphi* No. (%)
2002(64	58 (90.62)	6 (9.37)
2003(60)	45 (75)	15(25)
2004(52)	45(86.53)	7(13.46)
2005(50)	38(76)	12(24)
2006(37)	25(67.56)	12(32.43)
2007(24)	11 (45.83)	13(54.16)
2008(22)	18(81.81)	4(18.18)
Total (309)	240(77.66)	69(22.34)

common causative agent. It was 13.46% in 2004 and increased to 54.16% in 2007 as shown (Table 1, Figure 6). In our study isolation rate of *S. Typhi* is almost the same from 2003 - 2006. In 2007, the incidence of S. typhi has increased to a great extent. However the incidence of S. Paratyphi 'A' has drastically increased that is (9.3% in 2002 - 54.16%) in 2007.

A comparison of the blood culture and the Widal test reports was also done. In our study, 60 - 83% of S. typhi cases were both blood culture and Widal test positive, 15.83% were blood culture positive and Widal negative and in 23.33% cases, Widal was not done due to lack of blood samples. Among the S. paratyphi 'A' cases which were isolated, 40.57% were blood culture and Widal positive, 37.68% were blood culture positive and Widal negative and in 21.73% cases, Widal was not done due to lack of blood samples.

The antimicrobial sensitivity pattern to commonly used antibiotics is as shown (Tables 2 - 5). Regarding *S. Typhi,* the reports of antibiotic sensitivity are variable from time to time. The antimicrobial sensitivity pattern against Ampicillin (AMP), cotrimoxazole (COT) Chloramphenicol (CHL), Ciprofloxacin (CF) and ceftriaxone (CEF) was found as shown in the table and resistance was seen to AMP, CHL and COT, more so from 2003 - 2005. From 2006 onwards the resistance has decreased. Regarding ciprofloxacin, S. typhi showed 3 - 5% resistance over the years 2003 - 2008. There is decline in resistance after that. MIC for CF of 25 isolates was collected. 3 showed MIC < 0.125 µg/ml, 20 showed 0.5 µg/ml and 2 showed 2 µg/ml. All the 309 isolates were sensitive to ceftriaxone as shown (Table 6). In case of Paratyphi-A, 100% resistance to AMP was seen in 2002 and showed a decreasing trend till 2005. After that there was a sudden increase in resistance to 66.6% in 2006 followed by a decrease in resistance to 24% in 2007. The resistance ultimately went up to 50% in 2008.Chloramphenicol resistance was high in 2002 and 2003 which decreased from 2004 onwards. COT resis -tance was high initially and then decreased to 15 - 25% in our study. Regarding, CF is concerned, isolates were 100% sensitive in 2002 - 2006 and showed resistant trend in 2007 and 2008. MIC showed two strains having MIC 2 µg/ml that is intermediate sensitivity. Regarding ceftriaxone, all isolates are sensitive. The result of high sensitivity of all isolates to ceftriaxone is similar to the study of Madhulika et al. (2004).

DISCUSSION

Enteric fever continues to be a public health problem in India. Isolation of Salmonella species occurs throughout the year. This means that drinking water conditions and sanitation have not improved or a large number of carriers are present in the society. However there is a slight trend towards increased isolation in the summer months. The situation is compounded by the emerging resistance to antibiotics that were effective earlier. Wide variation in the sensitivity pattern of various strains circulating in different geographic regions in India makes it necessary to assess the sensitivity of typhoid bacilli to antibiotics before instituting therapy. This also calls for compilation of data from specific zones for epidemiological analysis. Numerous outbreaks due to MDR salmonella are reported from different parts of world in general and India in particular.

An important factor associated with this increase in multidrug resistance among particular *Salmonella* spp. is the national and international spread of certain clonal genotypes, the most recent being the global epidemic spread of multidrug-resistant *S. typhimurium* DT104, since the early 1990s."

Enteric fever is mainly caused by *S. enterica* serotype typhi while that due to Salmonella paratyphi A has been reported less frequently. But the incidence of S paratyphi A is on the rise since 2004 as seen in our study. This is similar to the study of S. S Thankhi wale (Tankhilwala et al., 2003). Isolation rate of *S. typhi* has decreased to a greater extent where as the incidence of *S. paratyphi* 'A'

Table 2. Resistance pattern of *S. typhi* isolates.

Year	Amp.	CoT	Chlor	Cipro	Ceftriaxone	S to all
2002	31	43	33	4	0	3
(58)	(53.44%)	(74.13%)	(56.89%)	(8.88%)		(5.17%)
2003	28	37	35	1		1
(45)	(62.22%)	(82.22%)	(77.77%)	(1.72%)	0	(2.2%)
2004	27	24	28			8
(45)	(60%)	(53.33%)	(62.22%)	5	0	(17.77%)
2005	24	25	21	(11.11%)		9
(38)	(63.15%)	(65.78%)	(55.26%)	0	0	(23.68%)
2006	10	6	6	1		8
(25)	(40%)	(24%)	(24%)	(4%)	0	(32%)
2007	4	3	1	2		4
(11)	(36.36%)	27.27%)	(9.09%)	(18.18%)	0	(36.66%)
2008	3	1	3	1		13
(18)	(16.66%)	(5.55%	(16.66%)	(1.55%)	0	(72.22%)

Table 3. Resistance pattern of *S. paratyphi* 'A'.

Year	Amp.	CoT	Chlor	Cipro	Ceftriaxone	S to all
2002	6	2	4	0	0	0
(6)	(100%)	(33.33%)	(66.66%)	0	0	0
2003	6	12	12	0	0	0
(15)	(40%)	(80%)	(80%)	0	0	0
2004	2	2	1	1	0	5
(7)	(28.570%)	(28.57%)	(14.28%)	(14.28%)	0	(71.42%)
2005	2	7	4	2	0	3
(12)	(16.66%)	(58.33%)	(33.33%)	(16.66%)	0	(25%)
2006	8	8	2	2	0	1
(12)	(66.66%)	(66.66%)	(16.66%)	(16.66%)	0	(0.8%)
2007	3	2	0	5	0	5
(13)	(23.07%)	(15.38%)	0	(38.46%)	0	(38.56%)
2008	2	1	0	2	0	1
(4)	(50%)	(25%	0	(50%)	0	(25%)

has drastically increased. The reason might be due to widespread use of vaccines which are effective only against *S. typhi*. Regarding the sensitivity pattern, the reports of antibiotic sensitivity pattern for *S. typhi* are variable in our study. Resistance was more to ampicillin, chloramphenicol and cotrimoxazole from 2002 - 2005. Many cases are reported for chloramphenicol resistant strains of S. typhi in Mexico and Kerala simultaneously. In Hubli 100% resistance to chloramphenicol has been reported in 1997 (Hemalatha et al., 1999) and more than 95% resistance to chloramphenicol was reported from Hyderabad in 1999(13). Resistance to 3 conventially used antibiotics mainly CHLOR, AMP, COP is seen in our study. Similar is the result of many other studies (Ceraj et al., 1999; Sanghavi et al., 1999). In 2006 onwards, the resistance of S. typhi has decreased in our study (Chopra et al., 1992). The attributable reason may be due to non-usage of the drug form a long time.

Table 4. MDR *S. typhi* isolates.

Year	MDR_4 (ACCOTCF)	MDR_3 (ACCOT)	MDR_2
2002 (58)	1(1.72%)	31(53.44%)	10 (17.24%)
2003 (45)	2(4.44%)	20(44.44%)	11(24.44%)
2004 (45)	1(2.22%)	15(33.33%)	5 (11.11%)
2005 (38)	1(2.63%)	16(42.10%)	14 (36.84%)
2006 (25)	1 (2%)	4 (16%)	2 (8%)
2007 (11)	--	--	3 (27.27%)
2008 (18)	--	--	3 (16.66%)

Regarding ciprofloxacin, S. typhi showed 3 - 5% resistance (2003 - 2008) in our study and this is similar to

Table 5. MDR *S. paratyphi* 'A' isolates.

Year	MDR_4 (ACCOTCF)	MDR_3 (ACCOT)	MDR_2
2002 (6)	--	--	3 (50%)
2003 (15)	--	2 (13.33%)	9 (60%)
2004 (7)	--	--	2 (28.57%)
2005 (12)	--	--	2 (16.66%)
2006 (12)	3 (25%)	--	2 (16.66%)
2007 (13)	--	--	2 (15.38%)
2008 (18)	--	--	2 (11.11%)

the study in Nagpur (Ceraj et al., 1999). Out of 25 isolates of *S. typhi*, 3 showed MIC of Ciprofloxacin as 0.125 µg/ml, 20 showed 0.5 µg/ml and 2 showed 2 µg/ml. The development of resistance is due to the overuse of Ciprofloxacin in the treatment of enteric fever. Incomplete treatment may also be a factor contributing to the development of resistance. This provides a strong case for Ciprofloxacin, reconsidering the use of the first line of antibiotics for treatment viz, AMP, CHLOR, COT and this has been reported by others (Fule et al., 1988; Hemalatha et al., 1999; Sood et al., 1999). Similarly in a study from New Delhi, 32% of the isolates of *S. Paratyphi* A were found to have decreased sensitivity to ciproflo - xacin. The results are similar to what our study presents.

All the 309 isolates were sensitive to ceftriaxone in our study. This underlies the importance of this drug for treating MDR and Ciprofloxacin resistant Enteric fever cases. Emphasis has to be laid on the sparing use of the drug to prevent the occurrence of resistance to ceftriaxone. It should be used only if the 1^{st} and 2^{nd} line antibiotics have failed to evoke a satisfactory response or if the isolate is resistant to Ciprofloxacin. Regarding, S. paratyphi A, antibiotic sensitivity pattern is highly variable, showing absolute (100%) resistance to AMP in 2002 followed by a decreasing trend till 2005(16.66%). This was followed by a sudden increase in resistance to 66.66% in 2006 (Tankhilwala et al., 2003) followed by a decrease in resistance to 23% in 2007 and 50% in 2008. The profile of resistance pattern is comparable to the study of Tankhiwal et al. (2003).

Chloramphenicol sensitivity is documented in literatures ranges from 19.7 - 100% (Anandi et al., 1997; Chopra et al., 1992; Sanghavi et al., 1999; WHO, 1969). There is a similar occurrence in our study. The sensitivity was 100% in 2007 and 2008. Thus there was not even a single *S. paratyphi* in 2007 which showed a resistance to chloram-phenicol. The reason is not far to seek. The less we use a particular drug, the probability of the organism becoming sensitive to the drug increases. Compared to this, the development of resistance to ciprofloxacin may be due to overuse of ciprofloxacin in the treatment of enteric fever.

Regarding Cotrimoxazole there is a decrease in resistance to ciprofloxacin (15 - 25%) as compared to 2002 - 2006 when the concerned isolates were 100% sensitive. It showed resistant trend in 2007 - 2008. MIC was 2 µg/ml that is intermediate sensitivity. CF is the drug of choice for EF in India. In a recent study from New Delhi 32% of isolated of S. paratyphi 'A' were found to have decreased sensitivity to CF and our study is comparable to this study. All isolated were sensitivity to ceftriaxone in our study and is similar to study of others (Madhulika et al., 2004).

Conclusion

The findings of the present study indicate that MDR S. typhi is on the rise. The first line antibiotics however may still have a role to play in the treatment of typhoid fever as suggested by the re-emergence of chloramphenicol sensitivity. A simple disk diffusion test can rapidly pro-vide information that can predict treatment failures and obviates the need for the more time consuming dilution tests. Thus, sensitivity pattern of causative organism must be sought before instituting appropriate therapy to prevent further emergence of drug resistance. With ceftriaxone emerging as the sole defense against NARST strains, physicians should be advised against using this drug empirically, and it should be instituted only in the event of non-responsiveness to ciprofloxacin. As there is a trend for the development of Ciprofloxacin resistance as (shown by rising MIC values), indiscriminate use of ciprofloxacin or ceftriaxone should be strongly discouraged and they should be used in an event of non responsiveness to the three conventional agents. Also, the treatment must not be completely dependent on the Widal test. Blood culture, along with the Widal test and clinical history, must be taken into consideration for treatment.

When looking for reasons for the rapidly increased quinolone resistance in our Salmonella isolates, three issues must be considered: transferable resistance, mutational resistance, and clonal spread. Until now, transferable resistance to the quinolone antimicrobial group has been described in one preliminary report

Table 6. MIC values of *S. typhi* and *S. paratyphi* (Total of 13 dilutions).

No of isolates	MIC of each isolate ciprofloxacin (ug/ml)	MIC of isolate ceftriaxone (ug/ml)
	S. typhi	
1	0.5	<0.125
2	0.5	<0.125
3	0.5	<0.125
4	<0.125	<0.125
5	2	<0.125
6	0.5	<0.125
7	0.5	<0.125
8	<0.125	<0.125
9	0.5	<0.125
10	0.5	<0.125
11	0.5	<0.125
12	0.5	<0.125
13	0.5	<0.125
14	2	<0.125
15	0.5	<0.125
16	0.5	<0.125
17	0.5	<0.125
18	<0.125	<0.125
19	0.5	<0.125
20	0.5	<0.125
21	0.5	<0.125
22	0.5	<0.125
23	0.5	<0.125
24	0.5	<0.125
25	0.5	<0.125
	S. paratyphi 'A'	
26	0.5	<0.125
27	0.5	<0.125
28	0.5	<0.125
29	<0.125	<0.125
30	2	<0.125
31	0.5	<0.125
32	0.5	<0.125
33	<0.125	<0.125
34	0.5	<0.125
35	0.5	<0.125
36	0.5	<0.125
37	0.5	<0.125
38	0.5	<0.125
39	2	<0.125
40	0.5	<0.125
41	0.5	<0.125
42	0.5	<0.125
43	<0.125	<0.125
44	0.5	<0.125
45	0.5	<0.125
46	0.5	<0.125
47	0.5	<0.125
48	0.5	<0.125
49	0.5	<0.125
50	0.5	<0.125

Table 7. Sensitivity Range (NCCLS, vol.21, No.1; 2001).

Ciprofloxacin	MIC (ug/ml)	Ceftriaxone	MIC (ug/ml)
Sensitive	≤ 1	Sensitive	≤ 8
I. sensitive	2	I Sensitive	16-32
Resistance	> 4	Resistance	> 64

(Hakanen, Ronald). However, transferable fluoroquinolone resistance appears to be rare in bacteria in vivo. Thus, either clonal spread or resistance due to mutations in chromosomal genes remains the potential mechanism accounting for the high level of reduced fluoroquinolone susceptibility in North India. The possibility of clonal spread as a major contributing factor was excluded by identification of 13 serotypes among the quinolone-resistant isolates. In addition, some of these serotypes contained different antimicrobial resistance patterns. Based on these data, we conclude that the reduced fluoroquinolone susceptibility of salmonellae in North India primarily involves mutations in chromosomal genes. By no means does this finding exclude the presence of any other additional resistance mechanisms. The emergence of mutation-based resistance may be fostered by selection pressure caused by the use of antimicrobial agents in either human medicine or agriculture. Treatment with first generation quinolones (e.g., nalidixic acid) is known to further rapid emergence of resistance in the family of Enterobacteriaceae (D'Alessio, Ronald).

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