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ORIGINAL ARTICLE

The Changing Scenario Of The *Salmonella* Serotype And Its Drug Resistance Pattern

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ABSTRACT

Enteric fever continues to be a global health problem because of its high morbidity and mortality and presently, due to the increased incidence of multi drug resistant salmonella. So, the present study was planned to evaluate the change in frequency of the isolated *salmonella* serotype and to know its drug resistance pattern.

Key Words: enteric fever, multi- drug resistant *salmonella*

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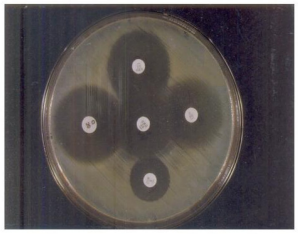
Introduction

Enteric fever continues to be a global health problem and it is endemic in developing countries including India. Enteric fever occurs in all parts of world where the water supplies and sanitation are sub-standard. Enteric fever includes typhoid fever caused by S. typhi and paratyphoid fever caused by S. paratyphi A, B and C. As enteric fever causes high morbidity and mortality, it is of great threat to the public and also to the clinician. There is evidence of the emergence of multi- drug resistant salmonella strains, which has been seen in many patients. .Salmonella typhi has rapidly developed resistance to commonly used drugs like ampicillin, chloramphenicol and cotrimoxazole, which were formerly the mainstay of treatment. To some extent, this problem was resolved with the advent of fluoroquinolones like ciprofloxacin. However, of late, the efficacy of this antibiotic too has been questioned, mainly due to increasing reports of increasing defervescence time and poor patient response. This indicates that the organism has begun to develop resistance to fluoroquinolones and is corroborated by a steady increase in the minimum inhibitory concentration (MIC) of ciprofloxacin. So, the present study was undertaken to know the frequency of isolation of *salmonella* serotypes and their antibiotic susceptibility in KMC, Manipal, Karnataka, a tertiary hospital.

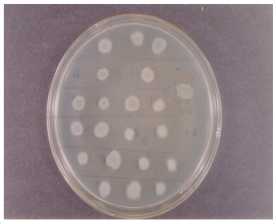
Material and Methods

Blood and bone marrow samples were collected from suspected cases of enteric fever who were admitted to KMCH. Their blood culture was done by standard microbiological methods. Antibiotic testing was done by the Kirby-Bauer Method [Table/Fig 1]. The tests were interpreted by comparing their results with the Kirby-Bauer table and the control strain used was *Escherichia coli* (ATCC 25922). 50 strains of salmonella were used to check for the minimum inhibitory concentrations (MICs) of ciprofloxacin and ceftriaxone by the agar dilution method. The E test was carried out using 3 strains for ciprofloxacin with dilutions ranging from 0.125µg/ml to 512 µg/ml (doubling dilutions), which has been shown in, (Table/Fig 2 ,3,4,5) refer to [Table/Fig 2]

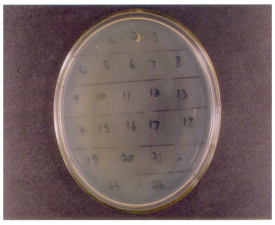


(Table/Fig 1) Kirby- Bauer method of sensitivity

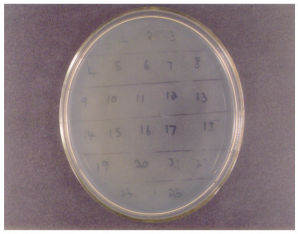
(Table/Fig 2) E- test for MIC calculation



(Table/Fig 3) MIC of ciprofloxacin <0.125 µg/ml



(Table/Fig 4) MIC of ciprofloxacin 0.5 µg/ml



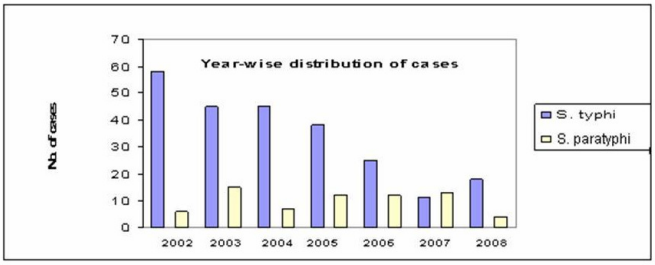
(Table/Fig 5) MIC of ceftriaxone <0.125 µg/ml

Results

309 strains of salmonella serotypes were isolated over a period of 6yrs and 9 months i.e. from Jan. 2002-Oct.2008. Out of 309 cases, 240 were caused by S. typhi and 69 were caused by S. paratyphi A. Enteric fever caused by S. typhi was prominent in 2002 – 2003 and then, from 2004 onwards, S. paratyphi A was the most common causative agent, (13.46% in 2004 to 54.16% in 2007) as shown (Table/Fig 6) ref to [Table/Fig 3]. In our study, the isolation rate of S.typhi was almost the same in all the years from 2003-2006. In 2007, the incidence of S. typhi increased to a great extent, whereas the incidence of S. paratyphi 'A' increased drastically i.e. (9.3% in 2002 to 54.16% in 2007).The antimicrobial sensitivity pattern of commonly used antibiotics are as shown in (Table/Fig 7) refer to [Table/Fig 4] , (Table/Fig 8) refer to [Table/Fig 5],(Table/Fig 9) refer to [Table/Fig 6], (Table/Fig 10) refer to [Table/Fig 7] . The reports of the antibiotic sensitivity of S.typhi are variable from time to time. The antimicrobial sensitivity pattern is as shown in the table against Ampicillin (AMP), cotrimaxozole (COT) Chloramphenicol (Chl), Ciprofloxacin (CF), ceftriaxone (CEF) and resistance was seen in AMP, ChL, COT more, from 2003-2005. Then, from 2006 onwards, the resistance decreased. S. typhi showed 3-5% resistance to ciprofloxacin from 2003-2008 and later on, there was a decrease in its resistance.

(Table/Fig 6) 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)



(Table/Fig 7) Resistance pattern of S. typhi isolates

Year	Amp.	CoT	Chlor	Cipro	Cefotaxim	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/Fig 8) Resistance pattern of S. paratyphi 'A'

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

(Table/Fig 9) 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%)

(Table/Fig 10) 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%)

In case of paratyphi-A, it showed 100% resistance to AMP in 2002, followed by a decreasing trend in resistance till 2005 and then there was sudden increase in resistance to 66.6% in 2006, followed by a decrease in resistance to 24% in 2007 and an increase to 50% in 2008. Resistance to chloramphenicol was high in 2002 and 2003, which showed a decrease from 2004 onwards. Resistance to cotrimaxozole was high, which then decreased to 15-25% in our study. The isolates were 100% sensitive to ciprofloxacin from 2002 to 2006 and showed a resistant trend in 2007 and 2008. After calculating MIC, it was found that 2 strains of *S. typhi* were intermediately sensitive, with MIC values of 2 µg/ml. 3 strains showed an MIC of < 0.125 and the remaining showed an MIC of 0.5µg/ml. The same patterns of MIC were observed with paratyphi 'A' strains also. All 50 isolates were sensitive to ceftriaxone, with an MIC of < 0.125 µg/ml [17].

By the disc diffusion method, it was found that 6 out of the 240 (2.5%) MDR strains of S.typhi and 3 out of the 69 (43%) MDR strains of S. Paratyphi 'A' were resistant to four drugs (ACCOTCF), but MIC determination showed that only 2 strains (0.85%) of S.typhi and 2 strains (2.8%) of S. paratyphi 'A' were intermediately resistant, with MIC values of 2 µg/ml. This may be due to a technical error.

Out of 240 cases caused by S. typhi , 191(79.58%) were adults and 49(20.41%) were children and out of 69 cases caused by S. paratyphi 'A', 63(91.30%) were adults and 6(8.69%) were children, as shown in (Table/Fig 11) refer to [Table/Fig 8]

(Table/Fig 11) Age-wise distribution of S. serotypes

Year (S.typhi + S.paratyphi)	S.typhi		S.paratyphi 'A'	
	Adult No. (%)	Paediatric No. (%)	Adult No. (%)	Paediatric No. (%)
2002 (58 + 6)	44 (78.56)	14 (28.13)	6 (78.56)	0
2003 (45 + 15)	35 (77.77)	10 (22.22)	12 (78.56)	3 (20)
2004 (45 + 7)	41 (91.11)	4 (8.88)	7 (78.56)	0
2005 (38 +12)	22 (57.89)	16 (42.10)	10 (78.56)	2 (16.66)
2006 (25 + 12)	22 (88)	3 (12)	12 (78.56)	0
2007 (11 + 13)	11 (100)	0	12 (78.56)	1 (7.69)
2008 (18 + 4)	16 (88.88)	2 (11.11)	4 (78.56)	0
	191 (79.58)	49 (20.41)	63 (91.3)	6 (8.69)
Total	240		69	

Among the cases caused by S. typhi, 187(77.%) were males and 53(22.08%) were females and among the 69 cases of S. paratyphi 'A' which were isolated, 61(88.40%) and 8(11%) were females in our study as shown (Table/Fig 12), [Table/Fig 9].

(Table/Fig 12) Sex -wise distribution of S. serotypes

Year (S.typhi + S.paratyphi)	S.typhi		S.paratyphi 'A'	
	Male No. (%)	Female No. (%)	Male No. (%)	Female No. (%)
2002 (58 + 6)	42 (72.41)	16 (27.58)	5 (83.33)	1 (16.66)
2003 (45 + 15)	35 (77.77)	10 (22.22)	14 (9.33)	1 (16.66)
2004 (45 + 7)	39 (86.66)	6 (13.33)	4 (57.14)	3 (42.85)
2005 (38 +12)	24 (63.15)	14 (36.84)	12 (100)	0
2006 (25 + 12)	21 (84)	4 (16)	10 (83.33)	2 (16.66)
2007 (11 + 13)	8 (72.72)	3 (16)	12 (92.30)	1 (7.69)
2008 (18 + 4)	18 (100)	0	4 (100)	0
Total	1871 (77.91)	53 (22.08)	61 (88.4)	8 (11.59)
	240		69	

Fever was observed throughout the year the peak incidence was seen in Jan, Feb, April, July and Nov.

A comparison of the blood culture and the widal test reports was also done, as shown in (Table/Fig 13) refer to [Table/Fig 10]. 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.

(Table/Fig 13) Comparison of blood culture and Widal test

Year	No.of	S.typhi			No.	S.paratyphi 'A'		
		C +ve W +ve	C +ve W – NR	C +ve W-NR		C +ve W +ve	C +ve W –ve	C +ve W-N
2002	58	29	16	13	6	1	5	0
2003	45	23	10	12	15	1	9	5
2004	45	30	5	10	7	1	6	0
2005	38	19	5	14	12	6	1	5
2006	25	20	1	4	12	11	0	1
2007	11	11	0	0	13	6	5	2
2008	18	14	1	3	4	2	0	2

Discussion

Enteric fever (EF) is one of the most common causes of pyrexia of unknown origin (PUO) in most parts of the world and it continues to be a major health problem despite the use of antibiotics and the development of newer antibacterial drugs. EF occurs in all parts of the world where water supplies and sanitation are sub- standard. In other words, EF continues to be global health problem.

Numerous outbreaks due to MDR salmonella have been reported from different parts of world and even from India [2], [3], [4]. EF is mainly caused by the S.enterica typhi serotype and paratyphi A'has been reported less frequently. But the incidence of paratyphi 'A' is increasing since 2004 and this is similar to the reports from the study by S.S Thankhiwale [17] The isolation rate of S.typhi has decreased to a greater extent whereas the incidence of S.paratyphi 'A' has drastically increased. The reason may be due to the usage of the typhi -oral vaccine which is effective only against S. typhi. The reports of the antibiotic sensitivity pattern of S. typhi are variable in our study.S. typhi was more resistant to ampicillin, chloramphenicol and cotrimaxozole. From2002-2005, many chloramphenicol resistant strains of S. typhi were reported simultaneously from Mexico and Kerala [1],[5] In 1997, 100% resistance to chloramphenicol was reported from Hubli [9] and in 1999, more than 95% resistance to chloramphenicol was reported from Hyderabad [10]. Resistance to 3 conventionally used antibiotics, mainly Chlor, AMP and COP was seen is our study and these results correlate with that of other studies [2], [6], [13.] From 2006 onwards, in our study, the resistance of S. typhi was found to decrease and this was also comparable to the findings of other studies[17]. The reason attributable to this, may be the non- usage of the drug form a long time.

S. typhi showed 3-5% resistance to ciprofloxacin in 2003-2008 in our study and this is similar to the findings of a study done at Nagpur.(17) Out of 25 isolates of S. typhi, 3 showed an MIC of 0.125 µg/ml for CF, 20 showed an MIC of 0.5 µg/ml and 2 showed an MIC of 2 µg/ml. The developing resistance is due to the overuse of CF in the treatment of EF. Incomplete treatment may also be a factor which contributes to resistance. This provides a strong case for CF, reconsidering the use of the first line of antibiotics for Rx viz, AMP, Chlor and COT and this has been reported by others also [9],[10],[11],[18]. All the 309 isolates were sensitive to ceftriaxone as found in our study. This underlines the importance of this drug for treating cases with multidrug, EF and CF resistance.. Emphasis has to be laid on the sparing use of the drug. It should be used only if the 1st and 2nd line antibiotics failed to evoke a satisfactory response, or it the isolate is resistant to CF.

The antibiotic sensitivity pattern is highly variable in S. paratyphi 'A'. It was found that resistance to AMP was 100% in 2002 which decreased in 2005 and suddenly increased to 66.66% in 2006 (17), followed by a decrease to 24% in 2007 and an increase to 50% in 2008 and this is comparable to the findings from the study by Tankhiwal *et al* [17]. In literature, Chloramphenicol.sensitivity was found to range from 19.7- 100% [12],[13],[14],[15] and it was the same case in our study. The sensitivity was 100% in 2007 and in 2008. There was a decrease in resistance (15-25%) to COT. The concerned isolates were 100% sensitive to CF from 2002 to 2006. It showed intermediate sensitivity from 2007 to 2008 (MIC 2 µg/ml) CF is the drug of choice for EF in India. In a recent study from New Delhi, 32% of isolated S. paratyphi 'A' were found to have a decreased sensitivity to CF and the results of our study are comparable to this study [19]. All isolated strains were sensitive to ceftriaxone in our study and this was similar to the findings of others [17].

With the emergence of MDR S. typhi, quinolone, particularly fluoroquinolones, have been widely used and recommended as alternative drugs for typhoid fever where the first- line drug is no longer in use. Fluoroquinolones which are available since 1980, have good in vitro susceptibility and in vivo efficacy against *salmonella* species, including S. typhi. Nalidixic acid, the prototype and the first member of the quinolone group, is now seldom used. Resistance to Nalidixic acid as a screening test for detecting reduced susceptibility to the quinolone group of drugs merits consideration because there are strains with a decreased susceptibility to ciprofloxacin but which are susceptible to nalidixic acid. The current NCCLS recommendations to check for Nalidixic acid resistance is easy to implement, but may have a reduced specificity. So resistance can be detected by Standard disc diffusion and MIC can detect fluorquinolone resistance [22], [23].

Out of the 240 cases caused by S .typhi , 191(9.58%) were adults and 49 (20.41%) were children. Out of the 69 cases caused by S. paratyphi 'A', 63(91.30) were adults and 6(8.69%) were children [20]. The % incidence in the middle aged group may be due to their working conditions where they are forced to eat outside their homes. School going children were affected due to their eating habits ie: eating on the road side 77.9% of the patients were males and 22.08% were females. More cases reported are males than females probably as a result for increase of exposure to infection due to their eating habits outside the house [21] But the carrier rate is more in females.

EF due observed through out the years. The peak incidence was in Jan, Feb, April, July, Nov ., This coincides with the hot climate and the rainy season. Increase in flies in the mango season (April) and in the hot season, the consumption of ice-creams in which the typhoid bacilli survive for over a month, contributed to it. It is more in moist winter conditions because of the survival of the bacteria [16] in soil irrigated with sewage. .

A comparison of the blood culture and the Widal test in our study showed that blood culture is a more sensitive method for the diagnosis of EF than the Widal test. We isolated salmonella spp. only after 72-96 hours of incubation. Culture positivity was also less. This was due to following reasons viz. KMCH is a tertiary Hospital. The patient might have taken antibiotics before coming to this hospital.

In many suspected cases, the blood culture as well as the Widal test was negative ie: the patient may not be suffering from enteric fever. In some cases, the blood culture was positive but the Widal test was negative. This occurs when the blood for the Widal test is drawn at a very early stage of the disease, if it is within a week.

Conclusion

309 strains of salmonella serotypes were isolated over a period of 6yrs and 9 months i.e. from Jan. 2002- Oct. 2008. Antibiotic sensitivity testing was done by the Kirby-Bauer Method and the present study indicates that the first line of antibiotics still have a role to play in the treatment of enteric fever. As there is a trend for the development of Ciprofloxacin (CF) resistance,,it is concluded that the indiscriminate use of the drug should be strongly discouraged and that it should be used in an event of non responsiveness to the three conventional drugs. 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.

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