



CHANGING TRENDS IN THE ANTIBIOGRAMS OF SALMONELLA ISOLATES IN NORTHERN AREA OF PUNJAB

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ABSTRACT

Typhoid fever continues to remain a health problem as the causative organism *Salmonella Typhi* has developed resistance to many of the antibiotics used. The present study was undertaken to compare the changing trends of antibiograms of *Salmonella enterica* serovar *Typhi* and *Salmonella enterica* serovar *Paratyphi A* isolates to be rationalized. 200 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. *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. With increasing resistance to ciprofloxacin and the possibility of re-emergence of sensitivity to chloramphenicol, the policy of empirical treatment of enteric fever needs to revise.

Keywords: 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 *S. Typhi* and Paratyphoid fever caused by *S. Paratyphi A, B & C*. 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 [5-6]. 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 [7]. In the recent past, fluoroquinolones and cephalosporins have gained importance for the treatment of enteric infections. Given the variation in the susceptibility patterns reported for *S.Typhi*, it is important to constantly monitor it so as to provide suitable guidelines for treatment.

MATERIALS AND METHODS

This study was carried out in Adesh Medical college & Hospital. A tertiary care hospital located in northern India over a period of five years. *Salmonella enterica serovar typhi & paratyphi A* isolated to determine their in vitro susceptibility to various antimicrobial agents. (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. After identification, Antibiotic sensitivity testing was done by Kirby-Bauer. Antibiotic sensitivity was tested using Disc diffusion method on Mueller- Hinton Agar (Hi-Media, Bombay, India). Antibiotics discs used were-chloramphenicol-30mcg/disc, ampicillin-10mcg/disc, ciprofloxacin 5mcg/disc, ceftriaxone-30mcg/disc, Nalidixic acid-30mcg/disc, amikacin-30mcg/disc, and ofloxacin-5mcg/disc. Commercially available, six mm discs (Hi Media) were used. The tests were interpreted by comparing with the Kirby-Bauer table. The control strain used was *E. coli* (ATCC 25922). 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 (Fig-1,2,3,4)



Fig.1: E- test for MIC calculation

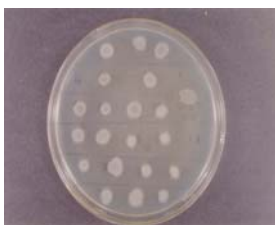


Fig.2: MIC of ciprofloxacin <0.125 µg/ml



Fig. 3: MIC of ciprofloxacin 0.5 µg/ml

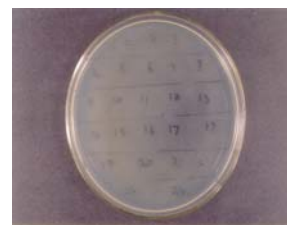


Fig. 4: MIC of ceftriaxone <0.125 µg/ml

RESULTS

200 strains of *salmonella* serotypes were isolated over a period of 5 years i.e. from Jan 2004-jan 2008. Out of 200 cases, 143 were due to *S. Typhi* & 57 were due to *Paratyphi-A*. Enteric fever caused by *S. Typhi* was prominent in 2004 - 2006. After that *S. Paratyphi A* showed an increasing trend from 2006 onwards and was the most common causative agent in case of enteric fever (table 1).

In case of *salmonella typhi* the antimicrobial sensitivity pattern against Ampicillin (AMP), cotrimoxazole (COT) Chloramphenicol

(CHL), Ciprofloxacin (CF) & ceftriaxone (CEF) was found as shown in the table and decreased sensitivity was seen to AMP, CHL & COT, more so from 2004 to 2006 from 2006 onwards the sensitivity has increased. Regarding ciprofloxacin, *S. typhi* showed 95-99 % sensitivity over the years 2004 to 2006. Then there is decrease in sensitivity after that (2007-2008). In our study we did MIC for 25 isolates of *S.typhi* and 25 isolates of *S.paratyphi*. After calculating the MIC it was found that 2 strains of *S.typhi* were intermediate sensitive to CF with MIC 2 µg/ml, 3 showed MIC <0.125 µg/ml, and

remaining showed 0.5 µg/ml. same pattern was seen with paratyphi. All the isolates were sensitive to ceftriaxone. (Table 4)

In case of Paratyphi-A, less sensitivity to AMP was seen in 2004 & showed an increasing trend till 2005. In 2006, again there was a dip in sensitivity to 34% in 2006 followed again by an increase to 77% in 2007. The resistance ultimately went up to 50% in 2008. Chloramphenicol sensitivity was low in 2005(67%) but since

then sensitivity is steadily increasing and ultimately touched 100% in 2007-08.. COT resistance was high initially in 2005-06 but since 2007 sensitivity has increased.

Regarding CF, isolates were highly sensitive in 2004 to 2006 and showed decreased sensitivity in 2007 and 2008. MIC showed two strains having MIC 2 µg/ml. i.e. intermediate sensitivity. Regarding ceftriaxone, all isolates are sensitive.

Table 1: Year-wise distribution of cases

Year	S.typhi.No. (%)	S. para typhi.No. (%)
2004(50)	42(60)	8(14.03)
2005(40)	30(43)	10(17.54)
2006(45)	33(47)	12(21.05)
2007(35)	22 (31)	13(22.80)
2008(30)	16(23)	14(24.56)
Total (200)	143(71.5)	57(28.5)

The antimicrobial sensitivity pattern to commonly used antibiotics is shown in Table 2 and 3.

Table 2: Sensitivity pattern of S. typhi isolates

Year	Amp.	CoT	Chlor	Cipro	Ceftriaxone	S to all
2004	17	20	16	41	42	35
(42)	(40%)	(46%)	(37%)	(95%)	(100%)	(83%)
2005	12	11	13	30	30	23
(30)	(38%)	(35%)	(44%)	(100%)	(100%)	(76%)
2006	20	25	25	32	33	23
(33)	(60%)	(76%)	(76%)	(96%)	(100%)	(68%)
2007	13	16	20	18	22	14
(22)	(63%)	(72%)	(91%)	(82%)	(100%)	(63%)
2008	14	15	14	13	16	5
(16)	(84%)	(95%)	(84%)	(80%)	(100%)	(28%)

Table 3: Sensitivity pattern of S. paratyphi 'A'

Year	Amp.	CoT	Chlor	Cipro	Ceftriaxone	S to all
2004	6	6	7	7	8	2
(8)	(72%)	(72%)	(86%)	(86%)	(100%)	(29%)
2005	8	4	7	8	10	8
(10)	(84%)	(42%)	(67%)	(84%)	(100%)	(75%)
2006	4	4	10	10	12	12
(12)	(34%)	(34%)	(84%)	(84%)	(100%)	(99.2%)
2007	10	11	13	8	13	8
(13)	(77%)	(85%)	100	(62%)	(100%)	(62%)
2008	7	10	14	7	14	10
(14)	(50%)	(75%)	(100%)	(50%)	(100%)	(75%)

Table 4: Sensitivity Range (NCCLS, vol.21, No.1; 2001)

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

DISCUSSION

Salmonella typhi infects only human and human transmission occurs through consumption of contaminated food and water. Multiple serotypes of *Salmonella* cause the syndrome of enteric fever, of which typhoid fever is the best studied and described. India, being compounded by 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. The incidence of S paratyphi A is on the rise since 2006 as seen in our study. This is similar to the study of Arora et al^[8]. Isolation rate of *S.Typhi* has decreased to a greater extent where as the incidence of S. paratyphi 'A' has drastically

increased. The reason might be due to widespread use of vaccines which are effective only against *S. typhi*. Many cases are reported for chloramphenicol resistant strains of *S. typhi* in Mexico, South India, Vietnam, Korea and Thailand.^{5 [9]} & Kerala simultaneously. In Hubli 100% resistance to chloramphenicol has been reported in 1997^[10] and more than 95% resistance to chloramphenicol was reported from Hyderabad in 1999^[11] There was decreased sensitivity to 3 conventionally used antibiotics mainly CHLOR, AMP, COP is seen in our study. Similar is the result of many other studies^[12]. Regarding ciprofloxacin, *S. typhi* showed 100% sensitivity in 2005 which dropped to 82% in 2007 in our study and this is similar to other studies^[13,14]. 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^(15,16). All the 200 isolates were sensitive to ceftriaxone in our study which correlates with the study of others^(17, 18). This underlies the importance of this drug for treating MDR & 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 1st and 2nd 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 (84%) resistance to AMP in 2005 followed by a decreasing trend till 2006(34%). This was followed by a sudden decreasing in resistance to 50% in 2008. The profile of resistance pattern is comparable to the study of Tankhiwal *et al*⁽¹⁹⁾.

Chloramphenicol sensitivity is documented in literatures ranges from 19.7- 100%^(20, 21). 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 chloramphenicol. 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.

Regarding ciprofloxacin there is a decrease in sensitivity to ciprofloxacin to 50% in 2008 as compared to 2004 when the concerned isolates were 86% sensitive. I MIC was 2 µg/ml i.e. 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.⁽¹⁴⁾ The development of resistance to ciprofloxacin may be due to overuse of ciprofloxacin in the treatment of enteric fever. All isolated were sensitivity to ceftriaxone in our study and is similar to study of others^(17, 22)

Nalidixic acid resistance is a marker for predicting low-level resistance to ciprofloxacin among *S.typhi* and also an indicator of treatment failure to ciprofloxacin.^[22,23] However, system that we used for interpreting the susceptibility results, follows the NCCLS guidelines and does not incorporate the analysis with reference to the relation between nalidixic acid and ciprofloxacin resistances. In many cases ciprofloxacin sensitivity is shown to be high (83 - 96%) inspite of very high resistance to nalidixic acid among the *S.typhi* isolates. Madhulika *et al* who also found a high resistance to nalidixic acid (92%), documented high MICs (>0.5 µg/ml) to ciprofloxacin in majority of their isolates.^(22,23) So, to document the true susceptibility to ciprofloxacin, determination of the exact MICs of all *S.typhi* isolates to ciprofloxacin would be of clinical relevance.

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. Thus, sensitivity pattern of causative organism must be sought before instituting appropriate therapy to prevent further emergence of drug resistance. 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 agent the policy of empirical treatment of enteric fever needs to be rationalized. The changing trends in the antibiograms of *S.typhi* and *S.paratyphi A*, probably demands reconsideration for the use of chloramphenicol in typhoid fever, instead of ciprofloxacin or third and fourth generation cephalosporins to prevent the emergence of multidrug resistance. chloramphenicol can be a useful and cost effective alternative in select cases. Constant surveillance and antibiotic sensitivity testing

is required from different geographical areas in the country, to keep abreast with emerging patterns of drug sensitivity in enteric fever.

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