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Microbiology Section

Antibiogram of *Salmonella* Isolates: Time to Consider Antibiotic Salvage

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ABSTRACT

Introduction: Enteric fever is a major problem especially in developing countries. Timely and appropriate treatment plays a very important role in reducing the mortality. Fluoroquinolones and cephalosporins are the treatment options for enteric fever. Recent studies have shown that it is time to reconsider the use of earlier antibiotics.

Aim: The study was aimed to know whether salvage is possible and to avoid treatment failures following fluoroquinolone usage.

Materials and Methods: A one year retrospective data of *Salmonella* species isolated from 319 blood samples from our hospital and other diagnostic centers were studied. Demographic data, organism isolated and their changing pattern of antibiogram were analysed.

Results: Out of 319 Salmonella isolates, 52.4% (167) was *Salmonella typhi (S. typhi)* and 47.6% (152) *Salmonella paratyphi A (S. paratyphi A),* with a male preponderance. Most of the salmonellae were isolated in the months of June and July, with the majority being in the 1-10 and 21-30 years age groups. Both species were highly susceptible to chloramphenicol (95.2% and 100%) followed by third generation cephalosporins (97% and 98%), cotrimoxazole (95.8% and 98.6%) and ampicillin (94.6% and 93.4%) respectively. Highest resistance was seen for nalidixic acid (90.4% and100%) among both *S. typhi* and *S. paratyphi A* isolates followed by ciprofloxacin (62.2% and 54.6%) respectively. MDR to first line drugs was observed in a small proportion of *S. typhi* (1.7%) only.

Conclusion: The frequency of isolation of *S. typhi* and *S. paratyphi A* are in equal proportion and enteric fever is more prevalent in younger age group. It is ideal to adopt bivalent vaccination in Universal immunization schedule. The isolates show sensitivity to first line drugs, paving the way for salvage of the earlier drugs. Cephalosporins still remain the treatment of choice in MDR salmonella isolates.

Keywords: Cephalosporins, MDR, NAR, *Salmonella paratyphi A, Salmonella typhi*

INTRODUCTION

Enteric fever caused by *Salmonella enteric* serovar *typhi (S. typhi)* and *Salmonella enteric* serovar *paratyphi A (S. paratyphi A)*, is a global public health problem which is more common in developing countries [1]. Around the world, 21.6 million typhoid fever cases with 2,50,000 deaths [2] and 5.4 million paratyphoid fever cases occur annually [3]. In India, the incidence of enteric fever ranges from 102-2219 cases/ lac population [2]. Approximately 80% of cases and deaths are in Asia, the rest in Africa and Latin America [4]. The mortality rate for typhoid fever without, timely and appropriate treatment was estimated to be 30%; with specific therapy, the rate reduced to 0.5% [1].

Chloramphenicol, ampicillin and co-trimoxazole were considered as first line drugs for enteric fever. In 1972 an outbreak of chloramphenicol resistant strains, also resistant to ampicillin were reported from India. Multidrug Resistant (MDR) strains- resistant to all three first line drugs, were reported from Mumbai and New Delhi in 1988, from Bangalore in mid-1990's and Manipal in 1999 [2].

As per the WHO guidelines 2003, treatment of MDR typhoid depends on quinolone susceptibility pattern-in quinolone sensitive strains, treatment of choice is Fluroquinolones (FQ), for quinolone resistant strains, third generation cephalosporins are recommended [5].

FQ remained the drug of choice for enteric fever, which were soon replaced by FQ resistant strains [6]. Similarly even ceftriaxone resistant strains have been reported from various parts of India [6]. In contrast, recent studies have shown that strains previously resistant to the first line drugs are showing very low or no resistance at all [7].

Variations in the sensitivity patterns reported for *Salmonella* isolates, stress the significance of continuous monitoring of antibiotic susceptibility pattern of locally prevalent strains. This study was conducted to- detect antimicrobial susceptibility of local *Salmonella* isolates, for salvage of first line drugs and also to avoid treatment failures following fluoroquinolone usage.

MATERIALS AND METHODS

In this multicentre retrospective study, one year (January to December 2013) data of *Salmonella* species isolated from blood samples from our hospital (60) and collaborated diagnostic centers (259) were collected. Demographic data which includes the name, age, sex, address, date along with organism isolated and its antibiotic susceptibility profile for the following six drugs - ampicillin, co-trimoxazole, ciprofloxacin, ceftriaxone, chloramphenicol and nalidixic acid (NA) an indicator drug were analysed.

Total 319 blood samples were processed either by conventional blood culture, identified and confirmed by biochemical reactions and slide agglutination tests or automated methods i.e. BACTEC and VITEK systems, done as per the standard laboratory procedures [8]. The antibiotic susceptibility testing was performed at different centers by the Kirby Bauer's disc diffusion technique (a standard strain of *E. coli* ATCC 25922 was included as quality control) and interpreted using Clinical and Laboratory Standards Institute (CLSI) recommendations [9] or VITEK system.

RESULTS

A total of 319 *Salmonella* isolates were analyzed, which include 52.4% (167) *S. typhi* and 47.6% (152) *S. paratyphi A.* Male patients accounted for 63% (105) of *S. typhi* and 71% (108) of *S. paratyphi A*. Majority of the positive cases (81%) belonged to the 1-30 year age group, in which *S. typhi* was 55% and *S. paratyphi A* 45%. Though, positive cases were seen distributed throughout the year, most of the enteric fever cases were isolated in the summer months (March to July) [Table/Fig-1a,b].

Month/age group	*S. typhi* (1-10 years)	*S. typhi* (11-20 years)	*S. typhi* (21-30 years)	*S. typhi* (31-40 years)	*S. typhi* (41-50 years)	*S. typhi* (51-80 years)
Jan	1	2	5	2	1	-
Feb		1				
Mar	5	3	5	2	2	
Apr	2	5	2	-	1	
May	3	3	2	2	-	1
Jun	11	8	6	4	-	1
Jul	12	6	14	3	1	1
Aug	4	1	6	1	-	1
Sep	3	3	2	-	1	
Oct	2	3	1			1
Nov	4	3	3	1		
Dec	4	1	5			
Total no %	51 (30.5)	39 (23.3)	51 (30.5)	15 (8.9)	6 (3.5)	5 (2.9)

[Table/Fig-1a]: Age wise distribution of *S. typhi* cases.

Month/ age group	*S.para-typhi A* (1-10 years)	*S.para-typhi A* (11-20 years)	*S.para-typhi A* (21-30 years)	*S.para-typhi A* (31-40 years)	*S.para-typhi A* (41-50 years)	*S.para-typhi A* (51-80 years)
Jan	1	3	6	1	-	-
Feb	5	-	6	2	-	-
Mar	2	1	2	-	-	-
Apr	-	5	5	8	2	-
May	5	2	6	3	3	-
Jun	6	2	11	3	2	1
Jul	10	3	3	3	2	-
Aug	4	1	3	3	-	-
Sep	5	1	-	-	1	-
Oct	-	1	2	-	-	-
Nov	-	2	5	1	-	1
Dec	2	1	5	-	-	-
Total no %	40 (26.3)	22 (14.4)	54 (35.5)	24 (15.7)	10 (6.5)	2 (1.3)

[Table/Fig-1b]: Age wise distribution of *S. paratyphi A* cases.

Antibiotic	No's tested	Sensitive	Resistance
Ampicillin	167	158(94.6%)	9(5.4%)
Cotrimoxazole	167	160(95.8%)	7(4.2%)
Ciprofloxacin	167	104(62.2%)	63(37.8%)
3rd gen. Cephalosporin	167	162(97%)	5(3%)
Chloramphenicol	85	81(95.2%)	4(4.8%)
Nalidixic acid	136	13(9.5%)	123(90.5%)

[Table/Fig-2a]: Antibiotic susceptibility pattern of *S. typhi.*

Antibiotic	No's tested	Sensitive	Resistance
Ampicillin	152	142(93.4%)	10(6.6%)
Cotrimoxazole	152	150(98.6%)	02(1.4%)
Ciprofloxacin	152	83(54.6%)	69(45.4%)
3rd gen. Cephalosporin	152	149(98.1%)	03(1.9%)
Chloramphenicol	62	62(100%)	0(0%)
Nalidixic acid	117	0(0%)	117(100%)

[Table/Fig-2b]: Antibiotic susceptibility pattern of *S. paratyphi A.*

Out of 319 samples isolated [Table/Fig-2a and b] both *S. typhi* and *S. paratyphi A* isolates were highly susceptible to chloramphenicol (95.2% and 100%) followed by third generation cephalosporins (97% and 98%), cotrimoxazole (95.8% and 98.6%), and ampicillin (94.6% and 93.4%) respectively. Highest resistance was seen among both *S. typhi* and *S. paratyphi A* isolates for the indicator drug, NA (90.4% and100%) followed by ciprofloxacin (62.2% and 54.6%) respectively. MDR to first line drugs was observed only in a small proportion of *S. typhi* (1.7%).

DISCUSSION

Our study showed the ratio of isolation of *S. typhi* and *S. paratyphi A* is approximately 1:1 (52% and 48%). This increasing trend of isolation of *S. paratyphi A* (3-17%) is seen gradually since 1996 [10]. In a study by Sarika Jain et al., in Delhi, *S. paratyphi A* isolation was 23%, accounting for *S. typhi* to *S. paratyphi A* ratio of 4:1. As *S. paratyphi A* causes a milder disease, a strong clinical suspicion is essential for appropriate diagnosis and treatment. Such increasing isolation rates of *S. paratyphi A* have also been reported across India, which may be due to availability of the latest automated systems or to the replacement of trivalent typhoid vaccine by monovalent vaccine [3,11,12].

Clustering of cases was seen in summer due to scarcity and contamination of drinking water. Majority of the isolates (81%) were from the 1-30 year age group, which is proportionately high and alarming. This has to compel the health authorities to implement vaccination and health education regarding sanitary measures. Male preponderance was observed in our study-63% of *S. typhi* and 71% of *S. paratyphi A*, which may be due to more outdoor exposure.

Study across five endemic Asian countries- China, India, Indonesia, Pakistan and Vietnam in the year 2008 showed 7-65% prevalence of MDR *salmonella* isolates [2]. In our study, MDR was observed in 1.7% of *S. typhi* isolates, similar to studies reported by Indian Network for Surveillance, Gopal Muthu et al., in Madras, Shaik Mohammed et al., in Bangalore, World Health Organization, Jain et al., in Delhi [1,3,9,11]. The absence of MDR among *S. paratyphi A* strains was consistent with the reports from Walia M et al., in India and Arjyal et al., in Nepal [13,14]. A few studies report the occurrence of MDR among *S. paratyphi A* isolates also [1,9]. The low proportion of MDR may be due to restricted use of first line drugs leading to withdrawal of selective pressure, therefore reuse of first line drugs can be considered for management of enteric fever [15].

Nalidixic Acid Resistance (NAR) indicates low level resistance to ciprofloxacin and results in treatment failure. Strains that are already resistant to NA may require fewer exposures to FQ to develop high level resistance to ciprofloxacin, than the strains that are fully ciprofloxacin susceptible [1]. FQ resistance has to be confirmed by performing MIC to ciprofloxacin. In our study more isolates were resistant to NA and ciprofloxacin, but as it is a retrospective study we could not correlate the ciprofloxacin MIC values for all isolates.

In our study, a small percentage of isolates showed resistance to third generation cephalosporins (2.9% *S. typhi* and 1.9% *S. paratyphi A*), similar to study done by Jain et al., in Delhi (2% of Salmonella enteric strains). Therefore, cephalosporins continue to be a good option for treatment of MDR and NAR cases [11].

CONCLUSION

The present study indicates that both *S. typhi* and *S. paratyphi A* are in equal distribution. Reuse of the first line antibiotics can be considered for treating enteric fever cases. Increasing resistance to quinolones is alarming so it is necessary to determine MIC levels for ciprofloxacin to avoid treatment failures. Third generation cephalosporins still remain as a drug of choice for treatment of MDR enteric fever cases. If resistance develops against even the third generation cephalosporins, the treatment options available would be; fourth generation cephalosporins, penems, tigecycline or combination antibiotic therapy which will make treatment expensive. Due to variations in the antibiotic susceptibility pattern

of *Salmonella* isolates periodic monitoring of resistance pattern will remain essential for deciding treatment regimen. Immunization and health education should be mandatory. Immunization for enteric fever should be incorporated into the universal immunization schedule in our country, preferably using bivalent vaccine (*S. typhi* and *S. paratyphi A*), as many isolates are from younger age group. Adherence to WHO guidelines and practice of evidence based medicine, in the treatment of infectious diseases will salvage many antibiotics in future.

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