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Antimicrobial Susceptibility of Enteric Fever Salmonellae Isolated from Blood Culture.

Sudeepa Kumar M [#*1], Vijay Kumar GS[### 2], Prashanth HV[## 1], Prakash R [#1], Veena Krishnamurthy [#1], and Chandrashekar SC[#1]

[#]Assistant Professor, [##]Professor, [###]Professor and HOD.

[1]Department of Microbiology, Sri Siddhartha Medical College, Tumkur, Karnataka- 572 107

[2]Department of Microbiology, J.S.S Medical College, Mysore, Karnataka- 570 015

ABSTRACT

Enteric fever continues to be a major public health problem, especially in the developing countries. The problem worsened with the development of multidrug resistant Salmonella strains and the advent of nalidixic acid resistant Salmonella typhi and paratyphi A. So it is necessary to continually monitor the drug resistance pattern and understand the mechanisms involved. The venous blood was collected. All blood cultures were performed using fully automated Bact T Alert system. The Salmonella isolates were confirmed by slide agglutination using factor sera and subjected for antibiotic susceptibility test and MIC for ciprofloxacin by Agar dilution method. Antibiogram of these isolates revealed that all the S. typhi and paratyphi A isolates were sensitive to amoxicillin/clavulinic acid, cefrtiaxone, cefotaxime and amikacin. No MDR strains were isolated. Highest resistance was observed with nalidixic acid. All the nalidixic acid resistant strains of Salmonella typhi and paratyphi A had an MIC for ciprofloxacin between 0.5-1µg/ml. Any isolate that shows resistance to nalidixic acid should be reported as intermediately susceptibile to ciprofloxacin. The isolates showed high sensitivity to chloramphenicol (>96%). So the policy of empirical treatment of enteric fever needs to be rationalized and may necessitate a change towards evidence based treatment.

Keywords: Salmonella typhi, Salmonella paratyphi A, Minimum inhibitory concentration, Multidrug resistance, Nalidixic acid resistance.

Corresponding author

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INTRODUCTION

Enteric fever continues to be a major public health problem, especially in the developing countries of the tropics. It is endemic in all parts of India. *Salmonella typhi* and *Salmonella paratyphi A* are the predominant types of *Salmonella* responsible for enteric fever in India [1].

Salmonellosis including enteric fever has been effectively controlled in many developed countries. But in developing countries like India, it continues to manifest as gastroenteritis associated with fever and sometimes leading to sepsis. Failure to implement or delay in starting effective treatment is associated with high mortality (20%). Timely and effective treatment reduces the mortality rate to as low as 1% [2].

Until the development of multidrug resistant (MDR), *Salmonella enterica serovar Typhi* strains in 1989, chloramphenicol was the drug of choice for treating typhoid fever. Transferable plasmids were found to be responsible for the spread of multidrug resistance in S. typhi [3, 4]. Since then flouroquinolones, particularly ciprofloxacin was the drug of choice in the treatment of typhoid fever. The problem worsened with the advent of nalidixic acid resistant *Salmonella typhi* (NARST). Soon it was found that *S. paratyphi A* clinical isolates world over also showed similar resistance pattern and these were named as nalidixic acid resistance *Salmonella paratyphi A* (NARSPT). However towards the end of last decade, treatment failure with ciprofloxacin has been reported due to infection with NARST which were found to be susceptible to ciprofloxacin in disc diffusion test (5). Reports have indicated the emergence of *Salmonella enterica serovar Typhi* strains that exhibited decreased susceptibility to flouroquinolones [6].

So with the changing patterns in antibiogram it is necessary to continually monitor the drug resistance pattern and understand the mechanisms involved. Hence this study was undertaken to characterize the prevalence and resistant patterns, so that appropriate strategies can be adopted in the management of enteric fever.

MATERIALS AND METHODS

Patients attending the OPD or admitted at tertiary care hospital who are clinically suspected as enteric fever cases during Jan 2006 to Dec 2010 were included in the study. Non Salmonella species isolated from blood cultures and patient below one year of age were not included.

The venous blood was collected under aseptic precautions from these patients. 5ml from children and 10ml from adult respectively were collected. All blood cultures were performed using fully automated Bact T Alert system (BIOMERIEUX). The culture bottles after inoculating with patient blood were loaded in the chamber. The growth was indicated by flagging. The bottle which flagged was unloaded and subculture was made on Mac conkey's agar and Wilson and Blair media. Growth was subjected for a battery of presumptive

identification tests to identify the growth as *Salmonella typhi* or *Salmonella paratyphi A* [7]. The Salmonella isolates was further confirmed by slide agglutination using factor sera.

The Salmonella isolates were subjected for antibiotic susceptibility testing by Kirby Baur Disc Diffusion method according to CLSI guidelines [8]. The sensitivity was done to ampicillin, amoxycillin and clavulanic acid, ceftriaxone, cefotaxime, chloramphenicol, ciprofloxacin, co-trimoxazole, amikacin and nalidixic acid.

The Salmonella isolates were further subjected for MIC for ciprofloxacin by agar dilution method:

Sterility checked peptone water was inoculated with 2-5 morphologically similar colonies of Salmonella isolate. The broth incubated at 37^0C and turbidity matched to 0.5 Mac Farland standards. Then Mueller Hinton agar in 25ml quantity was sterilized and cooled to 50^0C and ciprofloxacin suspension was added to each agar containing tube to get a fixed concentration of 0.25µg/ml, 0.50µg/ml, 1 µg/ml and 2µg/ml. These drug containing agar tubes were immediately poured into petri dishes and allowed to set. Sterility of these plates was checked by overnight incubation at 37^0C. Grid was made on the drug containing Mueller Hinton agar and spot inoculation of each isolate was made separately. Spot inoculation of *ATCC E. coli* (27922) was also done as control in each plate. The plates were incubated aerobically in upright position at 37^0C for 24 hours. The drug containing plate was examined for growth/ no growth of the inoculated Salmonella isolate. The minimum concentration of ciprofloxacin inhibiting the growth of test strain was recorded as the MIC of Ciprofloxacin.

RESULTS

The present study was carried out for 5 yrs during 2006 to 2010. Fourteen thousand seven hundred and six (14706) blood samples were cultured and 292 yielded the growth of enteric fever Salmonellae. Out of 292 isolates of Salmonellae 160 were *S. typhi* and 132 were *S. paratyphi A.*

Antibiogram of these isolates revealed that all the *S. typhi* isolates were sensitive to amoxicillin/clavulinic acid, cefrtiaxone, cefotaxime and amikacin. Resistance was seen to chloramphenicol (2.5%), ciprofloxacin (1.2%), co-trimoxazole (1.2%) and ampicillin (7.5%) cases. Resistance to nalidixic acid was observed in 88% cases [Table 1]. Similar sensitivity pattern was seen in *S. paratyphi A* where it showed sensitivity to ceftriaxone, cefotaxime, amikacin, and resistance to ampicillin (3%), amoxycillin/clavulinic acid (1.5%), chloramphenicol (2.3%), ciprofloxacin (1.5%) and co-trimoxazole (1.2%). Highest resistance was observed with nalidixic acid (89.4%) [Table 2].

All 292 isolates were tested for MIC by Agar dilution method [Table 3]. 171 showed MIC of <0.25µg/ml, 94 showed MIC of 0.25 µg/ml, 23 showed MIC of 0.5 µg/ml and 4 had MIC of 1 µg/ml. Nalidixic acid resistant strains of both *Salmonella typhi* and *Salmonella paratyphi A* had MIC between 0.5-1 µg/ml and nalidixic acid sensitive strains had MIC ≤0.25 µg/ml [Table 4].

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Table -1: Antibiotic susceptibility pattern of S. typhi (N=160).

Sl. No.	Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
1	Ampicillin	148 (92.5)	1 (0.6)	11 (6.9)
2	Amoxycillin & Clavulinic acid	160 (100)	0	0
3	Ceftriaxone	160 (100)	0	0
4	Cefotaxime	160 (100)	0	0
5	Chloramphenicol	154 (96.3)	2 (1.2)	4 (2.5)
6	Ciprofloxacin	158 (98.8)	0	2 (1.2)
7	Co-trimoxazole	158 (98.8)	0	2 (1.2)
8	Amikacin	160 (100)	0	0
9	Nalidixic acid	19 (11.9)	13 (8.1)	128 (80)

Table-2: Antibiotic susceptibility pattern of S. paratyphi A (N=132).

Sl. No.	Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
1	Ampicillin	128 (97)	1 (0.7)	3 (2.3)
2	Amoxycillin & Clavulinic acid	130 (98.5)	0	2 (1.5)
3	Ceftriaxone	132 (100)	0	0
4	Cefotaxime	132 (100)	0	0
5	Chloramphenicol	129 (97.7)	0	3 (2.3)
6	Ciprofloxacin	130 (98.5)	0	2 (1.5)
7	Co-trimoxazole	130 (98.5)	0	2 (1.5)
8	Amikacin	132 (100)	0	0
9	Nalidixic acid	14 (10.6)	4 (3)	114 (86.4)

Table-3: MIC of *Salmonella* isolates by Agar Dilution Method.

Sl. No.	Organism	< 0.25 μg/ml		0.25 μg/ml		0.5 μg/ml		1 μg/ml	
		no	%	no	%	no	%	no	%
1	S. Typhi	85	53.1	60	37.5	13	8.1	2	1.3
2	S. Paratyphi A	86	65.2	34	25.7	10	7.6	2	1.5
		171	59.1	94	31.6	23	7.9	4	1.4

Table -4: Comparison of Nalidixic acid susceptibility and Ciprofloxacin MIC.

Antibiotic Susceptibility	Number of isolates		Ciprofloxacin MIC(μg/ml)
	Salmonella typhi	Salmonella paratyphi A	
Nalidixic Acid resistant	141	118	0.5-1
Nalidixic Acid sensitive	19	14	≤0.25

DISCUSSION

Enteric fever is a major public health problem in our country. National enteric fever data is found to be 2 to 3% in India. The present study also showed similar rate (2%). The last two decades have seen the emergence of multidrug resistance (MDR) against the conventional antibiotics (ampicillin, cotrimoxazole, and chloramphenicol) among the Salmonella species

especially in southeast Asia [9]. In the present study, of the 292 isolates of Salmonellae, 160 were *S. typhi* and 132 were *S. paratyphi A*. Although in our study *S. paratyphi A* was not the leading cause of enteric fever, there are reports of increasing isolation rates of *S. paratyphi A* from India as well as Asia [10, 11].

In the present study, *Salmonella enterica servar typhi* and *paratyphi A* showed high sensitivity to chloramphenicol (96%), co-trimoxazole (98%) and ampicillin (92%), the first line drugs for enteric fever. No MDR strains were isolated. The studies from Chennai [9] and Kolkata [12] have indicated similar sensitivities. In India, antibiotic resistance among *S. typhi* has been reported since 1960, and then the first outbreak of multidrug resistant *S. typhi* (MDRST) was reported in Calicut [13]. The incidence of MDR *S. typhi* has been reported to be as high as 60% but then decline in pune (1999), Nagpur (2001), Delhi (2004) and Calcutta (2000) [14,15,16,17]. Quinolones are highly effective against Salmonellae in vitro. Ciprofloxacin was considered the drug of choice for the treatment of multidrug resistant typhoid replacing chloramphenicol. Consequent to the wide spread use of ciprofloxacin especially in the community, resistance and treatment failure were being increasingly observed and reported. The quinolone resistance is due to altered DNA subunit. But recently plasmid mediated quinolone resistance has been reported [18]. Rampant use of ciprofloxacin not only for typhoid, but also for other infections gradually led to increased Minimum inhibitory concentrations (MICs) of ciprofloxacin to *Salmonella enterica serovar typhi* [9]. In our study, both *Salmonella typhi* and *paratyphi A* showed sensitivity to ciprofloxacin more than 98% by disc diffusion method. All the 192 isolated had an MIC of ≤ 1µg/ml and none of the isolates were in the resistant range of >2µg/ml.

However, nalidixic acid resistant (NAR) was seen among the isolates. All the NAR strains of *Salmonella typhi* and *paratyphi A* had an MIC for ciprofloxacin between 0.5-1µg/ml. The 4 isolates which showed resistance to ciprofloxacin by disc diffusion method had MIC of 1 µg/ml. Many workers have found that salmonellae with lower MIC falling within the CLSI sensitive range may also be NAR [19]. Nalidixc acid resistance is a marker for predicting low level resistance to ciprofloxacin among *Salmonella* and also an indicator of treatment failure to ciprofloxacin [20, 17, 21]. Single point mutation in the quinolone resistance – determining region (QRDR) of the topoisomerase gene *gyr* A (aminoacid 67to 122) in *Salmonellas* usually leads simultaneously to resistance against nalidixic acid, a nonfluorinated narrow spectrum quinolone, and to decreased ciprofloxacin susceptibility [22]. However many studies have found high resistance to nalidixic acid and increased MIC to ciprofloxacin in majority of their isolates [23, 24]. Any isolate that shows resistance to nalidixic acid should be reported as intermediately susceptible to ciprofloxacin [24]. Ideal antimicrobial treatment of patients with enteric fever depends on an understanding of local patterns of antimicrobial resistance and is enhanced by the results of antimicrobial susceptibility testing of the *Salmonella* isolated from the individual patients. Ciprofloxacin continues to be widely used because of advantage of oral route, tolerability and convenient dosage schedule. But clinicians need to be aware that patients infected with *Salmonella* with decreased ciprofloxacin susceptibility may not respond adequately. In this circumstance third generation cephalosporins such as ceftriaxone may be used. However, the resolution of fever and symptoms is slow and short course chemotherapy

has not proven satisfactory.The high cost and need for parenteral administration are further disadvantage of cephalosporin therapy [25].

In our study the Salmonellae isolates showed high sensitivity to chloramphenicol (>96%). Similar observation was done in other studies from India [25]. Re-emergence of chloramphenicol sensitive strains in previously resistant areas point towards the concept of antibiotic recycling, preserving the use of older antibiotics [26].

CONCLUSION

With increasing resistance to fluoroquinolones (ciprofloxacin), possibility of re-emergence of sensibility to chloramphenicol among Salmonellae, the policy of empirical treatment of enteric fever needs to be rationalized and may necessitate a change towards evidence based treatment for typhoid fever, instead of ciprofloxacin or third generation and fourth generation cephalosporins to prevent the emergence of multidrug resistance.

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