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Emerging Pattern of *Salmonella typhi* Drug Resistance

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Abstract: Enteric fever is a global health problem. The burden of enteric fever is increasing especially in developing countries like India, coupled with increasing emergence of Multi-Drug Resistant typhoid infections. We in the present study tried to evaluate the predominant type of S.*typhi* and S.*paratyphi A* in the patients with febrile illness attending Kakatiya Medical College and Hospital, Warangal, Telangana. In total of 89 suspected cases of enteric fever blood cultures were done from the period from Feb 2011 to Dec 2012. Collected blood samples introduced into brain heart infusion broth incubated aerobically at 37°C and then incubated for 48 hours. Subcultures were then made on blood agar and MacConkey agar, 24Hrs after collection. Phage typing was done and the sensitivity to antibiotics was done by Kirby Bauer disc diffusion method. Salmonella typhi was found in 35 samples and the phage types were E1 in 31 samples (88.57%) and type A was found in 4 samples (11.43%) of cases. The common Biotype was typed I in 33 cases (94.29%) and 2 cases were of Biotype II (5.71%) of cases. Similarly, S. *paratyphi A* was found in 10 samples all the samples were untypable for phages. Majorities were found to resistant to Cotrimoxazole (86.67%) followed by 73.33% isolates were resistant to Erythromycin. The isolates were most sensitive to Ciprofloxacin followed by chloramphenicol and Ampicillin. The findings of the study indicate that the predominant S.typhi phage was E1 and all the E1 strains were belonging to Biotype I. Although there is considerable resistance seen to antibiotics like cotrimoxazole, Erythromycin, Gentamycin, Norfloxacin but the isolates were still found to the susceptible to ciprofloxacin and chloramphenicol. However, such studies must be conducted regularly in order to find if any change in pattern of resistance of isolated strains is found in the population and physicians should use antibiotics judiciously in order to prevent the development of more drug-resistant organisms.
Keywords: S. *tyhpi*, S.*paratyphi A*, drug resistance.

INTRODUCTION

Typhoid fever is caused by Salmonella enterica subspecies enterica serovar Typhi, remains a major public health concern in developing countries. Approximately 13.5 million cases occur annually, and the disease is associated with 0.19 million deaths worldwide in 2010. [1] Globally, the WHO has estimated the annual incidence of typhoid fever as 21.7 million cases while the estimated crude incidence of typhoid fever in Southeast Asia is approximately 110/100,000 persons per year [2, 3]. S.*typhi* and S.*paratyphi A, B, C* are Gram-negative bacteria having the capability to invade the bloodstream and cause typhoid and paratyphoid fevers respectively. The main mode of transmission is the fecal-oral route. The common symptoms of patients with enteric fever are acute fever, headache, malaise, abdominal distress, diarrhoea, and 'rose spot' rash and about 3–5% of that infected progress to a chronic carrier state [4-6]. It was previously thought that the S.*paratyphi A* causes milder disease then S. *typhi* many studies have however contradicted these claims [7, 8]. There have been changes in the epidemiology of enteric fever as has been reported in some studies and there has been rising incidence of S.*paratyphi an* infection [9, 10]. Changes in antibiotic resistance patterns among salmonellae to conventional first-line antibiotics (Ampicillin, Cotrimoxazole, Chloramphenicol) has been reported from different parts of India [11-13]. There was emergence of chloramphenicol resistance and later emergence of multidrug resistance in S.*typhi* was again followed by chloramphenicol susceptible strains [12, 14]. Now with emergence of Nalidixic acid-resistant S.*typhi* (NARST) isolates this, along with the emergence of resistance to third and fourth generation cephalosporin has reduced the therapeutic options available for treatment to newer quinolones including extended spectrum Cephalosporins,

Azithromycin, and Carbapenems [15-19]. Patients infected with nalidixic acid resistant S.*typhi* require higher fluoroquinolone dosages and longer and more expensive treatment. They also tend to experience higher stool carriage rates and higher fatality rates [20, 21]. An increase in fluoroquinolone resistance in S.typhi strain will limit the value of the use of this class of antibiotic for empirical treatment in future. Recently isolated cases of high-level ciprofloxacin resistance in S. *paratyphi A* have been reported from South India [22]. With this background, we in the present study tried to evaluate the prevalence of salmonella stereotypes in enteric fever and study their antibiotic and resistance pattern in this group of the population.

MATERIALS AND METHODS

A total of 89 blood cultures were studied during Feb 2011 to Dec 2012. All the patients were admitted to Kakatiya Medical College and Hospital, Warangal, Telangana with febrile diseases and clinically suspected of having typhoid fever and those who were without any antibiotic therapy during the preceding week were included in the study. The Institutional Ethical Committee Approval for the conduction of the study was obtained. 5 ml of blood was drawn from each patient was introduced into 70ml BHI broth; it was then incubated aerobically at 37°C for 48 hours. Subcultures were then made on both blood agar and MacConkey agar 12Hrs and 24Hrs after collection. The identification of isolates was done using biochemical tests and specific antisera using standard methods [23]. Isolates which were Indole negative and methyl-red positive, Voges-Proskauer negative, Citrate Negative, Urease negative, TSI-K/A with slight H_2S, Ornithine, and Lysine Decarboxylase positive, xylose and d-tartarate fermenting without production of gas and sucrose and lactose non fermenting were identified as S.*typhi*. The further study was done by confirming serotyping. The confirmed isolates were sent to the National Salmonella Phage Typing Centre at Lady Hardinge Medical College, New Delhi for phage typing. Biotyping was done by xylose fermentation. Xylose positive strains were grouped as Biotype I and xylose negative strains as biotype II [24]. The S. Typhi colonies were tested as per standard protocol [25]. All isolates were subjected against Chloramphenicol (30 µg), Gentamycin (10µgm), Ampicillin (10µg), Co-Trimoxazole (1.25/23.75µg), Ciprofloxacin (5µg), and Erythromycin (15µg), Amikacin (10µgm), by Kirby Bauer's disc diffusion technique [26].

RESULTS

Forty-five Salmonella were isolated from samples collected during the period from Feb 2011 to Dec 2012 from the patients with symptoms of enteric fever. Salmonella typhi was found in 35 samples and the phage types were E1 in 31 samples (88.57%) and type A was found in 4 samples (11.43%) of cases. The common Biotype was typed I in 33 cases (94.29%) and 2 cases were of Biotype II (5.71%) of cases. Similarly, S. *paratyphi A* was found in 10 samples all the samples were untypable for phages given in table 1.

Table-1: showing phage types and biotypes of various salmonella isolates.

SEROTYPES	TOTAL	PHAGE TYPES				BIOTYPES	
		E1	A	O	Untypable	I	II
S. *Typhi*	35	31	4	0	0	33	2
S. *Paratyphi A*	10	0	0	0	10	0	0
Total	45	31	4	0	10	33	2

Antibiotic resistance pattern was performed from the cultures and majority were resistant to Cotrimoxazole (86.67%) followed by 73.33% isolates were resistant to Erythromycin. The isolates were most sensitive to Ciprofloxacin followed by Chloramphenicol and Ampicillin. More than 50% of isolates were found to be resistant to Gentamycin, Norfloxacin, and Cephalexin is given in table 2.

Table-2: showing the sensitivity pattern of culture isolates

Antibiotics	S.*typhi* [35]	S.*paratyphi A* [10]	Sensitive	%	Resistance	%
Ampicillin	27	6	33	73.33	12	26.67
Chloramphenicol	30	7	37	82.22	8	17.78
Gentamycin	18	5	23	51.11	22	48.89
Cotrimoxazole	4	2	6	13.33	39	86.67
Norfloxacin	20	4	24	53.33	21	46.67
Ciprofloxacin	32	8	40	88.89	5	11.11
Cephalexin	18	6	24	53.33	21	46.67
Erythromycin	8	4	12	26.67	33	73.33
Amikacin	21	6	27	60.00	18	40.00

DISCUSSION

In the present study, S. *typhi* isolates were found to be sensitive to ampicillin, chloramphenicol, and ciprofloxacin. Most of the isolates were resistant to cotrimoxazole. V Gupta *et al.* [27] in a similar study for 3 years from 2008 to 2010 found resistance to Nalidixic Acid (NA) and fluoroquinolones and complete sensitivity to ceftriaxone along with re-emergence of chloramphenicol sensitivity for Salmonella isolates. However, the worldwide emergence of multi-drug resistant strains of salmonella in the past two decades has to lead to the withdrawal of chloramphenicol and its replacement with fluoroquinolones and third-generation cephalosporins for treating enteric fever cases. In the present study, we observed an independent population dynamics and response to each antimicrobial agent. Although in our study 88.89% of the isolates of S. *typhi* and S. *paratyphi* A were sensitive to ciprofloxacin they showed resistance to Erythromycin as well as cotrimoxazole. V Gupta *et al.* [27] found 13.6% of isolates of the enteric fever were resistant to ciprofloxacin and in near agreement with the results obtained in the present study here we observed 11.11% resistance to ciprofloxacin. In one study by R. Raveendran *et al.* [28] at Sir Gangaram Hospital New Delhi, India found the number of isolates with ciprofloxacin resistance in 5.6% of the cases with S. *typhi* infection. They also found that the sensitivity to Ampicillin, chloramphenicol and cotrimoxazole resistance in S. *typhi* to be decreased from 14.9% from the previous 27%. There are emerging cases of therapeutic failures related to fluoroquinolone treatment in patients with enteric fever [29, 30]. In lab findings of these several cases were reported as susceptible by disc diffusion method using the recommended breakpoint to fluoroquinolones. These isolates were having smaller zones of inhibition to fluoroquinolones by Kirby Bauer disc diffusion method and MIC is almost tenfold higher than the fully susceptible stains [31, 32]. Renuka *et al.* [33] reported the isolation of S. *typhi* strains showing high resistance to ciprofloxacin. Studies have found that a single mutation in zyr a gene is sufficient to confer resistance to Nalidixic acid (NA) and reduce the susceptibility to fluoroquinolones and second mutation leads to high levels of fluoroquinolone resistance. Acker ML *et al.* in USA [34] found an increase in a number of MDR strains and NA resistance of S. *typhi* (NA) and overall isolates were sensitive to ciprofloxacin and ceftriaxone. Another study in Bangladesh reported a decrease in MDR isolates with no corresponding increase in sensitive strains. An increase in ciprofloxacin MIC has been reported in UK and India. [35-37]. In a study by U Madhulika *et al.* found that the MIC of >0.5gm/l of ciprofloxacin were sensitive to ampicillin, chloramphenicol, and cotrimoxazole and increasing numbers of isolates were showing high MIC to ciprofloxacin alone [38]. There is a clear indication that nowadays there is indiscriminate use of ciprofloxacin with typhoid as well as other unrelated infections and partial or incomplete treatments may be one of the factors causing an increase in development of drug resistance by Salmonella typhi.

CONCLUSIONS

The findings of the study indicate that the predominant S.*typhi* phage was E1 and all the E1 strains were belonging to Biotype I. Although there is considerable resistance seen to antibiotics like Cotrimoxazole, Erythromycin, Gentamycin, Norfloxacin but the isolates were still found to the susceptible to ciprofloxacin and Chloramphenicol. However, such studies must be conducted regularly in order to find if any change in pattern of resistance of isolated strains is found in the population and physicians should use antibiotics judiciously in order to prevent the development of more drug-resistant organisms.

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