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Current Trends of Antimicrobial Susceptibility of Salmonella Enterica Serovar Typhi and Paratyphi A from Blood Cultures in a Tertiary Care Center of Chennai, Tamil Nadu, India.

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ABSTRACT

Early detection and antimicrobial susceptibility surveillance are important for its effective management and prevention of complications in Enteric fever. The present study was conducted to find out the sensitivity pattern of the isolates to the commonly used antibiotics in Enteric fever.A total of 290 blood samples were collected from patients with pyrexia for isolation of the agent by blood and clot culture. Antimicrobial susceptibility was done by disc diffusion method and Minimum inhibitory concentration (MIC) was determined using agar dilution technique for Ciprofloxacin and Ofloxacin. Among the 290 cases, culture was positive in 40 patients (13.79%). Out of 40 Salmonella isolates, 29 isolates were Salmonella enterica serovar typhi and 11 were Salmonella enterica serovar paratyphi A. MIC of ciprofloxacin and ofloxacin were high for one strain of Salmonella enterica serovar typhi. All the isolates were sensitive to ceftriaxone. To conclude, presence of quinolone resistance among Salmonella isolates from blood needs continuous monitoring and further evaluation.

Keywords: Antimicrobial susceptibility, Blood cultures, Minimum inhibitory concentration, Quinolone resistance, Salmonella enterica serovar paratyphi A, Salmonella enterica serovar typhi.

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INTRODUCTION

Enteric fever continues to be a global health problem, with an estimated 21.6 million people being affected (incidence of 3.6 per 1,000 population) and kills an estimated 200,000 people every year.The disease is endemic in many developing countries, particularly in Indian subcontinent, Southeast Asia, South and Central America and Africa, with annual incidence rate estimated to be greater than 900 per 100,000 populations in India [1].

In the 1970s, epidemic typhoid fever caused by Chloramphenicol-resistant strains emerged in Mexico and the Indian subcontinent [2]. In the beginning of 1989, multidrug-resistant strain of *Salmonella typhi* emerged in the Indian subcontinent, Southeast Asia and Africa [3].

After the development of resistance to the agents like chloramphenicol, trimethoprim-sulfamethoxazole and ampicillin. Hence, fluoroquinolones, such as ciprofloxacin became the drug of choice for the treatment of this infection [4]. However, there have been reports of therapeutic failure of ciprofloxacin due to high level resistance in patients with enteric fever [5,6].

Early detection and antimicrobial susceptibility surveillance must be applied to prevent the complications as well as the emergence of multidrug resistance strains. In view of the above facts, the present study was undertaken to detect the sensitivity pattern of the isolates to the commonly used antibiotics in our Hospital.

Aim

A cross sectional study was done to find out the antimicrobial susceptibility pattern of agents causing enteric fever.

Objectives

- To characterize and identify the common agents causing enteric fever using clot culture and conventional blood culture method
- To find out the antimicrobial susceptibility pattern of agents causing enteric fever
- To compare the results with previous studies to find out any changes in the sensitivity pattern
- To determine the Minimum Inhibitory Concentration (MIC) of the resistant strains by agar dilution method

MATERIALS AND METHODS

The present study was conducted prospectively from August 2011 to January 2012 in the Department of Microbiology at Saveetha Medical College, which is a 500 bedded tertiary care hospital in Thandalam, Kanchipuram district in Tamilnadu. A total of 290 patients with pyrexia for more than seven days attending the Pediatric and Medicine departments were included in this study. Patients with respiratory tract infections (tuberculosis, pneumonia), urinary tract infections, malaria, Dengue fever, Leptospirosis and immune compromised

patients (AIDS) were excluded from this study. A questionnaire was filled up for each patient to document the age, sex, presenting illness, treatment history and investigations results. Institutional ethical committee clearance was obtained for this study.

Five ml of venous blood from adults and 2 ml from pediatric patients was collected aseptically for blood culture and clot culture. After clotting of blood in the sterile tube, the specimens were centrifuged for 5 minutes at 3000 rpm (rotation per minute). The clot was used for clot culture and serum for Widal test [7]. The specimens were transported within two hours to the laboratory. For whole blood culture 3-5 ml blood was added to the brain heart infusion medium without centrifugation [8]. The clot was broken up with a sterile glass rod and added to bottle of 50 ml bile broth. Streptokinase (100 units per ml) was added into the broth to facilitate lysis of the clot. Subcultures were done from whole blood and clot on to Blood agar & Mac Conkey agar after 48 hours, 72 hours and one week. Non-lactose colonies from the Mac Conkey agar plates were identified by Gram staining, oxidase test and conventional biochemical tests (such as indole, citrate, urease, triple sugar iron, mannitol motility) and slide agglutination test with high titre sera to confirm Salmonella [9]. Antimicrobial susceptibility testing was done with Kirby Bauer technique with ATCC *Escherichia coli* 25922 as control strain according to guidelines by Clinical and Laboratory Standards Institute [CLSI] [10].

Agar dilution was done to find out Minimum Inhibitory Concentration (MIC) of multi drug resistant strains. Minimum inhibitory concentrations were performed for ciprofloxacin and ofloxacin by agar dilution technique using Mueller Hinton agar according to the criteria of CLSI. The concentrations used for MICs were doubling dilutions from 0.5µg/ml to 8µg/ml for ciprofloxacin and 0.5 µg/ml to 16 µg/ml for ofloxacin. Spot inoculum was done with each isolate. For Salmonella, MICs of ciprofloxacin ≤ 1µg/ml considered as sensitive, ≥ 4µg/ml considered as resistant and ofloxacin ≤ 2µg/ml considered as sensitive, ≥ 8µg/ml considered as resistant [10].

Procedure

- Muller Hinton agar plates with different concentrations of ciprofloxacin and ofloxacin were prepared.
- The concentrations were 0.5 µg/ml, 1 µg/ml, 2µg/ml, 4µg/ml, 8 µg/ml for ciprofloxacin and 0.5 µg/ml, 1 µg/ml, 2µg/ml, 4µg/ml, 8µg/ml, 16µg/ml for ofloxacin.
- The broth culture was incubated at 37°C until it achieved the turbidity of the 0.5 McFarland standards (usually 2 to 4 hours)
- Isolated Salmonella strains and ATCC *Escherichia coli* 25922 were inoculated with standard loop as spot inoculum.
- Plates were incubated at 37^0C for overnight
- Reading was taken after incubation.

RESULTS

Total number of blood samples collected was 290.The study period was for six months. Enteric fever was diagnosed by blood culture and clot culture.

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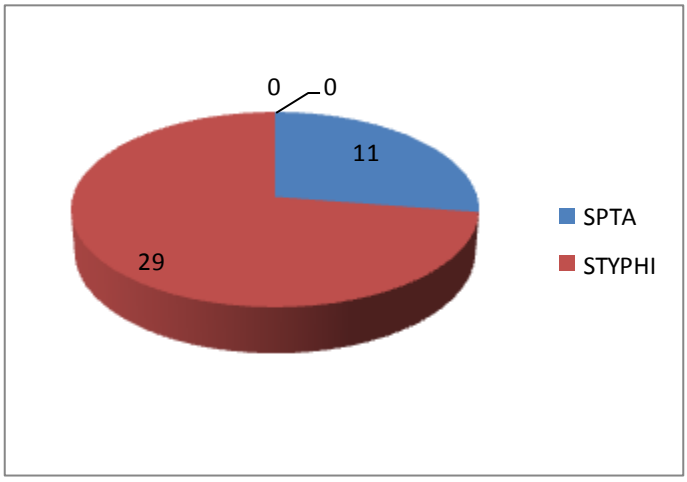
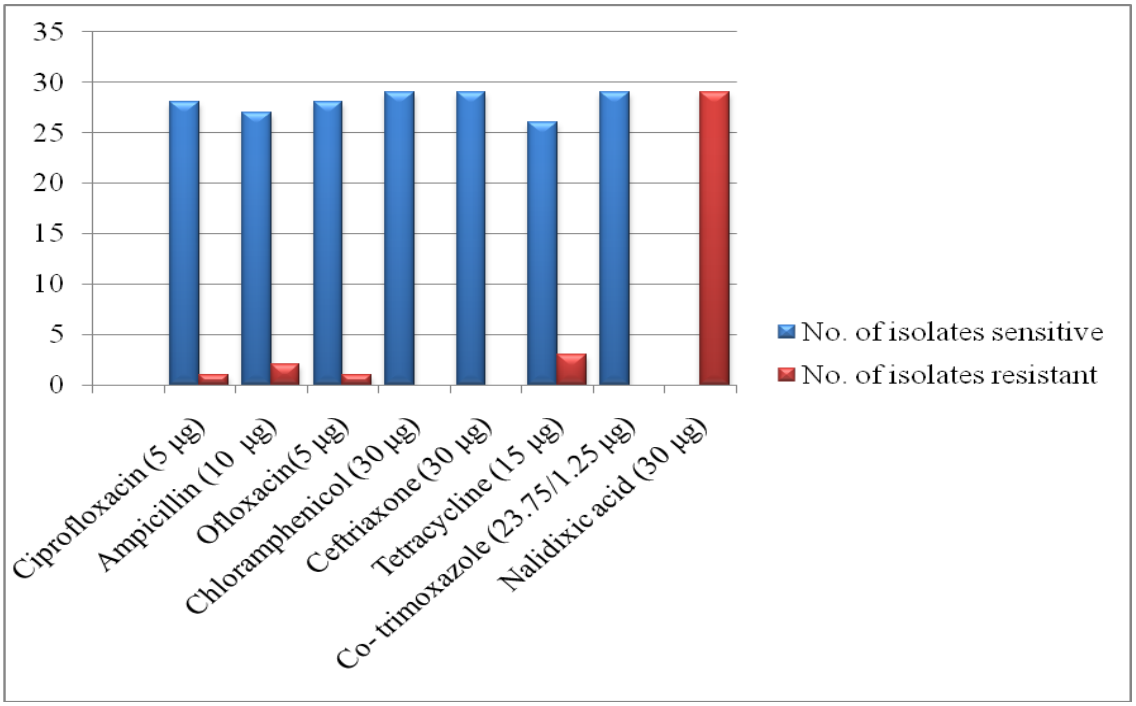
Among the 290 cases, culture was positive in 40 patients (13.79%). Out of 40 Salmonella isolates, 29 isolates were Salmonella enterica serovar typhi and 11 were Salmonella enterica serovar paratyphi A (Figure1).

Figure 1: showing distribution of Salmonella isolates



As indicated in Figure 2, all the 29 isolates of Salmonella enterica serovar typhi had shown 100% susceptibility to chloramphenicol, ceftriaxone and co-trimoxazole. But all the isolates were resistant to nalidixic acid. One isolate was resistant to ciprofloxacin and ofloxacin only. Similarly 3 isolates were resistant to tetracycline and 2 isolates were resistant to ampicillin.

Figure 2: showing Antibiotic sensitivity pattern of Salmonella enterica serovar typhi



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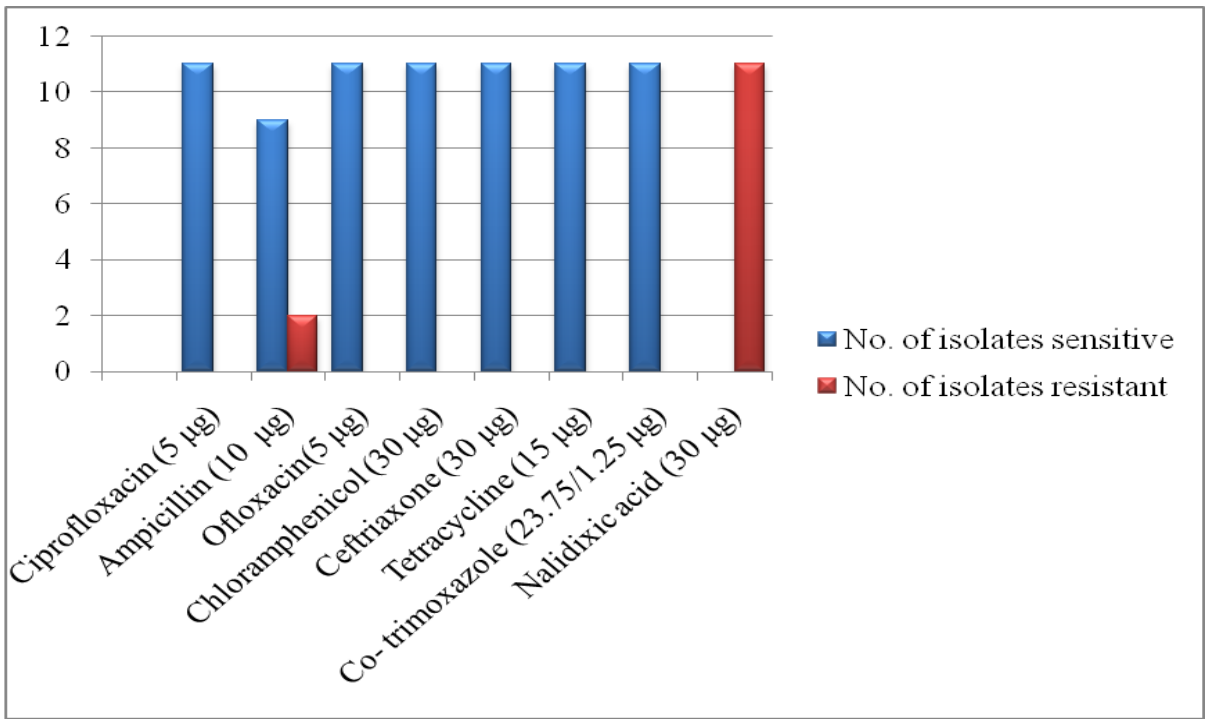
All isolates of Salmonella enterica serovar paratyphi A were sensitive to ciprofloxacin, ofloxacin, chloramphenicol, ceftriaxone, tetracycline, co-trimoxazole and 2 isolates were resistant to ampicillin as depicted in Figure 3. All isolates of Salmonella enterica serovar paratyphi A were also resistant to nalidixic acid.

Figure 3: showing Antibiotic sensitivity pattern of Salmonella enterica serovar paratyphi A



MIC was determined by agar dilution method for the isolates to confirm the resistance to ciprofloxacin and ofloxacin. ATCC *E.coli* 25922 was used as control.

Minimum inhibitory concentration (MIC) values of ciprofloxacin and ofloxacin to Salmonella isolates are shoen in Table 1 and 2 respectively.

Table 1: Showing Minimum inhibitory concentration (MIC) of ciprofloxacin to Salmonella isolates

MIC	Salmonella enterica serovar typhi	Salmonella enterica serovar paratyphi A	**Interpretation**
≤0.5 µgm/ml	17	7	Sensitive
1 µgm/ml	11	4	Sensitive
>8 µgm/ml	1	0	Resistant

Table 2: Showing Distribution of MIC value for ofloxacin

MIC	Salmonella enterica serovar typhi	Salmonella enterica serovar paratyphi A	**Interpretation**
≤1 µgm/ml	28	11	Sensitive
>8 µgm/ml	1	0	Resistant

Among the isolates, one strain of Salmonella enterica serovar typhi had shown resistance to ciprofloxacin and ofloxacin by Kirby Bauer and Stoke's method. Resistance was confirmed by determining the Minimum Inhibitory Concentration. The same strain had MIC of > 8µg/ml for ciprofloxacin and ≥ 16µg/ml for ofloxacin. The normal range of MIC for ciprofloxacin ≤ 1µg/ml was sensitive, ≥ 4µg/ml was resistant and for ofloxacin ≤ 2µg/ml was sensitive, ≥ 8µg/ml was resistant. Hence, the resistance of the above strain was confirmed.

DISCUSSION

In the present study, blood samples were taken from 290 patients with clinically suspected enteric fever.

Among the 290 cases studied, culture was positive in 40 (13.79%) patients of which 29 isolates were Salmonella enterica serovar typhi and 11 were Salmonella enterica serovar paratyphi A.

Isolation rate of Salmonella enteric serovar typhi and paratyphi A was 72.5% and 27.5% in our study. A similar study done by Krishnan P et al, on changing trends in antimicrobial resistance of Salmonella enteric serovar typhi and Salmonella enteric serovar paratyphi A in Chennai, 70 and 30% of isolates were Salmonella enteric serovar typhi and paratyphi A, which was in accordance with our study [11].

In our study, 2.5% of isolates showed resistance to ciprofloxacin and all the isolates were sensitive to ceftriaxone. A similar study done by Raveendran R et al, New Delhi high level ciprofloxacin resistance in salmonella enteric isolate from blood, 5% of isolates were resistant to ciprofloxacin and all isolates were sensitive to ceftriaxone which was comparable with our study [12].

There was no multi drug resistant isolates in this study, concurrently there has been an increase in the number of isolates sensitive to all antibiotics except nalidixic acid. A comparable observation was made by Madhulika U et al, Pondicherry who studied on current pattern in antimicrobial susceptibility of *Salmonella typhi* isolates in Pondicherry [6].

In the present study, all the Salmonella enteric serovar paratyphi A isolates were sensitive to chloramphenicol, ciprofloxacin, ceftriaxone and resistant to Nalidixic acid. Varsha Gupta et al had a similar study, where 100% of *Salmonella paratyphi* A were susceptible to ciprofloxacin, ceftriaxone and 90% isolates susceptible to chloramphenicol. Similarly 92.5% of isolates were resistant to Nalidixic acid. The results of this study correlated well with ours [13]. Though Nalidixic acid was not used for the treatment of enteric fever, nalidixic acid resistant phenotype is associated with an increased risk of fluoroquinolone treatment failure [4].

Our study showed 100% of Salmonella enterica serovar typhi isolates being sensitive to chloramphenicol. A study done by Goutam V et al, on sensitivity pattern of Salmonella serotype in Northern India, had 90% of *Salmonella typhi* isolates being sensitive to chloramphenicol. This result was in concurrence with our study [14].

Emergence of quinolone resistance was reported by recent studies by Ashwini Choudhary in which 13.66 per cent of *Salmonella typhi* isolates displayed reduced susceptibility to ciprofloxacin (MIC >0.5 µg/ml) [15].

In the present study, we encountered resistance to ciprofloxacin and ofloxacin in Salmonella enterica serovar typhi isolates. Quinlone resistance in Salmonella isolates has to viewed seriously and measures to detect and curtail resistance has to be taken immediately. The findings of this study emphasizes the need for further surveillance and evaluation in this area.

SUMMARY AND CONCLUSIONS

- All the isolates of Salmonella enterica serovar typhi and Salmonella enterica serovar paratyphi A were sensitive to Chloramphenicol and ceftriaxone.
- All the isolates of Salmonella were resistant to Nalidixic acid
- There were no Multi drug resistant strains among the isolates.
- Resistance to ciprofloxacin and ofloxacin was seen with one isolate of *Salmonella typhi* which was confirmed with MIC.

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