Antimicrobial Sensitivity Pattern Of Salmonella Spp. Isolated From Blood Culture Of Clinical Suspected Case Of Enteric Fever

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Abstract: Background: Enteric fever is one of the most common systemic infections in developing countries. The increasing rate of resistance to the commonly used antibiotics & changing trends of antibiotic susceptibility has been of great concern in the proper treatment and prevention of this disease. This study aims to investigate the current antibiotic susceptibility pattern of Salmonella typhi, Salmonella paratyphi A & B. Method: This study was carried out at GCS Medical College, Hospital & Research Centre in Microbiology Department, over the period of six months from March 2019 to August 2019. Blood culture samples from a suspected case of enteric fever sent in microbiology laboratory. Patients of all the age groups were enrolled during the study. Antibiotic susceptibility test of isolated Salmonella spp. was performed by the modified Kirby Bauer Disc Diffusion method and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Result**:** Total 116 blood culture samples were found positive for Salmonella species. Out of these, 106(91.37%) were Salmonella Typhi, 8(6.89%) were Salmonella paratyphi A & 2(1.72%) were Salmonella paratyphi B. Isolates were mainly from pediatric age group. Salmonella spp. shown 100% sensitivity, towards Ampicillin, Amoxicillin, Ceftriaxone, Cefixime, Cefepime, Doxycycline & Cotrimoxazole. Sensitivity pattern of S.Typhi was (81%) to Ciprofloxacin, & (84.90%) to Azithromycin. Conclusion**:** Early diagnosis and appropriate antibiotic therapy reduced the public health burden of typhoid fever. Third generation cephalosporins were found most effective & can be used as an empiric choice or First-line agents for the management of Enteric fever. Nowadays Fluoroquinolone resistance in Salmonella species is rising. [Patel I Natl J Integr Res Med, 2019; 10(6):19-22]

Key Words: Blood culture, Enteric fever, Salmonella spp

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Introduction: Typhoid fever is one of the commonest systemic infections of public health importance in developing countries. It is caused by Salmonella enterica subspecies enterica serovars typhi (S typhi) and paratyphi (S paratyphi). In India, about 494 children per 100,000, in the age group of 5-15 years, suffer from typhoid.[1]

The main cause of the spread of disease is substandard water supply, sanitation, and contaminated food. The disease places a significant burden on young children. After a few days of bacteremia, the bacilli localize mainly in the lymphoid tissue of the small intestine, resulting in typical lesions in Payer's patches and follicles. These lesions swell at first, then ulcerate and usually heal. After clinical recovery, about 5% of the patient's become chronic carriers and pass the bacteria intermittently in stool and rarely in urine.[2]

Antibiotic therapy constitutes the mainstay of management of enteric fever; mortality being as high as 30% in untreated cases, which falls to 1% with appropriate antibiotic therapy.[3] Nowadays antimicrobial resistance is a major issue in the management of enteric fever. Since then, an increasing frequency of antibiotic resistance has been reported from all parts of the world, but more so from the developing countries.

Drug resistance in Salmonella is of considerable importance to both clinicians and microbiologists and poses a major problem for public health authorities.[4] The uses of chloramphenicol, ampicillin, and cotrimoxazole have become infrequent. Azithromycin is viewed as an attractive alternative for the management of enteric infections.[5] Nowadays third-generation cephalosporins such as ceftriaxone have become the first line of treatment.[6]

Materials And Methods: A study was carried out from March 2019 to August 2019 (six-month duration), at GCS Medical College, Hospital & Research Centre in Microbiology Department. Blood culture samples from suspected cases of enteric fever sent to the microbiology laboratory. Patients of all the age groups were enrolled during the study. Blood sample collections were done by aseptic precautions & proper labeling of the patient's details.

All Blood culture bottles were loaded to BACTEC 9050 and when it signaled positive taken out of machine and processing of samples were done by first direct microscopy & gram stain done for presumptive identification of bacteria. Then samples were subculture on chocolate agar and Mac Conkey agar plates. They were incubated overnight at 37^{o}C in the incubator. On the second day, all inoculated plates were observed and colony characteristics were noted.

Non-lactose fermenting (pale colonies) on Mac Conkey agar were suspected as salmonella species & further confirmed by Biochemical reaction & agglutination by specific antisera. All the isolates of Salmonella spp. were tested for antimicrobial susceptibility testing by modified Kirby Bauer method of disc diffusion technique on Mueller Hinton agar. Salmonella strains were tested for their sensitivity by using following anti-microbial agents Ampicillin(10mcg), Ceftriaxone(30mcg), Cefepime(30mcg), Ciprofloxacin(5mcg), Doxycycline(30mcg), Azithromycin(15mcg), Nalidixicacid(30mcg), Cotrimoxazole(25mcg). The strains were identified as sensitive, intermediate or resistant as per the diameter of zone of inhibition according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[7]

Results: Total no. of 116 blood culture positive for Salmonella spp. were recovered over the period of six months duration from march 2019 to august 2019. Out of these 106(91.37%) were Salmonella Typhi, 8(6.89%) were Salmonella paratyphi A & 2(1.72%) were Salmonella paratyphi-B. (Table - 1). The study population was divided into two different age groups which are, 1-15 years of age were categorized as pediatric group and above 15 years as an adult group.

Table 1: Distribution of various salmonella spp. amongst paediatric & adult age groups of patients

Age groups	S.Typhi	S.paratyphi A	S.paratyphi B
Pediatric age group (1-15yr)	94	6	2
Adult age group(>15yr)	12	2	0
Total no.(N=116)	106	8	2

The rate of isolation of Salmonella spp was higher (87.90%) among pediatric group as compared to the adult group (13.79%).

Table 2: Sensitivity pattern of S.Typhi & S.paratyphi A

Antibiotic	Salmonella Typhi(No.=106)	Salmonella Paratyphi-A (No.=8)
	Sensitivity	Sensitivity
Ampicillin/ Amoxicillin	100%	100%
Ceftriaxone	100%	100%
Cefixime	100%	100%
Cefepime	100%	100%
Ciprofloxacin	81%	25%
Doxycycline	100%	100%
Azithromycin	84.90%	12.50%
Nalidixic acid	81%	25%
Cotrimoxazole	100%	100%

All the Salmonella spp.(S.typhi, S.paratyphi A & S.paratyphi B) showing 100% sensitivity towards Ampicillin, Amoxicillin, Ceftriaxone, Cefixime, Cefepime, Doxycycline & Cotrimoxazole.

Out of the 106 culture-positive S. typhi isolates, 86 (81%) were sensitive to Ciprofloxacin, 90 (84.90%) were sensitive to Azithromycin. Out of the 8 culture-positive S. paratyphi-A isolates,2(25%) were sensitive to Ciprofloxacin,1 (12.25%) were sensitive to Azithromycin. S.paratyphi B is less common in India, it rarely isolates from blood culture. In our study 2 culture-positive S. Paratyphi-B isolates were sensitive to all antibiotics which are tested for salmonella.

Discussion: In present study, total 116 blood culture isolates of Salmonella enterica, 106(91.37%) S.Typhi, 8(6.89%) were S. Paratyphi A & 2(1.72%) were S. Paratyphi-B. In a similar study done by Sarika Jain et al from New Delhi, out of 344 isolates of Salmonella enterica, 266 (77.3%) were S. Typhi, 77 (22.4%) were S. Paratyphi A, and one (0.3%) was S. Paratyphi B.[8] The rate of isolation of Salmonella spp.in the present study was higher (87.90%) among pediatric group as compared to adult group (13.79%), the study done by Bulbul Hasan et al Rajshahi Medical College, Dhaka the rate of isolation of Salmonella spp. was noted

higher(87.54%) among pediatric group compared to adult group (13.95%) which is similar to our study.[9]

Increase clinical suspicion for enteric fever may also be attributed to the high and rapid recovery rate of organisms by the BACTEC blood culture system used in the present study, even in the presence of antimicrobials. A high proportion of enteric fever cases in children suggest a greater burden of enteric fever in a younger age group as compared to adults. S. Typhi was isolated mostly from children. The major issue in the management of enteric fever is antimicrobial resistance. Resistance to chloramphenicol emerged in S. Typhi strains in the early 1970s, later development of plasmid-mediated resistance was soon followed by resistance to ampicillin and co-trimoxazole.

Multidrug-resistant typhoid fever (MDRTF) is resistant to first-line recommended drugs for the treatment such as chloramphenicol, ampicillin & co-trimoxazole. In our study, case of MDRTF is not seen; but in the study of Sarika Jain, Tulsi Das Chugh BLK from New Delhi, India 3.4% of Salmonella Typhi strains were multidrug resistant & The absence of MDR in S. Paratyphi A.[8]

Following emergence of multidrug-resistant enteric fever, fluoroquinolones (FQ) or third-generation cephalosporins remained as first-line drug in the 1990s. Ciprofloxacin, as a standard treatment for typhoid, given both extracellular and intracellular mechanisms of action & safety profile is also good. Reduced susceptibility to ciprofloxacin emerged due to indiscriminate use, over the counter availability and cheap formulations that delivered subclinical dosages. As far back as 1997, Vietnam reported quinolone resistance in Salmonella typhi[10,11] The first case of fluoroquinolone treatment failure in Pakistan was reported in1993[12,13]

In our study, resistance to ciprofloxacin in S.Typhi was 20(18.86%), & S. paratyphi A was 6(75%) which is higher than S.Typhi. But in study by Sarika Jain et al, increasing trends of FQ resistance in S. Typhi (35.3%) was observed; & resistance in S. Paratyphi A (49.3%) was higher than S. Typhi (35.3%)[8] In study by Bulbul Hasan et al from Dhaka only 03(18.75%) S. Typhi were resistant to Ciprofloxacin.[9] In a study carried out at Karachi, an increased fluoroquinolone resistance in Salmonella was observed - from 84.7 % to 91.7 % using CLSI 2009 guidelines; 88.2% of S. typhi and 83.9 % S. paratyphi-A strains were resistant in that study.[14]

In present study, all strains were susceptible to third-generation cephalosporins ceftriaxone and cefixime. Other studies have also reported little or no resistance to these antimicrobials.[15,16,17,18,19]

In our study, resistance to azithromycin in S.Typhi was (15.09%), & S.paratyphi A was (87.50%); but in the study of Sanjaya Kumar Shrestha et al, Lalitpur, Azithromycin show 100% sensitivity. The study by Sarika Jain et al, New Delhi, India azithromycin resistance occurred at a slightly higher rate in S. Paratyphi A (10.4%, 8/77) than in S. Typhi (6.4%, 17/266).[8] In areas where FQ resistance is a problem and/or if the physician is disinclined to use quinolones such as in children, the macrolide azithromycin in combination with ceftriaxone may prove to be effective in the treatment of Salmonella infection.[20,21]

Conclusion: Rapid diagnosis and appropriate antibiotic therapy reduced the public health burden of typhoid fever. Blood culture can provide promising result for diagnosis & management of typhoid fever if timely collected & processed. The present study revealed that all strain of salmonella shows 100% sensitive to third-generation cephalosporins & show resistance to Fluroquinolones & Azithromycin was 19% & 15.1% respectively. In developing countries, first-line agent for empirical management of enteric fever is third-generation cephalosporin. Ceftriaxone is the most effective drug, particularly has been used with success in the treatment of typhoid fever, even in the treatment of ciprofloxacin-resistant strains. In Outpatient, Cefixime is a favorable empiric choice for management of enteric fever cases.

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