A study of Isolation of Salmonella species from blood culture & it's Antimicrobial Resistant Pattern.

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Abstract

Background: The main aim of this study was to monitor the antimicrobial resistant pattern of Salmonella isolates to select proper antibiotic & prevent drug resistance in Salmonella species. **Material and Methods:** Total 250 blood culture bottles were collected from patients clinically suspected enteric fever and loaded to BACTEC 9050. Out of them 200 signalled positive samples were inoculated on chocolate and Mac Conkey agar plates and incubated overnight at 37^0 C in the incubator and identified by colony characteristics, procedures like Gram staining, wet preparation for motility and bio-chemical reactions like oxidase test, catalase test, triple sugar iron agar, Citrate test, using Salmonella antisera like Poly O, O-9, and H-d. The clinical isolates were subjected to antibiotic sensitivity test on Mueller-Hinton agar, using modified Kirby Bauer disc diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines. **Results:** Total 61 Salmonella were isolated. 49 were Salmonella typhi and 12 were Salmonella paratyphi. Overall rate of resistance of 49 S. typhi isolates was 2 % to ampicillin, 4 % to Azithromycin, 4 % to fluoroquinolones and 2 % to co-trimoxazole. S. paratyphi isolates were 100% sensitive to ampicillin, co-trimoxazole, chloramphenicol and 3rd generation cephalosporins. **Conclusion:** Enteric fever is one such infection which poses challenges in antimicrobial resistance. Continuous surveillance is important to track bacterial resistance and to treat infections in a cost-effective manner.
Keywords: Antimicrobial resistance, Enteric fever, Salmonella

Introduction

Enteric fever is a global public health problem and is endemic in many developing countries, including India.[1] Enteric fever is a systemic infection caused by the human adapted pathogens Salmonella enterica serotype Typhi (S. typhi) and S. enterica serotype Paratyphi (S. paratyphi) A, B and C .[2] According to recently revised global estimate, above 22 million cases of typhoid fever occur each year round the world with 200,000 mortality cases per year have been reported worldwide [3]while 90% of the sufferers are from the South East Asia.[4] 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.[5] It predominantly affects children and young adults [6] and if not treated appropriately has mortality rate of 30%, whereas, with proper treatment the mortality reduces to as low as 0.5%. [7]The most common risk factors are contaminated drinking water or food with faeces from either acutely infected persons, persistent excretors, or chronic asymptomatic carriers.[8] Low socio-economic status and poor hygiene conditions [9,10]are responsible for the spread of the infection.[11,12]

Typhoid is unique to human, characterized by malaise, fever, abdominal discomfort, transient rash, splenomegaly, hepatomegaly, bradycardia, and leucopenia, the most prominent major complications

are intestinal hemorrhage, and perforation. However, non-typhoidal Salmonella can also cause a variety of life-threatening extra-intestinal infections.

Drug resistance is fast becoming a major problem in the management of this infection and the emergence of multi-drug resistance has great implications for the therapy, for example, patients infected with such strains are more ill at presentation, have a longer duration of illness and higher mortality. However, there are no pathognomic features to distinguish such infections from infections with fully sensitive S. typhi at presentation.[13] Chloramphenicol resistance became established globally in the S. typhi population after 1972 on plasmids of incompatibility group Inc H and Multi drug resistance (defined as resistance to all the first line antibiotics used to treat typhoid fever, i.e. chloramphenicol, ampicillin and cotrimoxazole) has been endemic, particularly in Indian sub-continent and South East Asian countries since 1984.[14] Though initially, individual plasmids were known to code for multidrug resistance to each of these antibiotics, since 1988 a single plasmid was known to code for multidrug resistance. This plasmid belongs to incompatibility group H 11 and is highly permissible. In addition to Multi Drug Resistance (MDR) S. typhi, now resistances to fluoroquinolones have emerged as the newer challenges to the treatment of typhoid fever.[15] Aside from irrational practices of antibiotic use, mutations in chromosomal genes encoding DNA gyrase and Topoisomerase IV and by plasmid mediated quinolone resistant (PMQR) genes are suggested mechanisms for the development of resistance to nalidixic acid and reduced susceptibility to ciprofloxacin.[16]

This study was undertaken to isolate and identify S. typhi and S. paratyphi and know their antibiotic sensitivity pattern .So with the changing patterns in antibiogram it is necessary to continually monitor the drug resistance pattern and understand the mechanisms involved and according appropriate strategies can be adopted in the management of enteric fever.

Material and Methods

The retrospective study was carried out at Department of Microbiology in GCS Medical College & Research Center Ahmedabad. The study was conducted from March 2021 to January 2022. Total 250 blood culture bottles were collected from patients clinically suspected enteric fever.

Blood culture from peripheral /central vein was collected after proper hand washing and wearing gloves with proper sterilization of skin area with iodine and isopropyl alcohol.

Isolation of Salmonella

Blood culture bottles were loaded to BACTEC 9050 . The Bactec 9050 system bottles have a detector at the bottom that emits a fluorescent light as the CO2 concentration increases that will pass via an emission filter to a light sensitive diode. The system measures the voltage every 10 minutes and compares the new value with the previous value and emits positive signals as soon as the threshold value is reached. When it signalled positive taken out of machine and processing of samples was done. Wet-mount preparation & Gram stain of signaled positive samples were done. They were inoculated on chocolate agar and Mac Conkey agar plates. The inoculated plates were incubated overnight at 37^0 C in the incubator.

Identification and confirmation of Salmonella

All inoculated plates were observed next day The colony growths were identified by colony characteristics , standard microbiological procedures like Gram staining, wet preparation for motility and bio-chemical reactions like oxidase test, catalase test, triple sugar iron agar, Citrate test Final identification of isolates was confirmed serologically according to Kauffman-White classification using Salmonella (somatic and flagellar) antisera like Poly O, O-9, and H-d.

Antibiotic sensitivity test

The clinical isolates were subjected to antibiotic sensitivity test on Mueller-Hinton agar, using modified Kirby Bauer disc diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines. The panel of antibiotics included were Ampicillin (10 µg), Nalidixic acid (5µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Pefloxacin (5µg), Cefuroxime (30 µg), Cefotaxime (30 µg), Cefixime (30 µg) ,Cefoperazone-Sulbactum (105 µg), Doxycycline (30 µg), Co-trimoxazole (25 µg), Chloramphenicol (30 µg) and Azithromycin (15 µg)

The negative samples were incubated for next seven days and then subcultured, before finally declaring them as culture negative.

Results

Total of 61 Salmonella were isolated from 200 signal positive blood samples out of 250 suspected enteric fever patients.30 isolates were from male patients and 31 isolates were from female patients. Culture positive patients fell into age group of 1-70 yrs. 41 isolates were from children of age group 1 to 10 yrs. 11 isolates were from age group 11 to 20 yrs. 9 isolates were from adults of age group > 20 yrs. Out of 61 Salmonella isolates, 49 were Salmonella typhi and 12 were Salmonella paratyphi. Antimicrobial susceptibility pattern of Salmonella typhi and Salmonella paratyphi during our study period has been described in Table 1 and 2 respectively.

Table.1 Antibiotic susceptibility pattern of Salmonella typhi

Antibiotic	Susceptible (%)	Resistant (%)
Ampicillin (10µg)	47 (96 %)	2 (4%)
Cefotaxime (30µg)	49 (100 %)	0 (0 %)
Cefixime (30µg)	49 (100 %)	0 (0 %)
Nalidixic acid	40 (81.63%)	9 (18.37%)
Ciprofloxacin (5µg)	47 (96 %)	2 (4%)
Co-trimoxazole (25µg)	48 (98%)	1 (2%)
Azithromycin (15 µg	46 (94 %)	3 (6%)
Tetracycline (30µg)	47 (96 %)	2 (4%)
Chloramphenicol (30 µg)	49 (100 %)	0 (0 %)

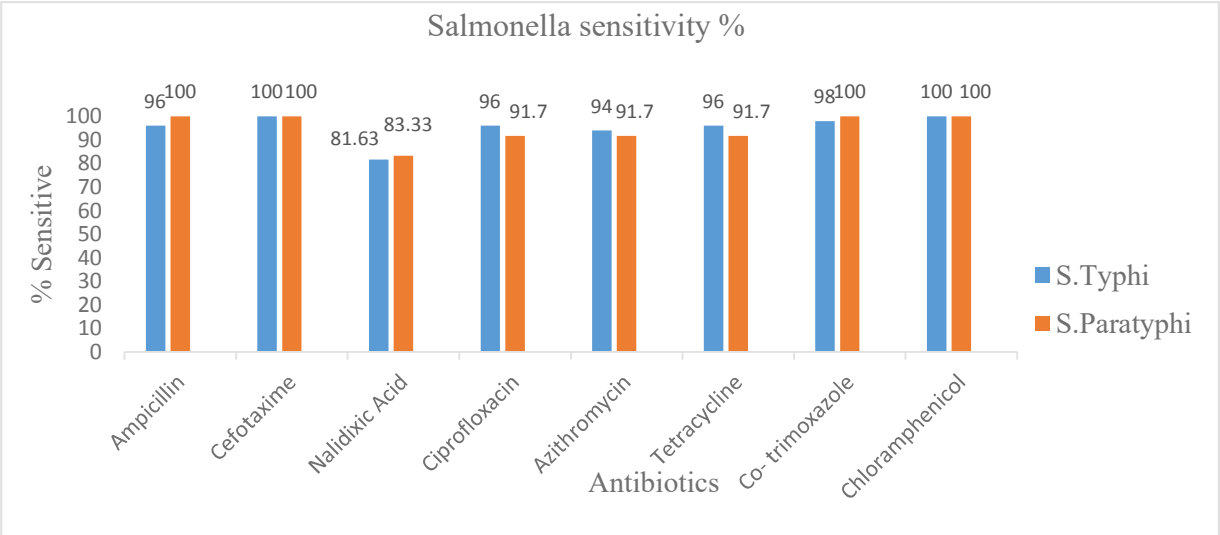
Table.2 Antibiotic susceptibility pattern of Salmonella paratyphi

Antibiotic	Susceptible (%)	Resistant (%)
Ampicillin (10µg)	12 (100 %)	0 (0 %)
Cefotaxime (30µg)	12 (100 %)	0 (0 %)
Cefixime (30µg)	12 (100 %)	0 (0 %)
Nalidixic acid	10 (83.33%)	2 (16.67%)
Ciprofloxacin (5µg)	11 (91.7%)	1 (8.3%)
Co-trimoxazole (25µg)	12 (100 %)	0 (0 %)
Azithromycin (15 µg	11 (91.7%)	1 (8.3%)
Tetracycline (30µg)	11 (91.7%)	1 (8.3%)
Chloramphenicol (30 µg)	49 (100 %)	0 (0 %)

Discussion

In the last fifteen years, the emergence of resistance to the antibiotics has lead to large epidemics. Typhoid is now encountered mostly throughout the developing world. Enteric fever is a major public health problem in India. Various studies document S. Typhi as the commonest serovar isolated over the years.[17] Management of this serious disease becomes complicated due to the resistance of antibiotics used for the treatment of enteric fever. It is becoming difficult to control the spread of multidrug resistant (MDR) Salmonella.[18] Out of 200 blood samples, 61 Salmonella were isolated. Our study also showed 80 per cent isolates of serovar Typhi while 20 per cent were serovar Paratyphi.[19] In our study period, S. typhi outnumbered S. paratyphi with almost 4 times higher rate of isolation in our region.

We have obtained blood samples from patients attending Gujarat Cancer Society Medical Collage, Hospital & Research Centre (GCSMCH & RC), Ahmedabad. This hospital is referral medical hospital for the region and, many patients seek treatment here, after receiving some treatment at local level. This factor may interfere with organism isolation rate. Blood sample collection time like before starting antibiotics or after may also affect isolation of Salmonella.



The highest incidence of typhoid occurs in paediatric age group. In the present study, 41 (67.21%) cases occurred between 1-10 years of age. 30 (49.18%) isolates were from male patients and 31 (50.82%) isolates were from female patients. There is no gender difference in our study.

In our study, overall rate of resistance of 49 S. typhi isolates was 2 % to ampicillin, 4 % to Azithromycin, 4 % to fluoroquinolones and 2 % to co-trimoxazole. S. paratyphi isolates were 100% sensitive to ampicillin, co-trimoxazole, chloramphenicol and 3rd generation cephalosporins.

Susceptibility pattern of S. typhi isolates to chloramphenicol (100%) were similar to study conducted by[20] GordanaMijovic et al., (2012)

In the last decade, there have been some reports of ciprofloxacin resistance in Salmonella.[21] It is believed that nalidixic acid resistance is a surrogate marker for ciprofloxacin resistance, as clinical failures have been documented in cases where ciprofloxacin has been used (based on susceptibility) for nalidixic acid resistant strains.[22]

Since its introduction in 1948, chloramphenicol has been the treatment of choice for typhoid fever and the treatment with chloramphenicol reduces death due to typhoid fever from about 20 to 1 per cent and the duration of fever from 14-28 days to 3-5 days.[23] However, chloramphenicol therapy has been associated with the emergence of resistance to chloramphenicol, a high relapse rate, bone marrow toxicity and high mortality rates in a recent study reported from the developing world.[24] Ampicillin and co-trimoxazole could be effective alternative drugs. In our study Salmonella sp. remained sensitive to chloramphenicol, amoxicillin, Ceftriaxone and co-trimoxazole (100, 96.72,100 & 98.36%, respectively) oral cephalosporins and macrolides are considered as the first-line agents for empirical treatment of enteric fever cases in cases of decreased susceptibility to fluoroquinolones.[25,26] However, few reports have also suggested resistance against azithromycin[27,28,29] and ceftriaxone[30,32] in S. typhi isolates. This may be due to production of drug-specific resistance genes, modification of target sites by enzymes (like methylases, esterases, phosphotransferases), or acquisition of an efflux pump in azithromycin-resistant cases [33] and production of beta lactamases (which inactivate cephalosporins by cleaving its beta lactam ring) in cephalosporin-resistant cases. Hence, the excessive use of these antibiotics should be limited so that their efficacy against Salmonella isolates is not jeopardized.

Azithromycin has prepared well in previously clinical studies for typhoid; however, there have been sporadic reports of azithromycin resistance.[34] All isolates in our study were sensitive to ceftriaxone in contrast to some studies that reported resistance to ceftriaxone.[35,36]

Conclusion

Enteric fever is one such infection which poses challenges in antimicrobial resistance. Hence, continuous surveillance is important to track bacterial resistance and to treat infections in a cost-effective manner. Azithromycin resistance appears to be emerging. However, isolates showed a high degree of susceptibility to ampicillin, co-trimoxazole and chloramphenicol. Thus, antibiotics like amoxicillin, cotrimoxazole, and third generation Cephalosporins (Cefotaxime) may once again be

helpful for the management of enteric fever in tertiary care hospitals. This study thus emphasizes the need for continuous evaluation and judicious use of antimicrobials, considering the ever-changing landscape. The surveillance of antimicrobial resistance in Salmonella spp. is very important. Also, it is important to maintain Salmonella active surveillance of resistance on an international and intersectoral level.

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