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Prevalence and Susceptibility Pattern of Salmonella Isolated from Blood Cultures at a Tertiary Care Centre

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Abstract: ***It is evident that enteric fever remains a pressing health concern in India, with its persistence rooted not only in environmental vulnerabilities - such as inadequate sanitation, unsafe water, and seasonal contamination during monsoons but also in the troubling evolution of antimicrobial resistance. In my view, the study's year-long surveillance of 3,116 blood cultures offers a revealing snapshot of the prevalence and resistance trends of Salmonella isolates in a tertiary care setting. The findings show an unsettling dominance of Nalidixic acid–resistant strains and emerging resistance to ceftriaxone, underscoring how overuse and misuse of antibiotics continue to fuel this problem. That said, the noted sensitivity to ampicillin, augmentin, and azithromycin provides a glimmer of hope, suggesting potential avenues for treatment in both community and hospital settings if used judiciously. The seasonal spike in isolates during August and September also raises another point typhoid control cannot be divorced from broader public health strategies addressing water quality and waste management. On further analyses, the absence of molecular resistance profiling in the study hints at a missed opportunity to link phenotypic resistance with genetic mechanisms, which could have strengthened long-term intervention planning. Overall, this work not only documents a local pattern of concern but also calls for cyclical surveillance, antibiotic stewardship, and infrastructural reforms if the cycle of recurrence is to be broken.***

Keywords: enteric fever, antimicrobial resistance, Salmonella typhi, blood culture, antibiotic stewardship

1. Introduction

Enteric fever is a major public health problem causing an estimated 11.9 to 26.9 million cases and 1,29 ,000 to 2,17, 000 deaths worldwide each year [1, 2]. There is a striking difference in various regions like it is more prevalent in India. Enteric fever is caused by important serovars Typhi and Paratyphi A, B and C of the Salmonella enterica species. India is endemic for enteric fever, where it is one of the main differential diagnoses for any pyrexia of unknown origin. In India, Salmonella enterica, serotype Typhi, remains the predominant Salmonella species causing enteric fever[3]. Humans are the only host for typhoidal salmonellas, and transmission is predominantly with contaminated water or food, polluted by infectious faeces. In India certain factors like limited or poor access to safe drinking water, poor sanitation and spreading urbanization has always being compelling.

There are various serological tests and rapid tests are available for the lab diagnosis of enteric fever, but the definite method of lab diagnosis remains culture of organism. Blood and bone marrow are the important samples which are used during the first week of Enteric fever. A specific diagnosis of typhoid requires access to a competent laboratory that can process blood cultures and such laboratories are uncommon in resource limited regions. Microbiologic culture of blood or bone marrow remains the mainstay of laboratory diagnosis [4].

The antimicrobial resistance in salmonella is increasing to several commonly used antimicrobial agents. Although fluoroquinolones (FQ) are the drugs of choice to treat invasive Salmonella infections, but the resistance to FQ is increasing quickly worldwide [5]. However, since the early 2000s, increasing fluoroquinolone nonsusceptibility (intermediate or full resistance to ciprofloxacin), especially in South Asia, has led to the use of third-generation cephalosporins. Ceftriaxone is a recommended first-line treatment. Typhi isolates are defined as MDR if they are resistant to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole. XDR Typhi isolates are MDR, nonsusceptible to fluoroquinolones, and resistant to third-generation cephalosporins.

These MDR and XDR Salmonella have increased the challenge in management by increasing morbidity and mortality. However, to control the spread of typhoid fever, surveillance for S. typhi and the assessment of antimicrobial susceptibility is essential.

Keeping these things under consideration present study is being planned to know the prevalence and susceptibility pattern of Salmonella isolated from blood cultures at a tertiary care centre.

2. Materials and Methods

Study area, duration and design

A cross-sectional retrospective hospital-based study was performed at a tertiary care center in central India of Punjab for a period of 12 months from 24 April 2023 to 20 April 2024.

Study population and inclusion criteria
All the fever cases visiting OPDs or admitted with advice of blood culture were included in the study. Their samples for blood culture were collected in the microbiology laboratory.

Exclusion criteria
Following categories were excluded from study
- Non consenting patients for blood culture
- Febrile patients who had received antibiotics within 1 week before presentation

Sample size and ethical consideration
A total of 3116 blood cultures samples were received in lab out of which salmonella was isolated from 61 samples. Clearance from the institutional ethical committee was taken.

Sample Collection and Transport
Under all the aseptic precautions, blood specimens were collected. The volume of blood sample was 8 mL–10 mL for adults and 2 mL–3 mL for pediatric patients, so that blood to media ratio was 1:5 to 1:10. After addition of blood, these automated blood culture bottles were mixed and further they were transferred to lab for incubation and further analyses.

Lab processing
Automated blood culture bottles were incubated at 37 ◦C for up to 5 days. After flagging of growth, subculture was done on blood agar (BA) and Mac'Conkey agar (MA). The inoculated culture plates were incubated at 37◦C for 18 to 24 h. The BA plates were used for the observation of non-hemolytic smooth white colonies, MA for nonlactose fermenting colonies [6-25]. Automated identification and susceptibility methods were used for rapid analyses and to reduce the turnaround time [7]. Serotyping of the isolates was further performed by agglutination method using Salmonella polyvalent antisera O [6-25].

Data analyses
Collected data was analyzed in Microsoft Excel 2016. Further statistical package for social science (SPSS) version 24.0 was used to analyze data. Chi-squared ($\chi 2$) test was used to predict the relationship between the variables in which a p value of <0.05 was considered as statistically significant.

3. Results

3,116 blood culture samples were received out of which 316 were growth positive at 10.14%. 61 samples from growth positive from fever cases had salmonella at 19.30%. From overall total samples of blood culture salmonella were isolated at 1.95%. Out of total 61 samples, 28 (45.90%) were from pediatric patients and 33 (54.1%) were from adults. Majority of patients in our study were adults. Salmonella were isolated from 44 males (72.13%) and 17 females (27.87%). 32 isolates (52.45%) were *Salmonella typhi*, 21 isolates (38.42%) were *Salmonella paratyphi A,* 5 isolates (8.19%) were *Salmonella enterica* and 3 (4.9%) isolates were detected from genus salmonella.

Antibiotic susceptibility was done with Ampicillin, Augmentin, Piperacillin-Tazobactum, Ceftriaxone, Amikacine, Meropenem, Nalidixic acid, ciprofloxacin and Azithromycin.

Ampicillin resistance was seen in 22 isolates (36.06%) out of 61, while only 02 isolates were Resistant to Augmentin and 96.72% were fairly sensitive. The same susceptibility pattern was seen with Piperacillin-Tazobactum (PIT). Out of 61, only 02 isolates were resistant, rest 96.72% were sensitive. Ceftriaxone resistance is on rise, 08 isolates (13.11%) were resistant and 53 isolates (86.89%) were sensitive. 55 isolates (90.16%) were Nalidixic acid resistant salmonella typhi (NARST). 54 isolates (88.52%) were resistant to Amikacine. Carbapenem resistance was seen in only one isolate and remaining 98.32% were sensitive to it. No resistance was seen with Azithromycine disc diffusion test.

4. Discussion

Isolation of salmonella from blood culture is the most definitive way of diagnosis in first week of illness. Positive blood culture results can help clinicians for diagnosis, the targeting therapy against the specific organism (s), and also provide prognostic value [8]. Adequate blood volume sampling is also crucial for positivity rate, with a 2% to 4% increase in positivity rate from each additional milliliter of blood [9]. Blood culture positivity has varied from 13.7% to 26.5% with the clinical condition of patient [10]. While at a larger study, out of 10,235 patients 1,082 (10.6%) were positive [11]. In present study also, from 3,116 blood culture samples 316 were growth positive at 10.14%. The percentage of positivity is in correlation with other studies. The overall average S. Typhi and S. Paratyphi A positivity rate for blood cultures performed at Joshi Laboratory between 2014 and 2018 was 3.1%, which is similar to the rate reported from Vellore, India (3.8%) over a comparable time period [12,13]. In the present study, 19.3% were isolated from the blood culture samples of fever cases.

Studies have already demonstrated that salmonella isolates were more common in an age group below 30 years [14] which implies that typhoid fever is most common in pre-school and school age children. In our study also, out of total 61 isolates, 28 (45.90%) were from pediatric patients which is correlating with the other studies.

This study was conducted over a period of one year, hence significance of water pollution in a rainy season can't be commented. In our study, there has been Enteric fever pathogens were isolated most in the month of September and August. August and September sees normal to above normal rainfall over large parts of the country. Water is more likely to be polluted in the wet season because the rains may wash debris and littered garbage into wells and streams used as domestic sources of water.

As per Woodward TE [15], First antibiotic to treat typhoid was chloramphenicol and it was introduced in 1948. After resistance to chloramphenicol, ampicillin and co-trimoxazole were introduced for treatment in 1970 [16]. Multi Drug Resistant salmonella are those strains which are resistant to ampicillin, chloramphenicol, and co-trimoxazole. The emergence of MDR strains was associated with

genotype change as discussed by Saha S etal [17]. In our study we could not do testing for chloramphenicol and co-trimoxazole. But we tested for ampicillin and augmentin. Both these drugs were fairly sensitive. Chowta MN et al [18] in Indian setting have shown the drug resistance in typhoid is a major factor leading to the morbidity and mortality of the disease. Ampicillin was used previously as a mainstay of treatment of typhoid. In our study Ampicillin resistance was seen in 22 isolates (36.06%) out of 61.

In case of salmonella, susceptibility of Nalidixic acid can be used as a marker for susceptibility of quinolones. Resistant and susceptible salmonella were known as Nalidixic Acid-Resistant Strains (NARST) and Nalidixic Acid-Sensitive Strains (NASST) [19]. The prevalence of NARST and NASST varies as per location. In our study we have very high count of NARST. We found 55 NARST isolates (90.16%) in six months at our center. There has been study conducted by Yashvant kumar et al [20] as Antibiogram Profile of Salmonella enterica Serovar Typhi in India – A Two Year Study. In which they have found 93.8% resistance to Nalidixic acid. In another study conducted by Sreenivasan srirangaraj [21] et al at pondecherry, found aot 100 % reisistance to fluroquinolones. The increase in resistance to Nalidixic acid or fluroquinolones is of concern because of over antibiotic abuse. Ceftriaxone resistance was seen in 08 isolates (13.11%), while remaining 53 isolates (86.89%) were sensitive. Sreenivasan srirangaraj,[21] has shown 100% susceptibility to ceftriaxone. In their study 93.75% were sensitive and 6.25% were intermediate sensitive. In our study no resistance was seen with Azithromycine by disc diffusion, however Sreenivasan srirangaraj has noted few isolates resistance to Azithromycine. Silvia argimon et al [22] has reported the persistent circulation of third-generation cephalosporin resistant Salmonella Typhi in Mumbai. They have linked to the acquisition and maintenance of a previously characterized IncX3 plasmid carrying the ESBL gene blaSHV-12 and the fluoroquinolone resistance gene qnrB7 in the genetic context of a triple mutant also associated with fluoroquinolone resistance. In our study these molecular characterization were not conducted hence further association with resistant plasmids or genes were not commented.

5. Conclusion

To conclude, though study was conducted in short time it has highlighted the alarming resistance pattern in salmonella. NARST isolates and quinolone resistance are at peak, hence drug holiday or recycling of the antibiotics to be considered. The return of sensitivity to Ampicillin and Augmentin is good for OPD patients at peripheral hospitals. Azithromycin has showed good susceptibility hence it can also be used for OPD uncomplicated patients. In case of admitted patients we recommend to go with cephalosporins. There is periodic requirement of studies on susceptibility pattern along with details of resistant gene association. There is also requirement of drug holiday and antibiotic roatation to reduce the antibiotic resistance in salmonella.

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