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"Prevalence and Antibiogram of *Salmonella species* isolated from blood specimen at a tertiary care hospital

Abstract:

Background: This retrospective study aimed to evaluate the prevalence and antimicrobial susceptibility patterns of *Salmonella* species isolated from blood specimens at a tertiary care hospital between September 2022 and September 2024.

Methods: Blood culture was done by automated system (BacT/ALERT, Biomerieux). Identification, antibiotic susceptibility and MIC value were done with the help of Vitek-2 compact (Biomerieux System). AST is also done by Conventional method (Kirby-Bauer's Disk diffusion) for some Antibiotics like Ampicillin, Azithromycin (only for *S.typhi*) and Chloramphenicol. Slide agglutination test using specific antisera (Sifin diagnostics gmbh) was also done to confirm the serotype.

Results: A total of 2045 blood cultures were processed, yielding 90 isolates of *Salmonella*, including 73 (89.36%) *Salmonella typhi*, 15 (16.67%) *Salmonella Paratyphi A*, and 2 (2.12%) *Salmonella enterica*. In our study out of total 90 isolates of salmonella, 22 (24.45%) isolates are MDRO and 68 (75.55%) isolates are non-MDRO. Among these, *Salmonella typhi* showed high susceptibility to amoxicillin/clavulanic acid (100%), ertapenem (100%), and meropenem (98.6%), with 0% susceptibility 64% intermediate & 36% resistance to ciprofloxacin. *Salmonella Paratyphi A* demonstrated 100% susceptibility to cefepime, ertapenem, imipenem, and colistin, but 0% susceptibility &100% resistance to ciprofloxacin. A notable decrease in susceptibility to fluoroquinolones, particularly ciprofloxacin, was observed. Third-generation cephalosporins, such as ceftriaxone, retained efficacy, showing

89% susceptibility for *S. typhi* and 73.5% for *S. Paratyphi A.* Carbapenems and colistin were found effective for multidrug-resistant infections.

Conclusion: This study highlights increasing antimicrobial resistance in *Salmonella typhi* and *Salmonella paratyphi A*, with reduced effectiveness of ciprofloxacin, in both *S. Typhi* and *S. Paratyphi A.* Third-generation cephalosporins like ceftriaxone remain effective. Amoxicillin/clavulanic acid and piperacillin/tazobactam are good empirical choices, with de-escalation based on susceptibility testing. Carbapenems should be used cautiously in severe cases, and colistin is effective against multidrug-resistant strains. The findings stress the need for continuous resistance monitoring and updated treatment guidelines to ensure appropriate antibiotic use and limit resistance.

Key words: Enteric fever/ Typhoid fever, Salmonella species, AST of Salmonella species, Blood culture

Introduction:

Salmonella species is a gram-negative, rod-shaped, facultative anaerobic bacteria that belongs to the family of Enterobacteriaceae and only humans are the reservoir for it. Typhoid fever, also known as enteric fever, is a potentially fatal systemic infection caused mainly by *Salmonella enterica serovar typhi* (*Salmonella typhi*). Typhoid is a widely occurring bacterial infection found around the world. People living in low- and middle-income countries are especially at higher risk of contracting it. In South Asia, every year more than 7 million people are infected, with a death rate of 10%. (1,2,3)

Enteric fever is a significant public health issue globally and is commonly found in low- and middle-income countries, such as India. Typhoid fever and Paratyphoid fever, which are both life-threatening illnesses, are caused by *Salmonella typhi* and *SalmonellaParatyphi A*, respectively. (3,4)

Typically, it spreads through contaminated food or water. *Salmonella typhi* multiplies and spreads through the bloodstream, affecting various organs in the body. The disease's signs and symptoms are likely to appear gradually, one to three weeks after contact. (5,6)

Enteric fever is mainly spread through the fecal-oral route and presents with a range of symptoms, including fatigue, fever, chills, nausea, abdominal pain, a temporary rash, and enlargement of the liver and spleen. In spite of increased sanitation, personal hygiene, and availability of effective treatment, enteric fever remains as a serious health problem in developing countries. (6)

Early disease management can be aided by quick diagnosis, and precise antibiotic susceptibility testing guiding the treatment protocol. Empirical therapy is usually followed when laboratory confirmation is not done in many out patients' setup. Typhoid fever morbidity and mortality have decreased dramatically in industrialised countries as a result of improved housing conditions and the use of drugs. The management of cases are hampered due to emerging of drug resistance of isolates because of rampant and misuse of antibiotics. (7)

Various methods are available for blood culture for isolation of *Salmonella typhi*and *Salmonella Paratyphi A* such as conventional methods, semiautomated methods and automated methods. Automated method is the best of them, like BacT/Alert. Conventional blood culture methods often yield poor results because of low bacterial load and increased chance of contamination.

Blood culture is most relevant in the first to third week from the onset of the illness. Isolation, prompt identification and accurate antibiotic susceptibility test helps in timely management of the illness. (7, 8)

Chloramphenicol, ampicillin, and cotrimoxazole were once the primary treatments for managing enteric fever. However, strains that are resistant to these commonly prescribed antibiotics have emerged. Currently, cephalosporins and macrolides are the preferred treatments for enteric fever. However, the growing resistance to these medications has become a challenge in developing countries. Therefore, surveillance of susceptibility patterns guides clinical management at the local level. (9)

The purpose of the present study is to see the prevalence and antibiotic susceptibility pattern of *Salmonella species* isolated from blood specimen by automated blood culture system.

AIM &OBJECTIVES:-

To evaluate the prevalence of *Salmonella species* in Blood specimen. To Study the antibiotic susceptibility pattern of *Salmonella species*.

Material and Methods:

The study was carried out after receiving approval from the institutional ethics committee. A retrospective time bound study was carried out by taking relevant data, maintained over a period from September 2022 to September 2024. Blood samples received in department for culture and susceptibility during this period and processed as per standard protocol were included in this study. (25) *Salmonella species* isolated from Blood culture during study period were included. Duplicate isolates from same patients or specimen were excluded. Blood culture bottle incubated in an automated system (BacT/ALERT, Biomerieux) for microbial detection. Sub-cultures were done on blood agar and MacConkey agar from positively flagged culture bottles. Blood culture bottles were reported as sterile after 5 days if there is no growth. After overnight incubation, MacConkey agar colony morphology was observed. After observation Gram staining and oxidase test was done from NLF colonies. If colony suggestive of gram-negative bacteria and gives Non lactose fermenting and oxidase negative colony were processed for identification, antibiotic susceptibility and MIC value with the help of Vitek-2 compact (Biomerieux System). AST is also done by Conventional method (Kirby-Bauer's Disk diffusion) for some Antibiotics like Ampicillin, Azithromycin (only for *S. typhi*) and Chloramphenicol because these Antibiotics are not available in Vitek's GN AST card (N405).Slide agglutination test using specific antisera (Sifin diagnostics gmbh, Germany) was also done to confirm the serotype. Lastly the data of *Salmonella* species identified on Vitek-2 compact system along with its AST pattern will be compiled and analysed to know the prevalence of various *Salmonella* species and their AST pattern.

Results

During the study period, a total of 2045 blood culture specimens were received from the patients suspected for enteric fever and PUO, out of which 90 isolates of *Salmonella* were obtained. Among these isolates, 82.20% were from paediatrics and 17.80% were from adults. Out of the total 90 isolates of *Salmonella*, 73 (89.36%) were *Salmonella typhi*, 15 (4.25%) were *Salmonella Paratyphi A*, and 2 (2.12%) were *Salmonella enterica*. (Fig.1)

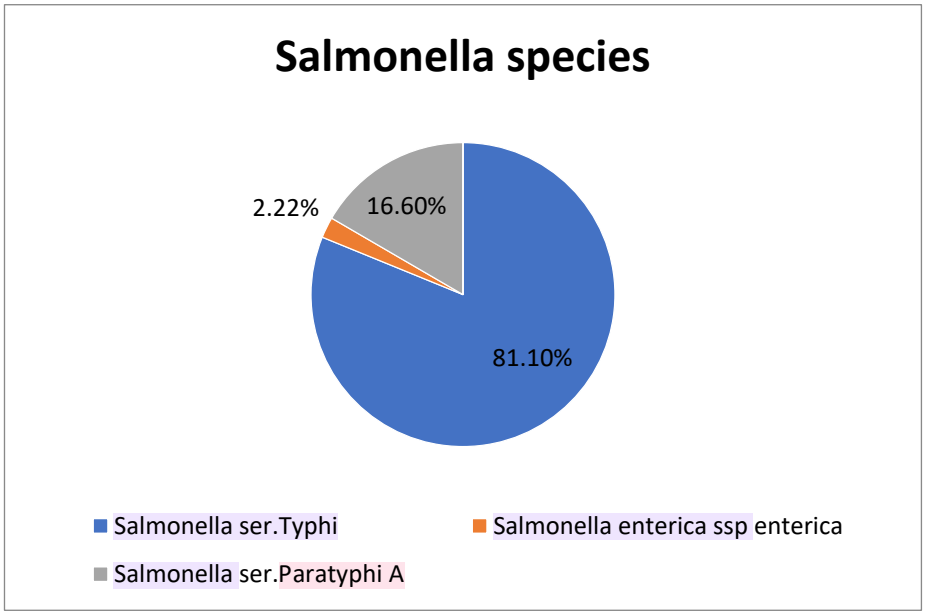

Figure 1. Species wise distribution of *Salmonella species.*

In our study out of total (90) isolates of salmonella, 22 (24.45%) isolates are MDRO and 68 (75.55%) isolates are non-MDRO. In *Salmonella serotype Typhi* (73), 14 (19.18%) isolates are MDRO and 59 (80.82%) isolates are non-MDRO. Out of 15 isolates of *Salmonella serotype Paratyphi A*, 6 (40%) isolates are MDRO and 9 (60%) isolates are non-MDRO. Out of 2 isolates of *Salmonella enterica ssp. Enterica*, 2 (100%) isolates are MDRO. (Table.1)

Table No. 1: MDRO *Salmonella species*

Salmonella species (n=90)	MDRO	Non-MDRO	
Salmonella serotype Typhi (n=73)	14(19.18%)	59(80.82%)	$\chi 2$=5.81 p-value ≈ **0.016**
Salmonella serotype Paratyphi A (n=15)	6(40%)	9(60%)	$\chi 2$=2.36 p-value ≈ **0.124**
Salmonella enterica ssp. Enterica(n=2)	2(100%)	0(0%)	$\chi 2$=6.37 p-value ≈ **0.012**
Total (n=90)	22(24.45%)	68(75.55%)	-

- ***Salmonella Typhi**:* Significant association ($p < 0.05$).
- ***Salmonella Paratyphi A***: No significant association ($p > 0.05$).
- ***Salmonella enterica***: Significant association ($p < 0.05$).

Salmonella typhi showed maximum susceptibility to Amoxicillin/Clavulanic Acid(100%), Ertapenem(100%) followed by Imipenem(98.6%), Meropenem(98.6%), Cefepime (98.6%), Trimethoprim/ Sulfamethoxazole (98.6%), Piperacillin/Tazobactam (97.2%), Chloramphenicol (95%), Cefoperazone/Sulbactam (94.5%), Colistin (91.7%), Ceftriaxone (89%), Ampicillin (87.5%) and Azithromycin (58.5%). *Salmonella typhi* showed 0% susceptibility, 64% Intermediate and 36% Resistance to Ciprofloxacin.

Salmonella Paratyphi A showed 100% susceptibility to Cefepime, Ertapenem, Imipenem, Colistin and Trimethoprim/ Sulfamethoxazole followed by Piperacillin/Tazobactam (93.5%), Meropenem (86.5%), Ampicillin (80%), Ceftriaxone (73.5%), Amoxicillin/Clavulanic Acid (73.5%), Chloramphenicol (68.66%), Cefoperazone/Sulbactam (53.3%). *Salmonella Paratyphi A* showed 0% susceptibility & 100% resistance to Ciprofloxacin.

3 As we had only two isolate of *Salmonella enterica ssp. Enterica* hence not much conclusion can be drawn from these results.

3 Antibiogram of common antibiotics for all the two strains are depicted in Table 2.

Table No. 2: AST pattern of *Salmonella* species

Antibiotics	*Salmonella serotype Typhi* (n=73)			*Salmonella serotype Paratyphi A* (n=15)		
	S (%)	*I* (%)	*R* (%)	*S* (%)	*I* (%)	*R* (%)
Ampicillin	64(87.5%)	3(4.20%)	6(8.30%)	12(80%)	1(6.66%)	2(13.33%)
Ciprofloxacin	0	47(64%)	26(36%)	0	0	15(100%)
Trimethoprim/ Sulfamethoxazole	72(98.6%)	0	1(1.4%)	15(100%)	0	0
Ceftriaxone	65(89%)	1(1.4%)	7(9.6%)	11(73.5%)	0	4(26.5%)
Azithromycin	43(58.5%)	1(1.5%)	29(40%)	-	-	-
Ertapenem	73(100%)	0	0	15(100%)	0	0
Imipenem	72(98.6%)	0	1(1.4%)	15(100%)	0	0
Meropenem	72(98.6%)	0	1(1.4%)	13(86.5%)	2(13.5%)	0
Chloramphenicol	68(95%)	2(3.07%)	3(4.10%)	13(68.66%)	1(6.7%)	1(6.7%)
Cefepime	72(98.6%)	SDD=1 (1.4%)	0	15(100%)	0	0
Amoxicillin/Clavulanic Acid	73(100%)	0	0	11(73.5%)	4(26.5%)	0
Piperacillin/Tazobactam	71(97.2%)	0	2(2.8%)	14(93.5%)	0	1(6.5%)
Cefoperazone/Sulbactam	69(94.5%)	3(4.10%)	1(1.4%)	8(53.3%)	2(13.3%)	5(33.3%)
Colistin	67(91.7%)	6(8.3%)	0	15(100%)	0	0

Discussion

Salmonella Typhi and *Salmonella Paratyphi A* are the main pathogens responsible for enteric fever.Changing trends of antimicrobial susceptibility pattern has been observed throughout different geographic regions of India which mandates constant surveillance and evaluation. (2)

The drug resistance in Enteric fever is considered one of the important factors in the morbidity and mortality from the disease. Ceftriaxone,Azithromycinand Ciprofloxacinhave been the main drugs used for treatment. There has been a wide variation in susceptibility to Ceftriaxone (MIC ≤1 mg/ml). In our study, susceptibility to Ceftriaxone was observed to be around 89% for *Salmonella typhi*. (2)

Our study has only 90 cases of typhoid fever (4.5%) out of 2045 blood samples processed during the study period. A total of 90 isolates of *S. Typhi* (73), *S. Paratyphi A* (15) and *Salmonella enterica ssp. Enterica* (2) were obtained by blood culture from suspected cases of enteric fever and PUO, giving an overall per cent positivity of 4.5. Almost 82.20 per cent of isolates were from paediatric population (55.40% boys and 44.60% girls). 17.80 per cent of isolates were from adults, among adults, 62.5 per cent were male whereas 37.5 per cent were female.

In our study out of total (90) isolates of salmonella, 22 (24.45%) isolates are MDRO and 68 (75.55%) isolates are Non-MDRO. In *Salmonella serotype Typhi* (73), 14 (19.18%) isolates are MDRO and 59 (80.82%) isolates are Non-MDRO. Out of 15 isolates of *Salmonella serotype Paratyphi A*, 6 (40%) isolates are MDRO and 9 (60%) isolates are Non-MDRO. Out of 2 isolates of *Salmonellaenterica ssp. Enterica*, 2 (100%) isolates are MDRO.

A fairly good susceptibility pattern was observed for third generation of Cephalosporins.*Salmonella typhi* shows 89% and *Salmonella Paratyphi A* shows

73.5%susceptibility to Ceftriaxone.In study done by Charu Jain et al. [1] stated that 100% of *Salmonella typhi* and 100% of *Salmonella Paratyphi A* isolates were reported susceptible to Ceftriaxone.In study done by Md. Badrul Islam et al. [5] stated that 91.95% of *Salmonella typhi* and 63.33% of *Salmonella Paratyphi A* isolates were reported susceptible to Ceftriaxone.In study done by Anu Maharjan et al. [22] stated that 95% of *Salmonella typhi* and 100% of *Salmonella Paratyphi A* isolates were reported susceptible to Ceftriaxone.

In our study *Salmonella typhi* showed 58.5% susceptibilityto Azithromycin.In study done by Charu Jain et al. [1] stated that 76.31% of *Salmonella typhi* isolates were reported susceptible to Azithromycin. In study done by Md. Badrul Islam et al. [5] stated that 45.98% of *Salmonella typhi* isolates were reported susceptible to Azithromycin.

In our study *Salmonella typhi* showed 87.5% and *Salmonella Paratyphi A* shows 73.5%susceptibilityto Ampicillin. In study done by Charu Jain et al. [1] stated that 72.30% of *Salmonella typhi* and 100% of *Salmonella Paratyphi A* isolates were reported susceptible to Ampicillin.

In our study *Salmonella typhi* showed 100% and *Salmonella Paratyphi A* shows 73.5%susceptibilityto Amoxicillin/Clavulanic Acid. In study done by Md. Badrul Islam et al. [5] stated that 81.22% of *Salmonella typhi* and 75.5% of *Salmonella Paratyphi A* isolates were reported susceptible to Amoxicillin/Clavulanic Acid.

In our study *Salmonella typhi* showed 97.2% and *Salmonella Paratyphi A* shows 93.5% susceptibility to Piperacillin/Tazobactam. In study done by Anu Maharjan et al. [22] stated that 95% of *Salmonella typhi* and 100% of *Salmonella Paratyphi A* isolates were reported susceptible to Piperacillin/Tazobactam. In study done by Md. Badrul Islam et al. [5] stated that 80.84% of *Salmonella typhi* and 53.33% of *Salmonella Paratyphi A* isolates were reported susceptible to Piperacillin/Tazobactam.

In the current study *Salmonella* isolates showed a decreased susceptibility towards FQ (**FLUOROQUINOLONES)**, especially Ciprofloxacin. As amongst *Salmonella typhi* isolates, 0% susceptible, 64% intermediate &36% of them were resistant and *Salmonella Paratyphi A* showed 0% susceptibility &100% Resistance to Ciprofloxacin. whereas all the isolates of *Salmonella enterica* were resistant to Ciprofloxacin. In study done by Upasana Bhumbla et al. [23] stated that 34% of *Salmonella typhi* and 50% of *Salmonella Paratyphi A* isolates were reported susceptible to Ciprofloxacin. In study done by Charu Jain et al. [1] stated that 29.23% of *Salmonella typhi* and 25% of *Salmonella Paratyphi A* isolates were reported susceptible to Ciprofloxacin. In study done by Anu Maharjan et al. [22] stated that 34% of *Salmonella typhi* and 0% of *Salmonella Paratyphi A* isolates were reported susceptible to Ciprofloxacin. Therefore, ciprofloxacin can no longer be considered to be the keystone for treatment.

In our study *Salmonella typhi* showed susceptibility to Ertapenem (100%)followed by Imipenem (98.6%), Meropenem (98.6%), Cefepime (98.6%), Trimethoprim/ Sulfamethoxazole (98.6%), Cefoperazone/Sulbactam (94.5%) and Colistin (91.7%).*Salmonella Paratyphi A* showed 100% susceptibility to Cefepime, Ertapenem, Imipenem, Colistin and Trimethoprim/ Sulfamethoxazole followed by Meropenem (86.5%), Cefoperazone/Sulbactam (53.3%). Results of Colistin are interpreted from the EUCAST guidelines.

In our study AST is also done by Conventional method (Kirby-Bauer's Disk diffusion) for some Antibiotics like Ampicillin, Azithromycin, and Chloramphenicol because these Antibiotics are not available in Vitek's GN AST card (N405). *Salmonella typhi* showed 95% susceptibility to Chloramphenicol followed by Ampicillin(87.5%) and Azithromycin (58.5%). *Salmonella Paratyphi A* showed 80%susceptibility to Ampicillin and 68.66% to Chloramphenicol.

In our study out of all *Salmonella* isolates two isolates of *Salmonella typhi* are showing resistance to Carbapenems.

The susceptibility of the isolates to Ampicillin, ceftriaxone and azithromycin is encouraging, as these antimicrobials are commonly used to treat *Salmonella* bloodstream infections. However, the high rates of resistance to other antimicrobials emphasize the need for ongoing antimicrobial susceptibility testing (AST) and careful selection of antimicrobials for treatment. The use of broad-spectrum antimicrobials, such as carbapenems, should be reserved for severe cases of *Salmonella* bloodstream infections, and their use should be guided by the results of AST.

However, increasing resistance can cause difficulty in clinical management. Therefore, AST data survey and Antimicrobial Stewardship policies are need of the hour to control Typhoid related morbidity and mortality.

Conclusion:

This study highlights the evolving antimicrobial resistance patterns of *Salmonella typhi* and *Salmonellaparatyphi A*, which are the primary causative agents of enteric fever. The findings indicate a significant decline in the efficacy of ciprofloxacin, with high resistance observed, particularly in *S. paratyphi A*. Conversely, third-generation cephalosporins, such as ceftriaxone, continue to show considerable effectiveness, with 89% susceptibility in *S. Typhi* and 73.5% in *S. paratyphi A*. Amoxicillin/Clavulanic Acid andPiperacillin/Tazobactam are showing good susceptibility to *Salmonella* species so we can use it as empirical therapy and de-escalation or escalation should be done after susceptibility report. Carbapenems could be considered in severe cases but should be used cautiously to avoid resistance development. Colistin is often reserved for multidrug-resistant infections, showed good efficacy against

both *S. Typhi* (91.7%) and *S. Paratyphi A* (100%). This confirms its potential as a treatment option for resistant infections.

The overall high rates of resistance to fluoroquinolones, combined with the emerging resistance to last-line antibiotics, underline the need for continuous surveillance of antimicrobial susceptibility. The study emphasizes the importance of implementing antimicrobial stewardship programs, guided by regular susceptibility testing, to ensure the appropriate selection of antibiotics and limit the spread of resistance. These findings call for an urgent need to reassess treatment guidelines for enteric fever, particularly in light of the increasing resistance to commonly used antibiotics, to minimize the impact of this disease on public health.From this study we suggest that antibacterial treatment should be carefully selected according to serotype and antimicrobial sensitivity results. Antimicrobial resistance monitoring for multi-drug resistant Salmonella is still required.

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