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RESEARCH ARTICLE

"PREVALENCE AND ANTIBIOGRAM OF *SALMONELLA* SPECIES ISOLATED FROM BLOOD SPECIMEN AT A TERTIARY CARE HOSPITAL"

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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 testing and MIC value were done with the help of Vitek-2 (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. To confirm the serotype, we had performed slide agglutination test using specific antisera (Sifin diagnostics gmbh, Germany).

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

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need for continuous resistance monitoring and updated treatment guidelines to ensure appropriate antibiotic use and limit resistance.

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Introduction:-

Salmonella species are gram-negative, rod-shaped, facultative anaerobic bacteria that belong to the Enterobacteriaceae family, with humans being the only known reservoir. Typhoid fever, also called enteric fever, is a serious systemic infection primarily caused 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, more than 7 million people are infected yearly with a death rate of 10%. (1,2,3)

Enteric fever is a major public health concern worldwide, particularly in low- and middle-income countries like India. Typhoid fever and Paratyphoid fever, both serious and potentially fatal diseases, are caused by *Salmonella Typhi* and *Salmonella Paratyphi A*, respectively. (3,4)

Typically, enteric fever spreads through contaminated food or water. *Salmonella Typhi* multiplies and enters the bloodstream, impacting various organs in the body. Symptoms usually develop gradually, within one to three weeks after exposure. (5,6)

Enteric fever primarily spreads through the fecal-oral route and is characterized by symptoms such as fatigue, fever, chills, nausea, abdominal pain, a temporary rash, and enlargement of the liver and spleen. In developing countries, enteric fever remained as a serious problem in spite of increased sanitation, personal hygiene, and availability of effective treatment. (6)

Early disease management can be done by early diagnosis, and precise antibiotic susceptibility testing guiding the treatment protocol. In outdoor patient setup practice of empirical therapy is usually followed when laboratory confirmation is not done. Improved housing conditions & use of drug had dramatically decreased morbidity and mortality of typhoid fever in developed countries. Practice of rampant & misuse of antimicrobial agents leads to emergence of drug resistance & hampered treatment of typhoid fever. (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 culture method is one of the best methods, like BacT/Alert. Conventional blood culture methods mostly yield poor results because of low bacterial load and there is increased chance of contamination.

Blood culture is the most relevant test advised in the first to third week from the onset of the illness. Isolation and correct identification of the organism 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, the strains which 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:-

Study was conducted after obtaining permission 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 Vitek2 (Biomerieux System). AST is also done by Conventional method (Kirby-Bauer's Disk diffusion) for some Antibiotics like Ampicillin (10µg), Azithromycin (15µg) (only for *S. Typhi*) and Chloramphenicol (30µg) because these Antibiotics are not available in Vitek's GN AST card (N405). Susceptibility Test (AST) was interpreted by using CLSI guidelines. [13, 26] Results of Colistin are interpreted from the EUCAST guidelines. Slide agglutination test using specific antisera (Sifin diagnostics gmbh, Berlin, Germany) was also done to confirm the serotype. Lastly the data of *Salmonella* species identified on Vitek2 system along with its AST pattern will be compiled and analysed to know the prevalence of various *Salmonella* species and their AST pattern.

Results:-

In this study, a total of 2045 blood culture samples were collected from patients suspected of having enteric fever or prolonged fever of unknown origin (PUO), From these, 90 *Salmonella* isolates were identified. Out of total 90 isolates, 82.20% were from paediatric patients and 17.80% were from adults. Among the 90 *Salmonella* isolates, 73 (81.11%) were *Salmonella Typhi*, 15 (16.66%) were *Salmonella Paratyphi A*, and 2 (2.22%) were *Salmonella enterica*. (Fig.1)

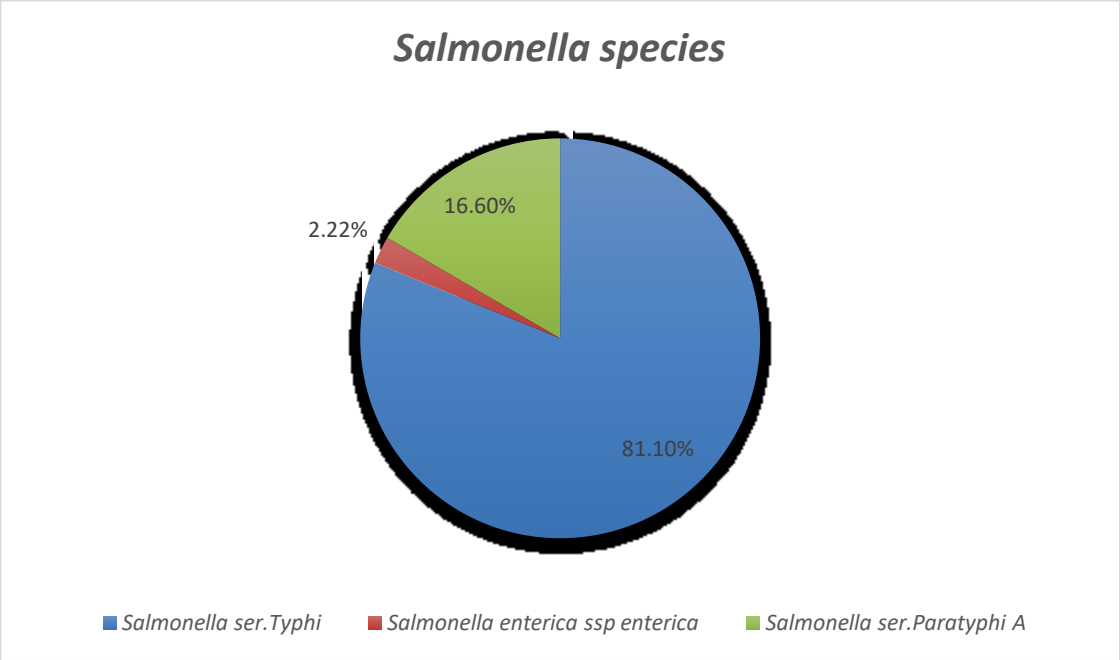

Figure 1:- Species wise distribution of *Salmonella* species.

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**	**Chi-squared & p-value**
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.

As we had only two isolate of *Salmonella*enterica ssp. Enterica hence we could not much conclude much about these results. The antibiotic susceptibility patterns of common antibiotics for the two strains are shown 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:-

The primary pathogens causing enteric fever are *Salmonella Typhi* and *Salmonella Paratyphi A*. There has been a noticeable shift in the antimicrobial resistance patterns across various regions of India, highlighting the need for ongoing monitoring and assessment.(2)

Drug resistance in enteric fever is a significant factor contributing to the morbidity and mortality associated with the disease. Ceftriaxone, Azithromycin and Ciprofloxacin have been the main drugs used for treatment. There has been significant variation in the susceptibility to Ceftriaxone (MIC ≤ 1 mg/ml). In our study, approximately 89% of *Salmonella Typhi* strains were found to be susceptible to Ceftriaxone. (2)

In our study, 90 cases of typhoid fever (4.5%) were identified from 2045 blood samples processed during the study period. A total of 90 isolates were obtained, including 73 of *S. Typhi*, 15 of *S. Paratyphi A*, and 2 of *Salmonella enterica ssp. Enterica*, through blood cultures from suspected cases of enteric fever and PUO. This resulted in an overall positivity rate of 4.5%. About 82.2% of the isolates were from the pediatric population, with 55.4% boys and 44.6% girls.Among the isolates, 17.8% were from adults. Within this group, 62.5% were male and 37.5% 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 *Salmonella enterica ssp. Enterica*, 2 (100%) isolates are MDRO.

A good susceptibility pattern was observed for third-generation cephalosporins. *Salmonella Typhi* shows 89% and *Salmonella Paratyphi A* shows 73.5% susceptibility to Ceftriaxone. In study done by Charu Jain et al. [1] It was observed that all isolates of *Salmonella Typhi* and *Salmonella Paratyphi A* were completely susceptible to Ceftriaxone. In study done by Md. Badrul Islam et al. [5] it was reported that 91.95% of *Salmonella Typhi* isolates and 63.33% of *Salmonella Paratyphi A* isolates were susceptible to Ceftriaxone. In study done by Anu Maharjan et al. [22] it was found that 95% of *Salmonella Typhi* isolates and 100% of *Salmonella Paratyphi A* isolates were susceptible to Ceftriaxone.

In our study *Salmonella Typhi* showed 58.5% susceptibility to Azithromycin. In the study conducted by Charu Jain et al. [1], it was found that 76.31% of *Salmonella Typhi* isolates were susceptible to Azithromycin. In study done by Md. Badrul Islam et al. [5] it was reported that 45.98% of *Salmonella Typhi* isolates were susceptible to Azithromycin.

In our study *Salmonella Typhi* showed 87.5% and *Salmonella Paratyphi A* shows 73.5%susceptibility to Ampicillin. In the study by Charu Jain et al. [1], it was observed that 72.30% of *Salmonella Typhi* isolates and 100% of *Salmonella Paratyphi A* isolates were susceptible to Ampicillin.

In our study *Salmonella Typhi* showed 100% and *Salmonella Paratyphi A* shows 73.5% susceptibility to Amoxicillin/Clavulanic Acid. The study by Md. Badrul Islam et al. [5] reported that 81.22% of *Salmonella Typhi* isolates and 75.5% of *Salmonella Paratyphi A* isolates were 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 the study by Anu Maharjan et al. [22], it was found that 95% of *Salmonella Typhi* isolates and 100% of *Salmonella Paratyphi A* isolates were susceptible to Piperacillin/Tazobactam. In the study by Md. Badrul Islam et al. [5], it was reported that 80.84% of *Salmonella Typhi* isolates and 53.33% of *Salmonella Paratyphi A* isolates were susceptible to Piperacillin/Tazobactam.

In the current study, *Salmonella* isolates demonstrated decreased susceptibility to fluoroquinolones (FQs), particularly Ciprofloxacin. Among the *Salmonella Typhi* isolates, 0% was susceptible, 64% were intermediate, and 36% were resistant. For *Salmonella Paratyphi A*, none of the isolates were susceptible, and 100% were resistant to Ciprofloxacin. Whereas all the isolates of *Salmonella enterica* were resistant to Ciprofloxacin. In the study by Upasana Bhumbla et al. [23], it was found that 34% of *Salmonella Typhi* isolates and 50% of *Salmonella Paratyphi A* isolates were susceptible to Ciprofloxacin.In the study by Charu Jain et al. [1], it was reported that 29.23% of *Salmonella Typhi* isolates and 25% of *Salmonella Paratyphi A* isolates were susceptible to Ciprofloxacin. In the

study by Anu Maharjan et al. [22], it was found that 34% of *Salmonella Typhi* isolates and none of the *Salmonella Paratyphi A* isolates were susceptible to Ciprofloxacin. Therefore, ciprofloxacin can no longer be regarded as the primary treatment option.

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, the growing resistance may complicate clinical management. Therefore, regular AST data monitoring and the implementation of Antimicrobial Stewardship policies are essential to control typhoid-related morbidity and mortality.

Conclusion:-

This study examines the changing patterns of antimicrobial resistance in *Salmonella Typhi* and *Salmonella Paratyphi A*, the main pathogens responsible for 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 consistently high resistance rates to fluoroquinolones, along with the rise in resistance to last-line antibiotics, emphasize the need for ongoing 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. Based on this study, we recommend that antibacterial treatment be carefully chosen based on the serotype and antimicrobial sensitivity results. Ongoing monitoring of antimicrobial resistance in multi drug-resistant *Salmonella* is essential.

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