RESEARCH ARTICLE OPEN ACCESS

Exploring the Resistance Pattern of Salmonella Typhoidal Species

Rehana F. Mehdi*, Zona Irfan, Nazia Qamar , Ambreen Rehman , Nadeem Nusrat , Ali Haider
Department of Pathology, Fazaia Ruth Pfau Medical College, Karachi, Pakistan

ARTICLE HISTORY

Received: April 04, 2024
Revised: July 19, 2024
Accepted: July 19, 2024

Citation: Mehdi FR, Irfan Z, Qamar N, Rehman A, Nusrat N, Hiader A. Exploring the resistance pattern of salmonella typhoidal species. Acad Res. 2024; 1(1): 6-10.

DOI: https://doi.org/10.70349/ar.v1i1.5

Abstract

***Background*:** Enteric fever/ typhoid, an infection caused by *Salmonella enterica* serovars: typhi and paratyphi, is continuously increasing with the emergence of antibiotic resistant Salmonella. According to WHO, an estimated 110,000 people die from it every year.

***Objective*:** The primary objective of this study was to investigate the antibiotic susceptibility pattern of typhoidal Salmonella species in blood cultures of patients.

***Methodology*:** This was a cross-sectional study, executed at Microbiology Laboratory (Diagnostic Centre), PAF Hospital Faisal Base Karachi, from August 2023 to March 2024. *Salmonella typhi* in all clinically suspected cases of typhoid fever was identified through conventional techniques following standard microbiological protocols. Assessment of antibiotic susceptibility was performed by Kirby-Bauer disk diffusion method.

***Results*:** Out of 1178 blood samples culture 54 were gram positive bacteria, 193 were gram negative bacteria and 931 samples showed no growth. Among the gram-negative sample, 140 tested positive for *Salmonella enterica* typhoidal species. API 20E was used to further differentiate and identify the Salmonella species, 131 cases were *Salmonella typhi* and 9 paratyphi. Out of 140 Salmonella isolates, 77% (108) were resistant to ampicillin, 49.2% (69) to Ceftriaxone, 35% (49) to Sulfamethoxazole, and 47% (66) to Ciprofloxacin. Azithromycin and Meropenem remained sensitive in all 140 isolates of Salmonella typhoidal species.

***Conclusion*:** This study identified the susceptibility profiles of Salmonella typhoidal species. Our findings underscore the importance of continuous surveillance and judious use of antibiotics to combat the emergence of antibiotic –resistant strains. Further research is warranted to continuously monitor and adapt treatment protocols in response to evolving resistance in Salmonella typhoidal species.

Keywords: Gram positive bacteria, Gram negative bacteria, frequency, *Salmonella typhi*, *E. coli.*

1. INTRODUCTION

Salmonella typhi, also known as *S. typhi*, is classified as a gram-negative enteric bacillus that falls within the family Enterobacteriaceae. Infections caused by Salmonellae can manifest in a variety of clinical presentations such as enteric fever, gastroenteritis, septicemia, and occasionally suppurative lesions. The transmission of this bacterium is typically linked to the consumption of food or water contaminated by individuals who are carriers excreting *Salmonella enterica* serotype typhi [1]. Global estimates indicate that approximately 22 million new cases of typhoid fever occur annually, resulting in around 200,000 deaths. The regions with the highest rates of morbidity and mortality are Africa, South Central and Southeast Asia, with Pakistan demonstrating the second highest incidence of typhoid fever at an estimated annual rate of 412.9 cases per 100,000 individuals [2]. A surveillance project focusing on the incidence of typhoid fever in low and middle-income nations like Bangladesh, Nepal, and Pakistan highlighted Pakistan as having notably elevated rates of typhoid fever within the region [3].

The trends in resistance patterns of *Salmonella typhi* species indicate a concerning rise in antimicrobial resistance (AMR) globally. Studies show that multidrug-resistant (MDR) strains are prevalent, with some regions reporting extensively drug-resistant (XDR) strains [4] Pakistan demonstrates 16% Multi drug resistant cases while 54% extensive drug resistant Salmonella typhoidal strains [5]. According to a study done in Pakistan, *Salmonella typhi* exhibited the greatest susceptibility to imipenem at 100% and azithromycin at 95%; conversely, it displayed the lowest sensitivity towards ciprofloxacin at 3.7%. Approximately half of the patients demonstrated resistance to ceftriaxone, while 48% exhibited resistance to meropenem. The incidence of multidrug-resistant cases documented was

* Address correspondence to this author at the Department of Pathology, Fazaia Ruth Pfau Medical College Karachi, Pakistan; E-mail: faryal@frpmc.edu.pk

20%, with 47% of strains being classified as extensively drug-resistant [6]. The appearance of XDR *Salmonella typhi* in India, Pakistan, Nepal and Bangladesh has resulted in azithromycin being the sole practicable oral therapy. The identification of a mutation linked to azithromycin resistance in a single *S. typhi* strain is documented in these studies [7-10]. This discovery carries significance as the potential dissemination of azithromycin resistance among *S. typhi* strains could render outpatient treatment extremely challenging, necessitating the use of injectable antibiotics [11]. The emergence of resistance to multiple antimicrobial classes underscores the urgency for continued surveillance to guide treatment decisions. These findings emphasize the critical need for judicious antibiotic use to prevent the further spread of resistant strains and ensure effective management of typhoid fever.

2. METHODOLOGY

This was a cross sectional study conducted at the Microbiology Laboratory (Diagnostic Centre), PAF Base Faisal Hospital, Karachi. It was carried out from August 2023 to March 2024. Non-probability consecutive sampling was used for the study. Inclusion Criteria included all blood samples for culture and sensitivity from patients and outpatients of the hospital. Blood samples for culture and sensitivity showing growth other than bacteria like fungus or yeast and repeat, duplicate samples from the same patient were excluded from the study. The consent form was also signed from the patients who agreed to be part of the study. All blood cultures were collected from a peripheral vein with proper aseptic precautions before starting any antibiotic therapy. The top of the blood culture vials were disinfected with 70% isopropyl alcohol, and allowed to dry. The venipuncture site was identified and cleansed with 70% isopropyl alcohol. The QC of Blood culture bottles was done through seeded suspension method by ATCC 25922 Strain of *Escherichia coli* and *Staphylococcus aureus* ATCC 25923. 0.1 ml dilution of both organisms are prepared and injected into blood culture bottles. They are then incubated at 37°C and confirmation is done by inoculation of isolates on the blood culture medium after they reveal positive growth.

The blood culture was collected in manual blood culture bottles. The sample were gram stained and sub cultured on 3rd and 5th day on 5% Sheep Blood agar, and MacConkey agar plates. They were incubated at 37°C for 18-24 hours for bacterial isolation.

The MacConkey agar plates were incubated aerobically in an ambient atmosphere while sheep blood agar were incubated in capnophilic atmosphere (5-10 % CO_2). Identification of typhoidal species of Salmonella isolates were done according to standard microbiological techniques with their characteristic appearance on gram staining, the oxidase test, the catalase test, motility, triple-sugar iron (TSI) fermentation, and colony morphology (Growth of colorless colonies of Non lactose fermenter, round and smooth in nature) completed for the final confirmation.

Biochemical tests embedded in the analytical profile index (API 20 E) were used to differentiate between the typhi and paratyphi strains. API test kits are designed to identify Gram positive and Gram negative bacteria and yeast. The API strips contain 20 miniature biochemical tests that are rapid and easy to perform. Likewise **API (Analytical Profile Index) 20E** is designed specifically for identification of members belonging to the family Enterobacteriaceae.

Antibiotic susceptibility of the isolates was determined by Modified Kirby Bauer disc diffusion method on Muller-Hinton-agar according to CLSI recommendations. The Muller-Hinton agar plates were incubated aerobically at 35°C ± 2 for 18-24 hours. Ampicillin, Aztreonam, Cefixime, Ceftriaxone, Chloramphenicol, Trimethoprim Sulfamethaxazole, Meropenem and Azithromycin were used as the antibiotic testing panel for Salmonella species. The control strains used were *Staphylococcus aureus* ATCC-25923® and *Escherichia coli* ATCC-25922®.

3. RESULTS

A total number of 1178 blood samples were received from August 2023 to March 2024. Out of which, 54 were of gram-positive bacteria while 193 gram negative bacteria were isolated from the total numbers of blood samples received for blood culture. The samples that did not show any growth of bacteria were 931 (Table **1**).

Table1: Total number of blood cultures, GRAM +VE (GPC), GRAM –VE (GNR) and NO GROWTH.

MONTH	TOTAL NUMBER OF			
	BLOOD C/S	GRAM +VE (GPC)	GRAM –VE (GNR)	NO GROWTH
AUG 2023	195	13	23	159
SEP 2023	145	09	18	118
OCT 2023	160	14	16	130
NOV 2023	153	04	37	112
DEC 2023	114	04	18	92
JAN 2024	127	03	33	91
FEB 2024	152	06	32	114
MAR 2024	132	01	16	115
Total	**1178**	**54**	**193**	**931**

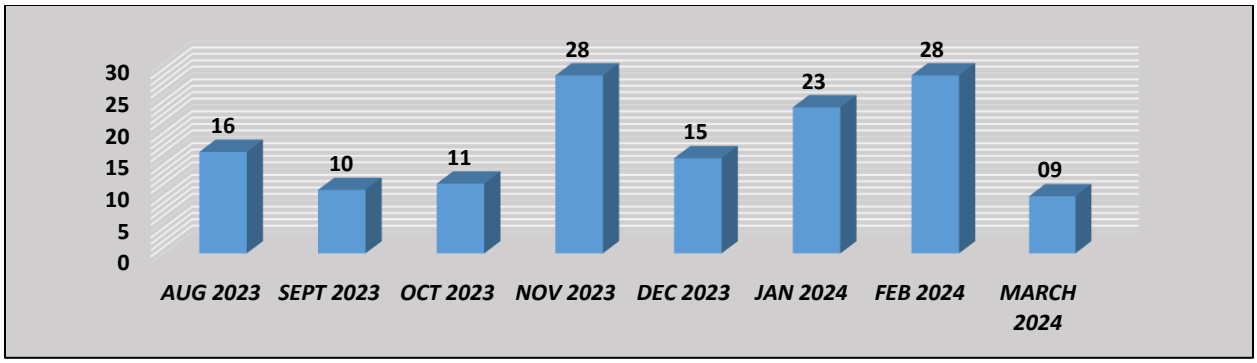

Figure 1: The monthly distribution of salmonella typhoidal species.

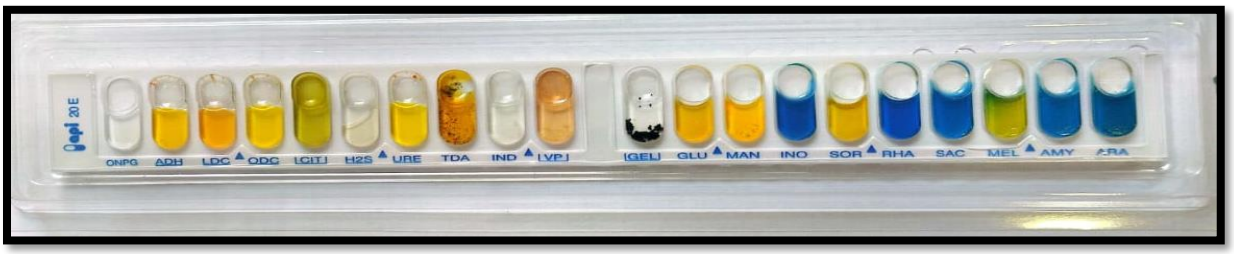

Figure 2: Biochemical reactions observed on API 20E.

In Fig. (**1**), the number of Salmonella typhoidal isolates in each month from August 2023 to March 2024 is mentioned. A total of 140 (72.5%) blood cultures were positive for Salmonella typhoidal species out of 193 gram negative bacteria.

To distinguish the *Salmonella typhi* from *Salmonella paratyphi*, API 20E was used. We found that out of 140 Salmonella isolates, 131(93.5%) were *Salmonella typhi* and 9 (6.4%) were *Salmonella paratyphi* (Fig. **2**).

Table 2: The resistance pattern of antibiotic panel of Salmonella typhoidal species.

Month	No. of *S. typhi* Isolate	AMP	CRO	SXT	CIP	MEM	AZM
AUG 2023	16	16	05	01	08	0	0
SEPT 2023	10	06	04	05	03	0	0
OCT 2023	11	09	05	04	04	0	0
NOV 2023	28	16	13	04	12	0	0
DEC 2023	15	12	08	12	10	0	0
JAN 2024	23	17	14	21	17	0	0
FEB 2024	28	25	12	02	03	0	0
MARCH 2024	09	07	08	0	09	0	0
TOTAL	**140**	**108 (77.1%)**	**69 (49.2%)**	**49 (35%)**	**66 (47.1%)**	**0**	**0**

AMP: Aampicillin; CRO: ceftriaxone; SXT: Sulfamethoxazole; CIP: Ciprofloxacin; MEM: Meropenem; AZM: Azithromycin

Our results showed among 140 isolates 77% (n=108) were resistant to ampicillin, 49.2% (n=69) were resistant to ceftriaxone, 35% (n=49) were resistant to sulfamethoxazole, and 47% (n=66) were seen to be resistant to ciprofloxacin. Our susceptibility profile highlighted no isolates were resistant to Azithromycin and Meropenem, all strains of salmonella typhoidal species showed sensitivity to Azithromycin and Meropenem.

4. DISCUSSION

The prevalence of antimicrobial-resistant bacterial infections is on the rise, resulting in a dwindling array of treatment options. Our investigation revealed a notable 72% incidence of Salmonella typhoidal isolates. In a study conducted among smoked fish vendors in the Tamale metropolis, a prevalence of 67% for Salmonella species was reported in smoked fish sold there [12]. Multiple factors contribute to the increased incidence of *S. typhi* isolates, including poor sanitation, compromised water quality, inadequate vaccination coverage, overcrowding, and, notably, antibiotic resistance. Our study underscored significant rates of enteric fever and a notable profile of Salmonella drug resistance, emphasizing the urgent need for intervention to combat antimicrobial resistance (AMR).

Our findings indicated a higher incidence of typhoid and paratyphoidal fever during November and February, with enteric fever cases occurring sporadically throughout the year, peaking during the summer and rainy seasons [13]. The shifting global climate, characterized by erratic rainfall patterns, has been implicated in instances of water flooding and sanitation challenges, potentially fostering the proliferation of Salmonella during these months. Our investigation noted a higher incidence of *S. typhi* isolates (93.1%) compared to Paratyphi *S. typhi* (6.4%), contrasting with some studies reporting a lower prevalence of Salmonella infection (47%) compared to Paratyphi infection (53%) [14]. Moreover, a study in Pakistan identified 62 Salmonella enterica isolates, with 83.87% being *Salmonella typhi* and 16.12% *Salmonella paratyphi* A [15]. Notably, typhoid fever has been linked to domestic contamination, while paratyphoid fever has been associated with flooding and contaminated food from street vendors [16], suggesting that food contamination may have influenced the patient samples in our study.

Regarding antimicrobial susceptibility, our analysis revealed that out of 140 isolates, 77% were resistant to ampicillin, 49.2% to ceftriaxone, 35% to sulfamethoxazole, and 47% to ciprofloxacin. However, all strains of Salmonella typhoidal species in our study exhibited sensitivity to both azithromycin and meropenem, (Table **2**) with no isolates demonstrating resistance to either drug. Similar resistance patterns were observed in a study conducted in Kenya, where Salmonella isolates exhibited strong resistance to ampicillin (72%), chloramphenicol (72%), and cotrimoxazole (70%), but high susceptibility to ceftriaxone (94%) and gentamicin (97%) [17].

Moreover, a study in Southern Pakistan highlighted sensitivity rates of 60.9% for cefixime, 65.8% for ceftriaxone, and 50.1% for ciprofloxacin among *S. typhi* isolates, with cases of multidrug-resistant (MDR) and extensively drug-resistant (XDR) typhoid reported [18]. Another study noted a higher prevalence of MDR strains in Iraq (83%) and Pakistan (52%) compared to other countries studied, with almost all isolates susceptible (99.7%) to ceftriaxone [19].

Our investigation of *Salmonella typhi* susceptibility patterns revealed sensitivity only to meropenem and azithromycin (Table **2**). However, Minimum Inhibitory Concentration of Azithromycin was not performed and can be taken as the limitation of this study. It further underscores the pressing need for further exploration of the factors contributing to multidrug resistance against ampicillin, ceftriaxone, and sulfamethoxazole.

The misuse of antibiotics stands as a paramount contributor to the emergence of multidrug resistance. Frequently, antibiotics are prescribed excessively for mild cases where treatment may be unnecessary. Furthermore, within the cohort receiving antibiotic treatment, the inadequate dosages and durations of prescribed courses significantly influence treatment outcomes. Concurrently, the low vaccination coverage against typhoid fever has exacerbated the prevalence of *S. typhi* infections. Consequently, the implementation of selective measures to counter multidrug resistance becomes increasingly imperative in such a context.

5. CONCLUSION

This study delineated resistance profiles of salmonella typhoidal species. Our results highlight the significance of ongoing surveillance and prudent administration of antimicrobial agents to address the rise of antibiotic-resistant strains. Additional research is necessary to consistently oversee and adjust therapeutic regimens considering the evolving resistance observed in Salmonella typhoidal species.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This study has received no financial support.

ACKNOWLEDGEMENTS

We authors acknowledge the efforts of our laboratory technicians, Samia Ehtesham and Mehak Qaisar Pervez.

AUTHOR'S CONTRIBUTION

RFM: Conception and design of the study
ZI, AR: Data collection
NQ, NN: Analysis and interpretation of the results
RFM, AH: Drafting of the manuscript

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