Original Article

Temporal Analysis of Antimicrobial Susceptibility in Salmonella: A Three-Year Surveillance Study

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Abstract

Objective: The study aimed to assess the antimicrobial susceptibility patterns of Salmonella sp. strains over a three-year period in a tertiary care hospital.

Methodology: It was a retrospective observational study. Electronic medical records were utilized for data retrieval from 2017 to 2019. The study parameters included individuals of all age groups, and genders diagnosed with typhoid based on positive blood cultures. Identification, speciation, and antimicrobial susceptibility testing adhered to standardized protocols, with statistical analysis conducted using SPSS 25.0.

Results: Among 769 Salmonella isolates, 709 cases were reported in 2019, making it the highest incidence over the study period. Extensively drug-resistant (XDR) strains peaked in 2019, comprising approximately 50% of cases, while multiple drug-resistant strains accounted for 25%. Notably, resistance to ampicillin, ciprofloxacin, co-trimoxazole, and ceftriaxone exhibited a consistent upward trend over the three-year span. Ciprofloxacin demonstrated the highest resistance, with only 4% sensitivity among Salmonella isolates.

Conclusion: The findings have highlighted a concerning escalation in antimicrobial resistance among Salmonella Typhi strains in Punjab, Pakistan, particularly evident in the prevalence of extensively drug-resistant strains. Multi-drug and extensively drug-resistant strains of Salmonella are difficult to treat and may give rise to even more drug resistance if not treated appropriately, leading to a vicious cycle of resistance.

Keywords: Typhoid fever, antimicrobial resistance, Drug-resistant strains, public health, Pakistan

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Introduction

Typhoid fever, caused by Salmonella enterica serovar Typhi, remains a persistent public health challenge, particularly in regions with inadequate sanitation and limited access to clean drinking water. The emergence of drug-resistant strains has further complicated the management of this infectious disease, making it essential to closely monitor antimicrobial susceptibility patterns.[1,2]

Antimicrobial susceptibility testing is essential for clinical management. This is especially due to multi-drug resistance developing over the years since its

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treatment with antibiotics started.[1] Chloramphenicol, Ampicillin, and Cotrimoxazole are considered first-line drugs for typhoid fever.[3] Typhoid fever causing Salmonella species became resistant to Chloramphenicol and Ampicillin in the 1970s. Cotrimoxazole-resistant species started making their appearance in the late 1980s.[4] Bacteria resistant to all three primary antibiotics were now known as multidrug-resistant strains. In the 1990s and onwards, this left the alternative drugs of Fluoroquinolones and Cephalosporins.[4,5] A 3-year review conducted from 2008 to 2011 revealed a high prevalence of antimicrobial-resistant strains at the beginning of this century. S. Typhi, for example, had Fluoroquinolone resistance in 88.2% of cases, followed by Ampicillin, Trimethoprim/sulfamethoxazole, and chloramphenicol at 66.8%, 66.5%, and 66.1%, respectively, and S. Paratyphi A, which had the highest Fluoroquinolone resistance at 83.9%. Trimethoprim/sulfamethoxazole and Ampicillin were at 83.9%, and 2.6% respectively.[6] The high incidence of typhoid in this area is caused by risk factors such as inadequate water supply, overcrowding, poor living conditions, and poor sanitation.[5,6]

The province of Punjab, Pakistan, has witnessed a troubling escalation in the prevalence of drug-resistant typhoid strains, after the initial outbreak in Sindh province in 2017.[1,7] The consequences of antimicrobial resistance are profound, as it diminishes the efficacy of conventional treatments, prolongs illness, and heightens the risk of severe complications.[7] Considering this evolving health crisis, concerted efforts have been launched to comprehensively study the antimicrobial susceptibility patterns of typhoid strains.[3]

Our goal was to provide a nuanced understanding of how Salmonella sp. strains have adapted to antimicrobial pressures over time. The findings of this study will be instrumental in shaping targeted therapeutic approaches, guiding antibiotic stewardship initiatives, and bolstering public health strategies to mitigate the burgeoning threat of antibiotic-resistant Salmonella sp. infections.

Methodology

The study adopted a cross-sectional descriptive design to analyse antimicrobial susceptibility patterns of typhoid strains in a tertiary care hospital in Punjab, Pakistan. The three-year study period spanned from 1st January 2017 to 31st December 2019. The study was approved by the Institutional Review Board at Shalamar Medical and Dental College (Approval No. SMDC-IRB/AL/59/2020).

A comprehensive dataset was compiled by extracting laboratory data from the hospital's electronic medical records. The primary focus was on data related to blood culture results, including the identification of Salmonella Typhi strains and their antimicrobial susceptibility profiles.

The blood samples were collected by venipuncture under aseptic conditions in BHI broth and then subcultured on solid medium such as MacConkey and Blood agar, after every 24 hours of incubation and subsequently, according to lab protocol. The recommended volume of blood to broth ratio is 1:5-1:10.[2,7]

The non-lactose fermenting colonies on MacConkey agar were subjected to identification following standard procedures. This included Gram staining, biochemical profile (using API 10S), and serotyping.

Isolates identified as Salmonella species were then subjected to antibiotic susceptibility testing. The Modified Kirby-Bauer disc diffusion technique was performed using eight common drugs, Ampicillin, Co-trimoxazole, Ciprofloxacin, Ceftriaxone, Chloramphenicol, and Meropenem, Colistin, and Azitrhromycin[8]. Although Colistin is not recommended in the reference, we included it in our study because it is used in other highly resistant gram-negative rods. A control strain of E. coli ATCC 25922 was included. The results of susceptibility testing were interpreted using the Clinical and Laboratory Standards Institute (2023) recommendation.[8] Multi-drug resis-

tant isolates were taken as those resistant to Ampicillin, chloramphenicol, co-trimoxazole, and ciprofloxacin. Extremely drug-resistant isolates were those in which all of the mentioned drugs with the inclusion of resistance to third-generation cephalosporins were present. The study included patients of all age groups who were diagnosed with typhoid fever based on positive blood cultures for Salmonella sp. The collected data was entered in Microsoft Excel, and subsequent graphs were also made on this software.

Results

Out of the total 769 isolates of Salmonella sp. from the predetermined three-year period, 95% (730) were S. Typhi. Most number of cases were encountered in the year 2019 (n=709). The lowest number of cases was calculated in the year 2017 (n=07). The number of females vs males is given in Table I. Generally, the number of males was greater than females, eventually infected males were more than the number of female patients except for the year 2018 (Males n=24, Females n=29). The total number of Salmonella isolates per year in blood cultures is given in table I. In this study, maximum number of cases infected were below fifteen years of age (n=654). Number of patients above 30 years of age are 38. Age-wise distribution of Salmonella sp. infected cases is given (table I).

The yearly distribution of extensively drug-resistant strains of Salmonella sp. is given in Table II. The maximum number of XDR strains was encountered in the year 2019 (n=392) followed by the year 2018. The numbers in 2017 are too low to be considered statistically significant. The yearly distribution of multidrug resistant strains of Salmonella sp. is given (Table II). The maximum number of MDR strains was calculated in the year 2019 (n=198). The antibiotic susceptibility profile of Salmonella sp. during these 3 years against Chloramphenicol, Co-trimoxazole, Ciprofloxacin, Ceftriaxone, Meropenem, Colistin, Azithromycin and Ampicillin are given (Figure 1). Salmonella species are 100% sensitive to the drugs Colistin and Meropenem in all three years. Of all the eight drugs, Ampicillin is the least sensitive with the percentage of sensitive isolates being as low as 14%, then 19% and 15% in the three years included in this study.

Table I: *Year, gender and age-wise distribution of Salmonella cases (n=769)*

	2017	2018	2019	Total
Gender				
• Male	5	24	428	457
• Female	2	29	281	312
Total	7	53	709	796
Age				
• <15	2	13	639	654
• 16-30	3	30	44	77
• >30	2	10	26	38
	7	53	709	769

Table II: *Yearly distribution of XDR and MDR strains of Salmonella sp. (n=769)*

Year	2017	2018	2019
XDR strains of *Salmonella sp.*	0 (0%)	24(45.3%)	392 (49.7%)
MDR strains of *Salmonella sp.*	0 (0%)	14(26.4%)	198 (25.1%)

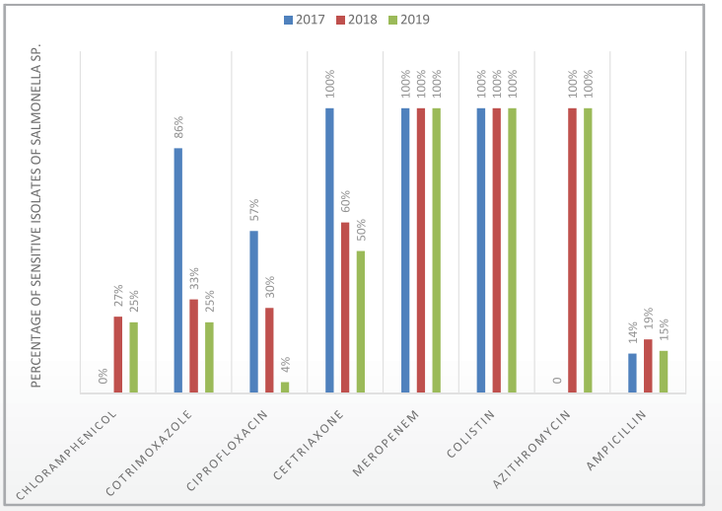

Figure 1: *Yearly distribution of Antibiotic susceptibility profile of Salmonella sp. against different drugs (n=769)*

Discussion

The results of our three-year surveillance study

revealed a concerning trend of increased antimicrobial resistance in Salmonella isolates during the years 2018 and 2019. Analysis of susceptibility patterns across the study period demonstrates a significant rise in resistance rates to multiple antimicrobial agents commonly used in the treatment of Salmonella infections. This escalation in resistance highlights a pressing issue that warrants attention and intervention to mitigate the potential consequences on public health.

There was a worrisome increase in XDR typhoid cases in 2019, from 45.3% in 2018 to about 50%. This surge may have been influenced by various factors, including antibiotic misuse, changes in disease transmission dynamics, or reduced access to healthcare as with all low to middle-income countries.[9,10] These findings are comparable to a study conducted in Hyderabad following an XDR outbreak.[1] The effects of the outbreak of XDR typhoid in Sindh reached Punjab and specifically our hospital, in 2018, as the numbers were significantly higher than in 2017. A comprehensive strategy to address the multifaceted challenges posed by XDR typhoid is required. Antibiotic resistance among Salmonella strains showed only a rising trend. Ampicillin and Co-trimoxazole showed resistance rates of 86% and 75%, respectively, at their highest.

Comparisons with other local and international studies shed light on the unique antibiotic resistance profiles in different regions. Our study found that S. typhi was the most common species isolated (92%), aligning with other local studies.[11,12,13] A research conducted in Karachi at PNS Shifa in 2019, showed the same results as our study, especially regarding Ciprofloxacin which was only 9%, susceptible compared to our 4%. This indicates that Ciprofloxacin should no longer be considered for treatment of Typhoid fever, especially in Pakistan.[14]

International studies show a discrepancy, as there are very scarce if any studies in the developed world regarding typhoid fever.[15] The studies are mostly about nontyphoidal salmonella as seen in a multinational review article in 2023 which focused on non-typhoidal salmonella in developed countries. This review shows that multidrug resistance is only 25% in these countries and there are no extensively drug-resistant strains.[16] These discrepancies emphasize the regional variations in antibiotic resistance and the importance of tailoring treatment strategies to local contexts. A multinational study conducted in Africa encompassing 6 countries showed an incidence of severe typhoid infection to be about 16%. This study which was conducted from 2016-2020 showed ciprofloxacin to be only 16.4% resistant, very different from our 96% resistant in 2019.[17]

Local studies within Pakistan have also demonstrated variable resistance patterns, with some reporting an increase in the MDR and XDR strains over the years. These differences may be influenced by factors like local antibiotic prescribing practices, vaccine coverage, and bacterial evolution.[18] The escalation of resistance presents noteworthy obstacles for clinical management, given that it curtails the efficacy of traditional antibiotic treatments and mandates the employment of substitute drugs that might possess more detrimental effects or restricted effectiveness. Moreover, the advent of Salmonella strains that are resistant to many drugs jeopardizes the efficacy of empirical treatment plans and could result in treatment setbacks, prolonged sickness, and higher medical expenses.

Collaborative efforts involving healthcare providers, policymakers, researchers, and the agricultural industry are necessary to address the complex challenges posed by antimicrobial resistance in Salmonella. By implementing comprehensive strategies that prioritize surveillance, stewardship, and infection control, we can work towards preserving the effectiveness of antibiotics and safeguarding public health against the threat of resistant bacterial infections.

Conclusion

The cases of MDR and XDR Typhoid can be seen as increasing drastically, after the outbreak in Sindh in 2017. Individual antibiotic resistance has also increased. The sensitivities of Chloramphenicol, Cotrimoxazole, Ciprofloxacin, Ceftriaxone, and Ampicillin are all 50% or below, rendering them useless for empirical therapy. Meropenem and Azithromycin are still 100% sensitive in our study.

Limitations: The study acknowledges certain limitations, including those inherent in retrospective data collection and the reliance on electronic medical records. While the findings offer valuable insights into the hospital's patient population, they may not be directly generalizable to broader populations.

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Conflict of Interest: None

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Authors Contribution

HR & MDI: Conceptualization and design of Project, literature search, Drafting and Revision.
MUN & SFD: Literature Search, Data Collection and Statistical Analysis.
SC & TMT: Writing of Manuscript**,** Drafting, Revision and approval of the final draft.

Authors agree to be accountable for all aspects of work in ensuring that questions related to the accuracy or integrity of any part of the manuscript are appropriately investigated and resolved.