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Rising Trend of Multidrug Resistant and Extensively Drug Resistant Strains of Salmonella Typhi: A Retrospective Study in a Tertiary Care Hospital of Peshawar

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Background: Typhoid fever is an infectious disease caused by the Typhi and Paratyphi serovars of Salmonella enterica. Over the years, there has been a surge in antibiotic-resistant strains of S. typhi, namely extensive (XDR) and multidrug-resistant (MDR), that have received significant amounts of public health concern in the developing world.

Objective: To determine the frequency of extensive and multidrug-resistant strains of Salmonella typhi obtained from positive blood cultures in a tertiary care hospital, MTI-LRH, Peshawar.

Methods: This cross-sectional study was conducted that involved collecting positive Salmonella Typhi blood culture reports from January 2021 to December 2021 at LRH. The data was provided through online medical reports using HMIS by the microbiology lab and analysed by SPSS 23. The culture reports were classified into 3 categories using the WHO criteria: NDR, MDR that is resistant to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole, and XDR that is resistant to five kinds of antibiotics (i.e., chloramphenicol, ampicillin, co-trimoxazole, fluoroquinolones, and third-generation cephalosporins).

Results: Of the 279 positive cultures, 201 were XDR, 78 were MDR, and 0 in NDR group. Males were predominant at 63.1%. 279 cultures were resistant to chloramphenicol, 278 to ampicillin, 274 to ciprofloxacin, 249 to cefiximen, 248 to ceftriaxone, and 231 to co-trimaxole. Among carbapenems, meropenem 100% sensitivity, followed by imepenem at 99.6%. Macrolides had a good sensitivity for 79.2% (221) strains.

Conclusion: The extensively drug-resistant species of Salmonella typhi are rising in number, with carbapenems showing the highest response. This would limit the use of oral antibiotics, necessitating the use of intravenous antibiotics.

Keywords: Enteric Fever; Extensive Drug Resistance; Multi Drug Resistance; Salmonella Typhi

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INTRODUCTION

Globally, 14.3 million cases of typhoid fever were recorded in 2017 among which South Asia accounted for the largest number of cases (more than 70%). The total number of deaths was approximately 13,600 in the same year [1]. Symptoms include fever, headache, malaise, loss of appetite, abdominal pain and constipation. Enteric fever can cause complications like pneumonia, meningitis, endocarditis, osteomyelitis, and arthritis while the most dreaded is intestinal perforation. Prompt treatment of enteric fever is necessary, failure of which can lead to an increase in mortality from 0.5 % to 30% [2].

Enteric fever based on antimicrobial resistance (AMR) falls into three categories namely, non-resistant, multidrug resistant (MDR), and extensive drug-resistant typhoid fever (XDR). At present, the MDR and XDR cases of *Salmonella typhi* are increasing every day with the highest occurrence in the province of Sindh and Punjab. The first-ever case of AMR in Pakistan was notified by the health department of disease surveillance, Sindh in 2016. Afterwards, a drastic rise in cases was up to roughly 9000 with 60% being XDR [3].

The first ever antibiotic used against typhoid fever was chloramphenicol in the year of 1946, but soon resistant strains emerged and as a result co-trimoxazole and ampicillin were introduced to treat the disease in the following decade despite their low efficacy. In the late 1980, multidrug-resistant (MDR) *S. typhi* strains appeared which were resistant to all three first line antibiotics (co-trimoxazole, chloramphenicol and ampicillin). These MDR strains of typhoidal *Salmonella* wreaked havoc spreading across the globe raising the fatality rate. To combat the multidrug-resistant *S. Typhi*, fluoroquinolones, mainly ciprofloxacin, was the drug of choice. However, it was not too long before misuse of fluoroquinolones gave rise to resistance as well. Studies reveal that approximately 80 to 90% cases of enteric fever in South Asia including Pakistan are resistant to ciprofloxacin. Due to the rise of fluoroquinolone-resistant *Salmonella typhi*, third-generation cephalosporin (ceftriaxone), carbapenems and macrolides have been considered for its treatment [4].

Pakistan ranks fifth among the most populated countries in the world with a population of 220 million according to the latest census [5]. Typhoid fever is a potentially life-threatening disease

and has become a challenge due to ongoing antimicrobial resistance and emergence of new strains leading to a significant rise in the morbidity and mortality ratios. Being an overly populated country, the incidence of enteric fever reported was 0.73 million cases with more than 8000 deaths in 2017 therefore, enteric fever remains endemic in the country [6].

One of the common suspected causes of the spread of *Salmonella typhi* is sewage contaminated water. A study by M. Israr et al. in September 2020 to September 2021 at a research laboratory of Abbottabad University of Science and Technology showed that out of 100 drinking water samples collected from different drinking sources in the Sub-Division of Hassan Khel Peshawar had an overall prevalence of *S. typhi* of 22% that were resistant to ampicillin (90.0%), chloramphenicol (22.7%), and trimethoprim-sulfamethoxazole (40.9%) and Cephalosporins (31.8%) [7]. A similar study in 2021 by Akram, J., et al. showed 64% samples positive for *Salmonella typhi* [8]. Both studies highlighted sewage contamination of drinking water with strong emphasis on improvement made by the government for water purification.

Keeping in view the continuously evolving *Salmonella typhoid* bacterium, there is considerable need to study patterns of antibiotic resistance in different locations to timely detect new strains and as a result select appropriate empirical antibiotics to help treat enteric fever. Aim of the present study was to determine the antibiotic resistance and sensitivity pattern of *Salmonella typhi* in a tertiary care hospital at Peshawar, KPK province, Pakistan.

MATERIAL & METHODS

This retrospective study was conducted at Lady Reading Hospital-MTI, Peshawar, from January 2021 to December 2021. 279 *Salmonella typhi* positive blood cultures were obtained from the Microbiology Department. The study followed standards of research ethics throughout the process of data collection and analysis including approval from the institutional review board (Ethical Committee) under reference number 347/LRH/MTI.

The department of microbiology at the hospital follows standard protocol for microbiological procedures. As an initial process, the blood was collected under aseptic measures in blood culture bottles and transported to the laboratory at the earliest. These culture bottles were then incubated

in BacT/ALERT 3D system (BioMerieux) till the detection of microbes or for five days maximum. Upon receiving a positive signal from the automated system, the culture bottles were taken out of the incubator. Positive blood cultures were further sub-cultured on different culture media such as Blood agar, MacConkey agar, and MSA agar plates for 24 hours at 37 °C [8]. In the presence of growth, bacterial identification tests were conducted to identify *Salmonella typhi* organisms through routine biochemical tests and serologically confirmed by slide agglutination with polyvalent antiserum.

Antimicrobial sensitivities were carried out by the laboratory using the disk diffusion method (Kirby-Bauer method) and were reported based on the measurement of the zone of inhibition that is followed by the Clinical & Laboratory Standards Institute (CLSI) guidelines. These blood cultures were tested against nine antibiotics namely ampicillin, azithromycin, ceftriaxone, cefixime, imipenem, meropenem, ciprofloxacin, chloramphenicol, and co-trimoxazole. Based on antibiotic-resistant pattern defined by the WHO, they were classified into three categories non-drug resistant (sensitive to all tested antibiotics), multi-drug resistant (resistant to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole), and extensively drug-resistant (resistant to chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones and cephalosporins) [9]. The reports for these samples were provided through the online medical records using HMIS (Health Management Information System) and analysed through Statistical Package for the Social Sciences (SPSS) version 23 for interpretation of data.

RESULTS

Out of 279 *Salmonella typhi* positive blood cultures, 10, 218 were XDR while 61 were MDR with 100% resistance to chloramphenicol, ampicillin, and ciprofloxacin (Figure 1). Males were predominant at 63.1 %, 44.1% aged between 6-10 years with majority samples received in the month of July. (Figure 2-3).

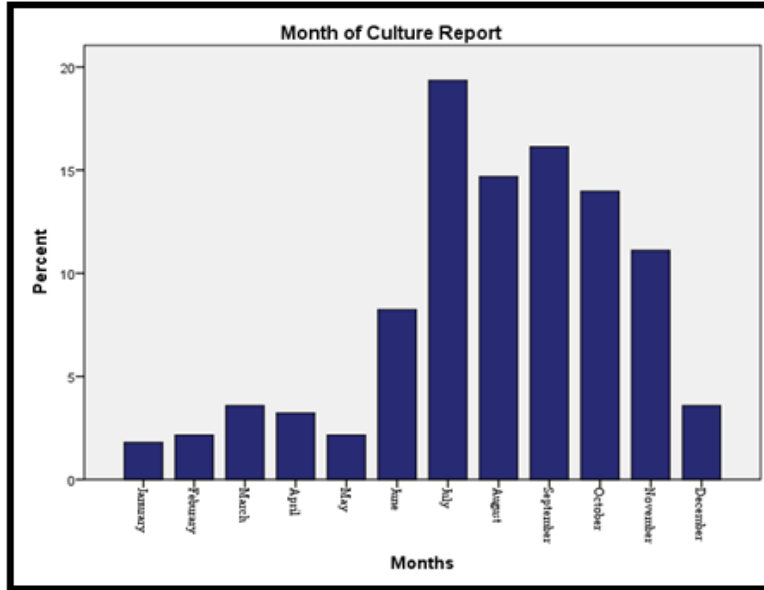


Figure 1: Percentage of Antimicrobial Resistance among enteric fever on Monthly basis

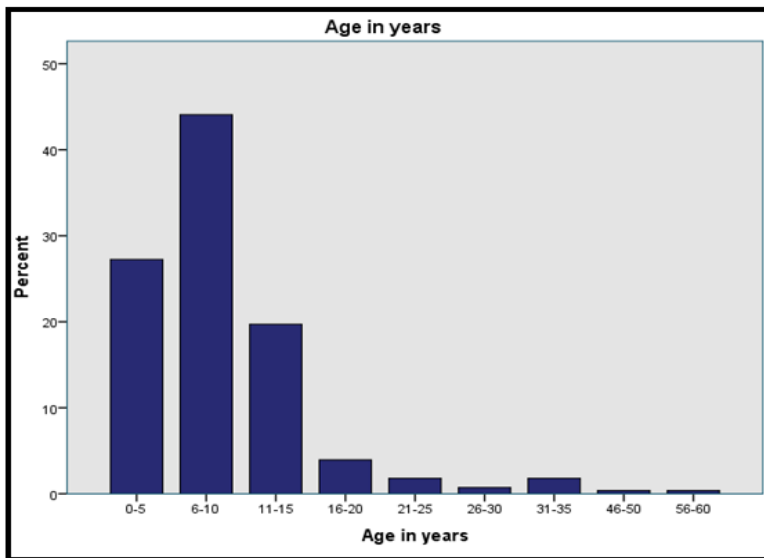


Figure 2: Percentage of Antimicrobial Resistance among enteric fever cases in relation to Age

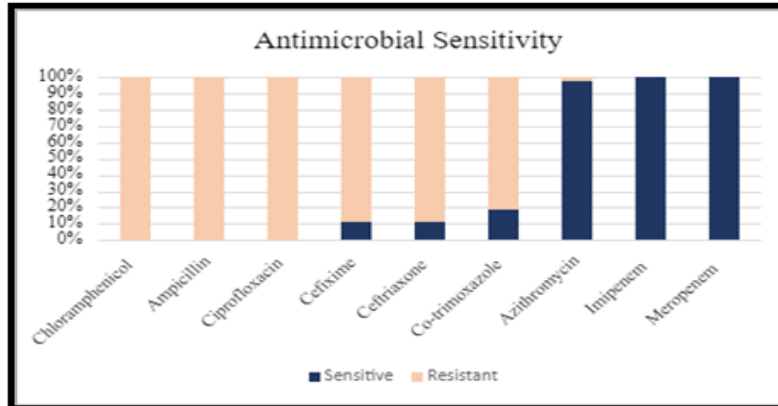


Figure 3: Antimicrobial sensitivity of *Salmonella typhi* positive cultures

DISCUSSION

Antimicrobial resistance against *Salmonella typhi* is a major issue in developing countries. Factors responsible include over-the-counter antibiotic availability, delayed diagnosis, poor medical practice, food and water sanitation. [10-11].

During summertime, in Pakistan temperatures range from 32-48 °C across the nation. *Salmonella typhi* is a temperature sensitive pathogen which survives and multiplies more in warmer weather as seen in a meta-analysis of available literature on bioclimatic models to explain how changes in temperatures, rainfall, and drought affects waterborne disease in Pakistan from 2000 to 2020 by Noureen et al. [12]. Similar results were obtained in our study showing a higher number of cases in the month of July as seen in Figure 1.

In comparison to previous studies conducted in Pakistan, males tend to be more affected than the female population. Henceforth, 63.4% of males were positive for enteric fever via blood culture which is roughly twice the females in our case. The possible reason could be higher incidence of outdoor dining and parents of male children seeking more healthcare [13]. Another explanation could be due to Th1 protective response in females as compared to males rendering them more susceptible to infection [14].

Also, this study stands by the existing fact that typhoid fever is a childhood illness that can sometimes be present in adults. The age group most affected was seen between 6-10 years, 44.1%, followed by 0-5 years (27.3%) and then 11-15 years (19.7%), respectively (Figure.2). Coinciding

results were observed in two studies led by Saeed et al and Chatham et al in 2019, stating that most of the affected population comes under the age of 15 years [15-16].

In the developing world, resistant *Salmonella typhi* strains have been on a constant rise with only sensitivities left such as Carbapenems and macrolides. Reportedly possible factors contributing to such an issue could be excessive antibiotic usage. In comparison to studies conducted by Tewari et al showed that first-line antibiotics used for *S. typhi* had the highest resistance among all the antibiotic classes for enteric fever, and similar can be appreciated in our case i.e., 48/279 and 1/279 were sensitive to co-trimoxazole and ampicillin (Figure 3), respectively [17].

In 1985, Fluroquinolones like ciprofloxacin was introduced to treat enteric fever but due to multiple uses of the antibiotic in other diseases, misuse because of clinical malpractice and easy availability in countries like Pakistan, Bangladesh, Nepal, and India resulted in the first ever outbreak, reported by 1997. Later, a survey in 2015 claimed that more than 90% strains of *S. Typhi* of the South Asian population had become resistant to fluoroquinolones. A very high resistance to fluoroquinolones was also seen in our study that is 97.5% whereas a recent review of blood cultures done by M. Khan et al in 2022 from a laboratory (Rehman Institute of Pathology) in Peshawar from December 2020 to January 2021 showed 100% resistance [18].

Cephalosporins replaced fluoroquinolones in 2014 and came out as the new drug for enteric fever. Favorable results were obtained and due to its broad-spectrum properties, patients benefited tremendously. Until late November 2016, strains resistant to ceftriaxone appeared in Pakistan that made the disease difficult to treat. These strains were identified as extensive drug resistant (XDR) species as they showed resistance to first line antibiotics, fluoroquinolones and cephalosporins. Our study has similar results to G. Fatima et al, who obtained 566 blood cultures in 2018 from a tertiary care hospital, Karachi that showed 85% resistance to cephalosporins [19].

At present, Carbapenems and Macrolides (azithromycin) stand as sole treatment against the XDR cases of *Salmonella Typhi*. Qureshi et al. Conducted a study in Karachi and Hyderabad from June 2017-18 comparing the cost effectiveness of meropenem and azithromycin used as monotherapy and dual antibiotic regimen. Their study suggested that monotherapy is effective over combined therapy. In addition, azithromycin was more economic than meropenem with an average cost per day of US\$5.87 in contrast to US\$88.46, respectively [20].

To our knowledge, these are the greatest number of XDR cases reported from any medical center in Pakistan. In 2022, A Ejaz et al conducted a study from 1st January to 31st December 2021 at Punjab Rangers Teaching Hospital, Lahore, Pakistan. The results were suggestive of 51.61% and 46.77% cases of XDR and MDR strains respectively. On the contrary, our review suggested 2.5 times the count for XDR strains (72%), marking it as the highest ever recorded case in Pakistan up till 2022 [21].

Limitations: The results for this review for antimicrobial sensitivity of *S. typhi* cannot be generalized to a larger scale due to being a single institution-based study. We also couldn't obtain details about the genotype of the *Salmonella Typhi* that was extensively resistant to the above-mentioned antibiotics. Therefore, more information should be collected from hospitals across the country to acquire definite data regarding current statistics for resistant strains.

Recommendations: To prevent pathogen dissemination, we need to take immediate precautionary measures by improvement of medical practice, reduction in over-the-counter medications availability, public health education, vaccination, food hygiene and lastly water sanitation.

CONCLUSION

XDR *Salmonella Typhi* made up the majority of the blood culture samples and had excellent sensitivity towards carbapenems with only under a fraction having intermediate resistance to macrolides such as azithromycin. Moreover, complete resistance to chloramphenicol, ampicillin, ciprofloxacin was noted, henceforth limiting the antibiotics for treatment.

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