EVALUATING THE ANTIBIOTIC SENSITIVITY AND RESISTANCE PATTERNS OF SALMONELLA TYPHI IN PEDIATRIC ENTERIC FEVER PATIENTS: A CROSS-SECTIONAL STUDY AT A TERTIARY CARE HOSPITAL

Bushra Noor[1*], Arshia Munir[2], Laiba Ali Khan[1], Arsalan Khan[1], Saif Ullah[1], Aroob Khan[3]

[1*]Khyber Medical College, Peshawar. (Email: bushra.noor289@gmail.com),
(Email: laibaalikhan510@gmail.com), (Email: eaglelion.21.ak@gmail.com), (Email: dsaif419@gmail.com),
[2]Khyber Teaching Hospital, Peshawar. (Email: drarshia@yahoo.com),
[3]Nawaz Sharif Medical College, Gujrat. (kgandapur890@gmail.com)

*Corresponding Author: Bushra Noor
*Khyber Medical College Peshawar. Email: bushra.noor289@gmail.com

ABSTRACT

Enteric fever, caused by Salmonella Typhi and Paratyphi, remains a formidable global health concern, particularly affecting developing nations. With an exceeding annual global incidence of significant mortality rates, the emergence of multidrug-resistant strains poses escalating challenges to effective treatment. This cross-sectional study was carried out at Khyber Teaching Hospital from October 2023 to July 2024 by using a non-probability purposive sampling method. The study focused on children aged 1 to 14 years who were admitted to the pediatric department and had blood cultures positive exclusively for Salmonella Typhi. Data were gathered from the patients' culture and sensitivity reports and analyzed using SPSS Software Version 21. Out of 359 cases, 206 (57.4%) were male and 153 (42.6%) were female. Antibiogram analysis revealed significant resistance to primary antibiotics, including co-trimoxazole (48%), chloramphenicol (96%), ceftriaxone (96.3%), and ciprofloxacin (95.9%). High resistance was also observed for ampicillin (98.9%), moxifloxacin (84.1%), and several others. In contrast, antibiotics like meropenem, doripenem, imipenem, ertapenem, tigecycline, and colistin showed 100% sensitivity. The combination drugs such as Pipercillin-tazobactam (99.2%) and Cefoperazone-sulbactam (98.5%) also showed higher sensitivities. The sensitivity of Azithromycin and Doxycyclin was 99.4% and 85.7% respectively. The study reveals a concerning trend of significant antibiotic resistance among Salmonella Typhi isolates, particularly against first-line antibiotics. Despite this, high sensitivity was noted for azithromycin, meropenem, doripenem, imipenem, and tigecycline, indicating their effectiveness in treating enteric fever. The healthcare professionals should consider these regional antibiotic resistance trends for better patient outcomes.

Keywords: Antibiotic Sensitivity, Pediatric Enteric Fever, Salmonella Typhi, Resistance.

Introduction

Enteric fever is a widespread bacterial infection acquired within communities globally, caused by Salmonella serotypes.[1] It presents a major health challenge, particularly in nations that are developing, where it contributes significantly to illness and mortality rates. If not promptly and

effectively treated, enteric fever can progress to severe complications such as low blood pressure, altered mental status, liver inflammation, brain inflammation, intestinal perforation, and potentially fatal outcomes.[2] In South and Southeast Asia, it ranks as the primary source of bloodstream infections acquired within communities. In developed countries including the United States, enteric fever is a notifiable disease and represents a serious health risk, second only to malaria among travel-related infections in terms of severity and potential for life-threatening illness.[3]

Each year, approximately 21 million individuals worldwide contract enteric fever, resulting in over 150,000 deaths.[4] Pakistan, as a developing nation, reports an annual incidence rate of 493.5 per 100,000 individuals, making it one of the nations most impacted by typhoid. The highest risk populations reside in the provinces of Punjab and Sindh.[2, 5] Asia bears the burden of about 80% of both cases and mortalities. The rate of enteric fever ranges from 102 to 2,219 cases per 100,000 individuals in India. When left untreated, typhoid fever poses considerable morbidity and death risks, with a predicted fatality rate of 30%.[6]

Antimicrobial resistance poses a significant challenge to modern medicine, contributing to over 35,000 deaths annually in the United States.[7] The management of enteric fever, guided by culture and sensitivity testing, differs across countries and has led to an increase in multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains in developing nations due to antibiotic misuse.[8] Initially, ampicillin, chloramphenicol, and trimethoprim/sulfamethoxazole were standard treatments, but their effectiveness has diminished due to bacterial resistance. The MDR strains ware first noted in 1970s, with widespread resistance observed in the 1980s and 1990s.[9] Subsequently, treatment shifted to quinolones and cephalosporins, which were effective until the emergence of XDR strains resistant to these drugs as well.[10] The initial outbreak of XDR typhoid began in Hyderabad in 2016 and rapidly extended to other cities within the province of Sindh, resulting in more than 10,000 reported cases only in Pakistan. This underscores the alarming spread of this extensively drug resistant strain.[11, 12]

The research aims to evaluate the sensitivity and resistance profiles (antibiogram) of Salmonella Typhi causing enteric fever. Understanding the antimicrobial susceptibility patterns of these isolates is crucial for optimizing treatment approacges and guiding health policies designed to manage the transmission of enteric fever. By evaluating the effectiveness of commonly used antibiotics against Salmonella Typhi, this study seeks to provide important information about the current state of antibiotic resistance in clinical settings. Such knowledge is essential for clinicians in selecting appropriate empirical therapies and for researchers in developing new treatment protocols to combat the rising challenge of antimicrobial resistance in enteric pathogens. By identifying antibiotics with high sensitivity rates and those exhibiting resistance, healthcare providers are able to make informed decisions, optimize treatment outcomes, and contribute to better management of enteric fever cases.

Materials and Methods

The Pediatric Department at Khyber Teaching Hospital, Peshawar, served as the setting for this cross-sectional observational study. The study was conducted over a period of 10 months, from October 2023 to July 2024. Using a non-probability purposive sampling method, we included a total of 359 positive blood cultures. The study focused on children aged 1 to 14 years who were admitted to the pediatric department and had blood cultures positive exclusively for Salmonella Typhi. To differentiate between various Salmonella enterica species, agglutination test was done at hospital laboratory. Positive blood cultures having Salmonella paratyphi (due to less cases) and blood cultures done for other condition were excluded from our study. The hospital lab grew Salmonella Typhi species using the BACTEC system, and tested antibiotic susceptibility on Muller-Hinton agar using the Kirby-Bauer disc diffusion technique.

Data Analysis

Data was recorded in Microsoft Excel and then was transferred to SPSS Software version 21. Analysis was done using this SPSS software. Antibiotic sensitive and resistant antiobiograms were made and frequency analysis was done for variables like age, gender and region. Quantitative variables were

assessed using the mean and standard deviation, whereas qualitative data was analyzed through frequencies and percentages. A combination of percentages and numerical figures were then used to convey the findings, providing a clear and succinct depiction of the data. This method not only makes the results more easily readable, but it also makes it easier to comprehend the complex patterns linked to antimicrobial resistance.

Ethical Consideration

The Ethical Approval was taken from Medical Director of Khyber Teaching Hospital and Director of Medical Education at Khyber Medical College (Ref: 647/DME/KMC). Both formally approved the data collection, guaranteeing compliance with ethical standards. Approval from institutional authorities guarantees that the research upholds the rights, dignity, and welfare of the pediatric patients involved in the study. This includes adherence to principles such as informed consent, confidentiality, and minimizing potential harm to participants.

Results

In our study out of 359 patients, 206 (57.4%) were male children and 153 (42.6%) were female children. This shows higher prevalence of enteric fever among males as shown in Figure 1.
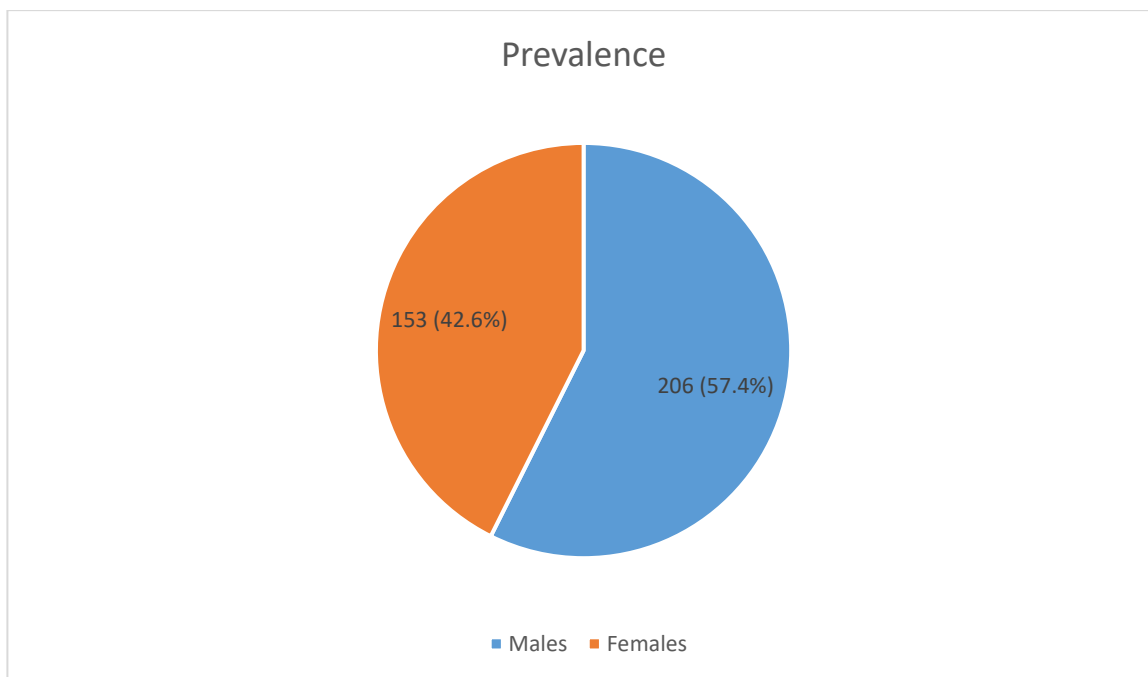
Figure 1: Gender-wise Prevalence of Enteric fever.

In our study, children's ages ranged from 1 to 14 years, with a mean age of 7.5 ± 3.3 years. The prevalence of enteric fever among children age group is shown in figure 2. The age and gender wise prevalence is shown in Table 1.
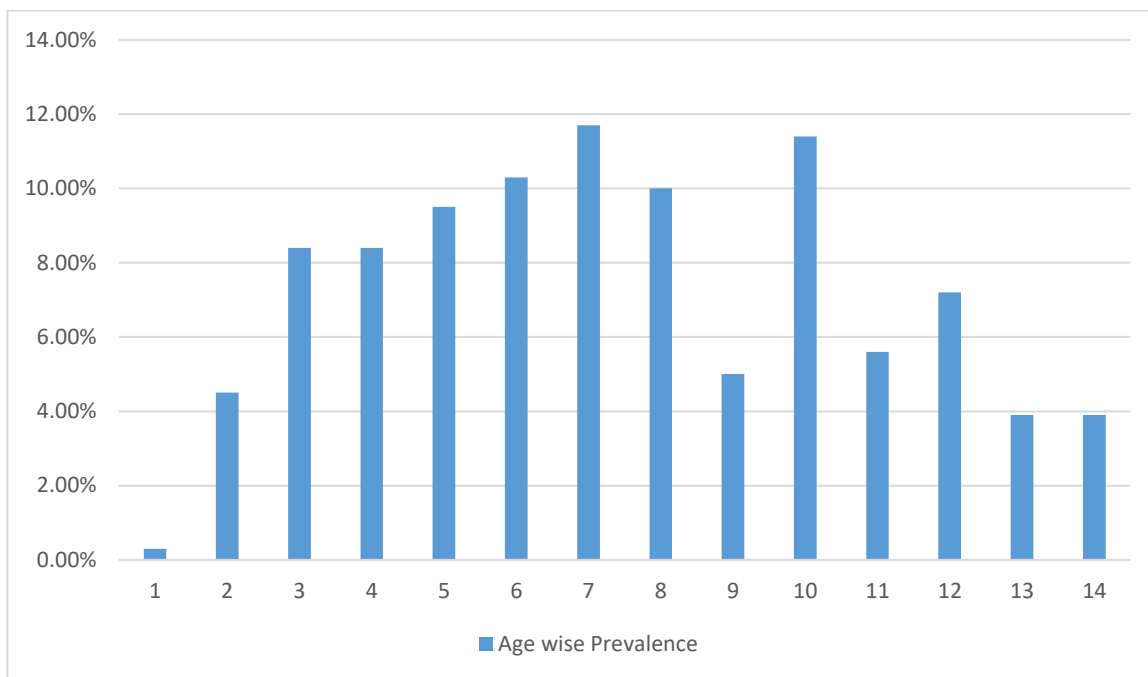
Figure 2. Age wise prevalence of Enteric fever.

**Table 1. Age and gender wise prevalence of Enteric fever.**

Age of Participants (Years)	Male, n (%)	Female, n (%)	Total, n (%)
1	1(0.3)	0	1(0.3)
2	6(1.67)	10(2.7)	16(4.5)
3	18(5)	12(3.3)	30(8.4)
4	16(4.5)	14(3.9)	30(8.4)
5	20(5.6)	14(3.9)	34(9.5)
6	23(6.4)	14(3.9)	37(10.3)
7	28(7.7)	14(3.9)	42(11.7)
8	18(5)	18(5)	36(10)
9	10(2.7)	8(2.2)	18(5)
10	17(6.5)	24(6.7)	41(11.4)
11	13(3.6)	7(1.9)	20(5.6)
12	16(4.5)	10(3.8)	26(7.2)
13	10(2.7)	4(1.1)	14(3.9)
14	10(2.7)	4(1.1)	14 (3.9)

The antibiogram analysis's findings shed light on the drug sensitivity and resistance patterns seen in the Salmonella typhi bacteria that cause enteric fever. A number of antibiotics showed significant efficacy against this bacteria. Significant resistance was observed in Salmonella Typhi isolates against first-line antibiotics, including co-trimoxazole (48%), chloramphenicol (96%), ceftriaxone (96.3%), and ciprofloxacin (95.9%). Resistance was also seen among other antibiotics such as Ampicillin (98.9%), Moxifloxacin (84.1%), Aztreonam (93.8%), Cefotaxime (93.1%), Co-amoxiclav (86.5%), Ceftazidime (93.6%), Levofloxacin (84.2%), Cefepime (92.9%), Gentamicin (89.7%). Salmonella Typhi resistant antibiogram is shown in figure 3.
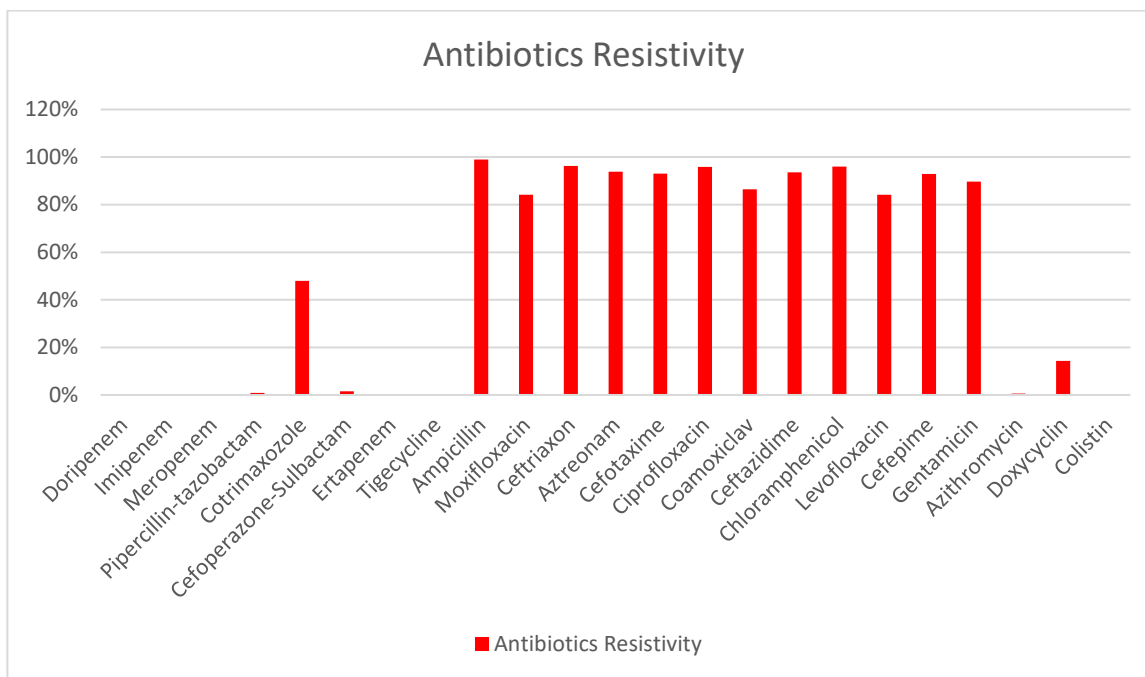
Figure 3. Salmonella antibiogram resistant pattern.

Regarding antibiotic sensitivities, certain antibiotics exhibited greater sensitivity patterns, including Meropenem (100%), Doripenem (100%), Imipenem (100%), Ertapenem (100%), Tigecycline (100%), Pipercillin-tazobactam (99.2%), Cefoperazone-sulbactam (98.5%), and Azithromycin (99.4%), Doxycyclin (85.7%). Despite a limited sample size, Colistin demonstrated a remarkable sensitivity of 100%. Salmonella's antibiogram pattern showed notable and dramatic changes. Salmonella sensitive antibiogram is shown in figure 4.
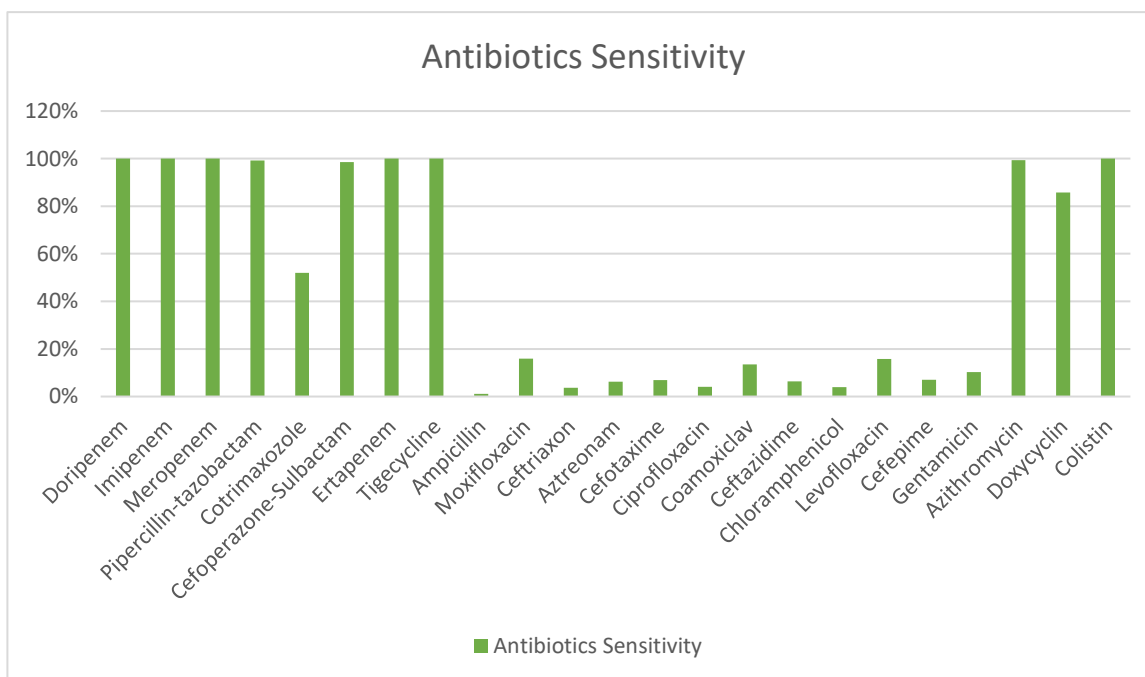
Figure 4. Salmonella antibiogram sensitivity pattern.

The results highlights the necessity of performing antibiogram assessments to guide the choice of suitable antimicrobial treatment. By pinpointing which antibiotics are likely to work and which may encounter resistance, healthcare professionals can make well-informed choices, ultimately

improving patient results and enhancing the management of enteric fever. Antibiograms details are given in table 2 and figure 3-4.

Table 2. Antibiotics sensitivity and Resistance.

Antibiotic	Number Tested	Sensitivity S, n (%)	Resistance R, n (%)
Doripenem	80	80 (100)	0
Imipenem	314	314 (100)	0
Meropenem	353	353 (100)	0
Pipercillin+Tazobactam	131	130 (99.2)	1 (0.8)
Cotrimaxozole	298	155 (52)	143 (48)
Cefoperazone+Sulbactam	131	129 (98.5)	2 (1.5)
Ertapenem	50	50 (100)	0
Tigecycline	106	106 (100)	0
Ampicillin	355	4 (1.1)	351 (98.9)
Moxifloxacin	44	7 (15.9)	37 (84.1)
Ceftriaxon	244	9 (3.7)	235 (96.3)
Aztreonam	129	8 (6.2)	121 (93.8)
Cefotaxime	131	9 (6.9)	122 (93.1)
Ciprofloxacin	340	14 (4.1)	326 (95.9)
Coamoxiclav	126	17 (13.5)	109 (86.5)
Ceftazidime	172	11 (6.4)	161 (93.6)
Chloramphenicol	298	12 (4)	286 (96)
Levofloxacin	57	9 (15.8)	48 (84.2)
Cefepime	126	9 (7.1)	117 (92.9)
Gentamicin	29	3 (10.3)	26 (89.7)
Azithromycin	308	302 (99.4)	2 (0.6)
Doxycyclin	28	24 (85.7)	4 (14.3)
Colistin	15	15 (100)	0

Discussion

The increasing issue of antimicrobial resistance (AMR) represents a serious global health challenge, particularly with regard to enteric fever caused by Salmonella species. Annually, over 14 million cases and 135,000 fatalities are attributed to enteric fever worldwide, highlighting the urgent need for effective treatment strategies amidst increasing AMR prevalence.[13]

Our study on enteric fever cases admitted to hospital in Peshawar, Pakistan, revealed a higher prevalence among male children compared to females aligns with another study conducted in southern Pakistan.[14] Our findings on antibiotic resistance patterns align with global trends, indicating widespread resistance across different geographical regions. This consistency underscores the global nature of the problem, although local variations in resistance rates were observed. These variations likely stem from differences in antibiotic usage practices and the prevalence of resistance mechanisms unique to each region.

Our study identified significant resistance to primary antibiotics including ciprofloxacin, co-trimoxazole, and chloramphenicol, echoing findings from a study conducted in London. However, our findings contrasted with this study by demonstrating notable resistance to ceftriaxone.[15] Factors such as geographical variations, changes in antibiotic prescribing practices, circulating pathogen strains, and differences in patient demographics contribute to these discrepancies. Similarly, study in the Kathmandu Valley, Nepal, has identified concerning trends in antibiotic resistance among Salmonella Typhi variant, particularly strong resistance to ciprofloxacin and gatifloxacin consistent with our study.[16] This finding is also in line with previous studies.[17, 18] Furthermore, in other study they observed decreased sensitivity rates for ceftriaxone and cefixime in their research.[14]

In North India, a study reported sensitivity to azithromycin, which aligns with our findings.[19] Similarly, isolates of S. typhi and S. paratyphi from East London remained sensitive to ceftriaxone and azithromycin in another study.[20] These varying susceptibility patterns underscore the importance of region-specific treatment guidelines and continuous surveillance to guide effective management strategies against AMR in enteric fever.

Our study found a sensitivity of 99.4% to Azithromycin, contrasting sharply with a study conducted in the same KTH and HMC Hospitals, Peshawar, in 2023. In that study, Azithromycin showed an overall sensitivity profile of 66.5% . This difference may be attributed to age variations. Age-related factors such as immune status or underlying health conditions could influence the drug sensitivity observed in these distinct patient cohorts.[18]

Our study, demonstrating a 100% sensitivity to meropenem, aligns well with a research performed in Germany, where meropenem and fosfomycin were successfully employed in the treating enteric fever. This consistency in sensitivity underscores the robustness of meropenem as a therapeutic option against pathogens associated with our study's conditions. The positive outcomes observed in the German study further substantiate the efficacy of meropenem in managing infectious diseases, particularly in scenarios where conventional treatments may be compromised by resistance patterns. These findings collectively support the notion that meropenem holds promise as a reliable and effective treatment choice, warranting its consideration in clinical practice and further exploration through rigorous clinical trials to validate its broader applicability and safety profile across different patient demographics and geographic regions.[21]

Our study enhances the understanding of antibiotic resistance in pediatric enteric fever patients in Pakistan. The findings highlight the necessity for customized treatment strategies that consider local resistance patterns and advocate for global efforts to combat AMR through prudent antibiotic use and the development of alternative treatment options.

Limitations
As the study was carried out at a single center, the findings may not reflect broader populations or different geographic regions. Variations in demographic characteristics, healthcare practices, and antibiotic resistance patterns in other settings may affect the generalizability of our results. Also the variations in laboratory methods for antibiotic sensitivity testing across different settings or over time could impact the consistency and comparability of results.

Conclusion
The study highlights a concerning trend of antibiotics sensitivity and resistance among our population. There is significant resistance observed among Salmonella Typhi isolates against first-line antibiotics such as co-trimoxazole, chloramphenicol, ceftriaxone, and ciprofloxacin. Resistance was also noted against a range of other antibiotics commonly used in clinical practice, including Ampicillin, Moxifloxacin, and Cefotaxime, among others. These findings highlight the evolving resistance patterns of Salmonella Typhi and the need for continuous monitoring. Certain antibiotics showed high sensitivity rates, including Azithromycin, Meropenem, Doripenem, Imipenem, and Tigecycline. These antibiotics can be considered as effective treatment options when managing enteric fever caused by Salmonella Typhi. Given the findings, healthcare professionals should consider these regional antibiotic resistance trends when designing therapeutic approaches for patients with enteric fever for better patient outcomes.

References

1. Das JK, Hasan R, Zafar A, et al. Trends, associations, and antimicrobial resistance of Salmonella Typhi and Paratyphi in Pakistan. Am J Trop Med Hyg 2018;99(3_Suppl):48–54.
2. Stanaway JD, Reiner RC, Blacker BF, et al. The global burden of typhoid and paratyphoid fevers: A systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect Dis 2019;19:369–81.
3. Meiring JE, Khanam F, Basnyat B, et al. Typhoid fever. Nat Rev Dis Primers 2023;9:71.
4. Rasheed MK, Hasan SS, Ahmed SI. Extensively drug-resistant typhoid fever in Pakistan. Lancet Infect Dis 2019;19:242–3.
5. World Health Organization. Joint external evaluation of IHR core capacities of the Islamic Republic of Pakistan: mission report: 27 April-6 May 2016. (2017) https://apps.who.int/iris/handle/10665/254614 (accessed on: November 15, 2021).
6. Sharvani R, Hemavathi, Dayanand DK, et al. Antibiogram of Salmonella isolates: time to consider antibiotic salvage. J Clin Diagn Res 2016;10
7. Centers for Disease Control and Prevention. About antibiotic resistance. (2022) https://www.cdc.gov/drugresistance/about.html (accessed on: December 22, 2022).
8. Maharjan A, Dhungel B, Bastola A, et al. Antimicrobial susceptibility pattern of Salmonella spp. isolated from enteric fever patients in Nepal. Infect Dis Rep 2021;13:388-400.
9. Levine MM, Simon R. The gathering storm: is untreatable typhoid fever on the way? MBio 2018;9
10. Akram J, Khan AS, Khan HA, et al. Extensively drug-resistant (XDR) typhoid: evolution, prevention, and its management. Biomed Res Int 2020;2020:6432580.
11. Ali Shah SA, Nadeem M, Syed SA, et al. Antimicrobial sensitivity pattern of Salmonella Typhi: emergence of resistant strains. Cureus 2020;12
12. Khan M, Shamim S. Understanding the mechanism of antimicrobial resistance and pathogenesis of Salmonella enterica Serovar Typhi. Microorganisms 2022;10:2006.
13. Browne AJ, Kashef Hamadani BH, Kumaran EAP, et al. Drug-resistant enteric fever worldwide, 1990 to 2018: a systematic review and meta-analysis. BMC Med 2020;18:1.
14. Dave J, Warburton F, Freedman J, et al. What were the risk factors and trends in antimicrobial resistance for enteric fever in London 2005-2012? J Med Microbiol 2017;66:698-705.
15. Arjyal A, Basnyat B, Nhan HT, et al. Gatifloxacin versus ceftriaxone for uncomplicated enteric fever in Nepal: an open-label, two-centre, randomised controlled trial. Lancet Infect Dis 2016;16:535-45.
16. Khan MI, Soofi SB, Ochiai RL, et al. Epidemiology, clinical presentation, and patterns of drug resistance of Salmonella Typhi in Karachi, Pakistan. J Infect Dev Ctries 2012;6:704-14.
17. Qamar FN, Yousafzai MT, Sultana S, et al. A retrospective study of laboratory-based enteric fever surveillance, Pakistan, 2012-2014. J Infect Dis 2018;218(suppl_4)
18. Laghari GS, Hussain Z, Hussain SZM, et al. Antimicrobial susceptibility patterns of Salmonella species in Southern Pakistan. Cureus 2019;11
19. Dahiya S, Katiyar A, Rai S, et al. Ceftriaxone-resistant Salmonella Typhi isolated from paediatric patients in north India: insights into genetic profiles and antibiotic resistance mechanisms. Indian J Med Microbiol 2023;46:100448.
20. Dave J, Sefton A, de Pinna E, et al. Trends in antibiotic susceptibility of enteric fever isolates in East London. Travel Med Infect Dis 2015;13:230-4.
21. Kleine CE, Schlabe S, Hischebeth GTR, et al. Successful therapy of a multidrug-resistant extended-spectrum β-lactamase-producing and fluoroquinolone-resistant Salmonella enterica subspecies enterica Serovar Typhi infection using combination therapy of meropenem and fosfomycin. Clin Infect Dis 2017;65:1754-6.