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Original Article

Prevalence and antimicrobial susceptibility of *Salmonella enterica* Typhi in febrile patients: a cross-sectional study

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Abstract
Introduction: Typhoid fever, caused by *Salmonella Typhi*, is a serious health problem, especially in developing countries like Pakistan where antibiotics are usually prescribed without susceptibility testing or epidemiological surveillance. Consequently, antibiotic-resistant typhoid bacteria appear, but are not reported to the authorities. There is limited research on the prevalence and antibiotic susceptibility patterns of *S. Typhi* among febrile patients in Swabi, Pakistan. This study aimed to address this gap at the Bacha Khan Medical Complex in Swabi.
Methodology: Laboratory records of hospitalized patients who received a blood culture from September 2022 to August 2023 were reviewed in this retrospective, cross-sectional study. Every isolate of *S. Typhi* underwent antibiotic susceptibility test using modified Kirby-Bauer disk diffusion and agar-dilution methods to measure the isolates' minimum inhibitory concentration (MIC) for ciprofloxacin and azithromycin. The data were analyzed using SPSS version 24.0.
Results: 4.85% of febrile patients were positive for *S. Typhi*, with a higher prevalence in the 0–14 years age group. Male gender and seasonal variation were significant factors. The isolates were resistant to ampicillin, amoxicillin, cefotaxime, and ciprofloxacin; and sensitive to azithromycin, and carbapenems. The MICs for ciprofloxacin were between 0.06 to 16 µg/mL. Among the isolates, 1.094% were sensitive and 98.90% were resistant to ciprofloxacin; and 100% isolates were susceptible to azithromycin.
Conclusions: Azithromycin and carbapenem were a suitable empirical therapy choice. However, the isolates were highly resistant to conventional first-line antibiotics (ampicillin, amoxicillin), second generation fluoroquinolones (ciprofloxacin), and third-generation cephalosporins (ceftriaxone, cefotaxime), that are considered vital in typhoid treatment.

Key words: *Salmonella Typhi;* typhoid; antimicrobial; susceptibility; febrile; Pakistan.

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Introduction

Salmonella enterica serovar Typhi (*S. Typhi*) is the primary cause of enteric fever worldwide. *S. Typhi* are facultative bacilli that are non-capsulated, non-sporulating, Gram negative, and have distinct flagellar, somatic, and outer coat antigens [1]. Typhoidal serovars of *Salmonella enterica* cause enteric fever, a dangerous bloodstream illness that disproportionately affects over 17 million people worldwide and has a documented annual fatality of 178,000,000 [2]. All age groups are affected, although children in low- and middle-income nations (LMICs), such as Pakistan, have the maximum recorded cases [2,3]. Antimicrobial resistance (AMR) is growing in importance globally and the World Health Organization (WHO) has classified *Salmonella* as a "priority pathogen" with drug resistance [4]. Additionally, LMICs were impacted by the epidemic caused by the H58 strain of *S. Typhi*, which is the most multi-drug resistant (MDR) pathogen among *Salmonella* [5,6]. As a result, the situation is concerning, and the residents in these areas have to face the combined difficulties of hard-to-treat MDR strains and financial limitations [2]. Numerous instances of MDR H58 have been recorded from the neighboring nations of Bangladesh, Pakistan, and India; and the potential transmissibility of MDR must be taken seriously [2,7].

Inability to obtain clean food and water due to inadequate sanitation leads to a high risk of illness [8]. Children between the ages of 5 and 19 years have been reported to have the highest incidence of infection in LMICs. Yet, research from South Asia indicate that the highest incidence of enteric fever is among children below 5 years of age [1]. Many regions of the world lack appropriate access to clean water and sanitary facilities. The lack of these basic necessities has a negative impact on typhoid fever and public health [9]. Typhoid fever is more common in areas with tainted water supplies and poor waste management [10]. Some individuals who excrete *S. Typhi* do not recall having typhoid fever, and its associated symptoms such as diarrhea or fever, recently. One to five percent of patients with acute typhoid infection go on to become chronic carriers of the sickness in their gall bladder; and the likelihood of patients with acute typhoid infection becoming chronic carriers of the disease in their gall bladder depends on their age, gender, and treatment regimen. The current selection and availability of medications, along with AMR in the most common strains, may have altered the predisposition of the bacteria, and as a result the affected individuals become chronic carriers [11].

According to WHO, typhoid is a serious public health concern that primarily affects children and young people [12]. It is the fourth most prevalent cause of death worldwide and the third most prevalent cause of death in Pakistan [13]. There were 573.2 cases of typhoid fever per 100,000 children aged 2 to 5 years annually, in Pakistan [13]. Treatment for *S. Typhi* infection is essential due to the acquisition of resistance genes by the bacteria, which is mainly facilitated by transmissible plasmids [14]. Antimicrobial therapy is the main treatment for the illness; however, treatment is becoming more challenging because of changes in the pathogen's susceptibility trend. Ironically, children in underdeveloped nations are still given empirical treatment, and some even self-medicate with easily accessible antibiotics; in spite of the lack of minimal fatal dose estimations, susceptibility testing, and periodic epidemiological censuses [15]. These may be the reasons for the high rates of illness and mortality recorded in the majority of emerging nations [16–18]. Therefore, assessing the isolate's antimicrobial susceptibility pattern against routinely prescribed antibiotics is essential for clinical management in these countries where economic restrictions prevent the assessment of distinct diagnostic and therapeutic methods [2].

This study determines the prevalence of *S. Typhi* infection and the antimicrobial susceptibility pattern among patients with a febrile illness. Typhoid fever continues to be a significant public health issue in Pakistan, and *S. Typhi* is becoming less susceptible to antibiotics. Extensively drug-resistant strains of *S. Typhi* that are resistant to ceftriaxone, ciprofloxacin, and first-line medications; have been reported in Pakistan [19]. Owing to this shifting pattern of antibiotic resistance in *S. Typhi*, and the cost of treating extensively resistant typhoid fever, it is imperative to regularly examine, and report, the resistance pattern of *S. Typhi* in various regions of Pakistan. Consequently, the purpose of this investigation was to determine the prevalence of *S. Typhi* infection and the pattern of its antibiotic susceptibility in patients who had a febrile illness, based on blood cultures from patients who presented to Bacha Khan Medical Complex (BKMC) in Swabi.

Methodology

Study design and sample population

BKMC is the largest healthcare facility in Swabi, Khyber Pakhtunkhwa. It is a 400-bed tertiary care teaching hospital, and was the site of this 1-year retrospective cross-sectional study conducted between September 2022 and August 2023. BKMC has cutting edge diagnostic and therapeutic treatments, and offers all major and minor specialties. This hospital receives patients from all around the Swabi district [20].

Febrile patients who were clinically suspected of having enteric fever were enrolled in our study. All participants remained anonymized during the course of the study. The BKMC, Swabi Institutional Review Board provided ethical clearance for the study. Informed consent was not required because the data for this retrospective analysis was sourced from routine medical checkups and patient privacy remained unaffected. Every procedure was carried out in compliance with the applicable rules and regulations.

Inclusion criteria

The following patients were enrolled in the trial because they met all of the eligibility requirements:

1) Individuals with fever (defined as a temperature higher than 37.5 °C) on presentation.

2) Individuals who, according to the treating physician, might have typhoid fever (as evidenced by the administration of the "Widal test," which has established restrictions) [1].

Exclusion criteria

The criteria for exclusion were

1) Individuals who had taken antibiotics in the 3 days before the consultation [1].

2) Repeat patients.

3) Individuals without complete test records or identity cards, which could compromise the ability to accurately identify and distinguish individuals, track their medical history, and ensure accuracy of study outcomes [21].

Blood specimen collection, culturing, and identification

Venous blood samples from each research

participant (n = 274) were taken using sterile disposable syringes by skilled laboratory professionals in accordance with the protocol (20 mL from adults, 1 mL for children under 1 year, and 1 mL/kg for children over 1 year) [22]. Sterile blood samples were collected and transferred to BACTEC™ (BD BACTEC, Sparks, Maryland, USA) culture bottles [23]. Demographic information about each patient, such as age and gender, was recorded.

The BACTEC™ culture bottles were incubated at 37 °C for up to 5 days. Following their development on BACTEC™, the isolates were re-inoculated on traditional culture media, including Mac Conkey agar (MA) and blood agar (BA). The inoculated culture plates were incubated at 37 °C for 24 hours. The non-hemolytic smooth white colonies were observed on BA plates, while the non-lactose fermenting colonies were observed on MA plates. Colony morphology, Gram staining, and biochemical assays such as the methyl red (MR), oxidase, catalase, Voges-Proskauer (VP), citrate utilization, and triple sugar iron (TSI) tests were used to identify the isolates [23,24]. Biochemical tests included in the API-10S biochemical kit system (bioMerieux, Lyon, France) were used for conclusive bacterial identification [25].

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was carried out using the modified Kirby-Bauer disk diffusion technique in compliance with the standards set forth by the Clinical and Laboratory Standard Institute (CLSI) [26]. Briefly, 3 to 5 morphologically identical colonies of pure culture bacteria were mixed with 5 mL of physiological saline (0.85% NaCl) to form a standard inoculum that was similar to the 0.5 MacFarland standard. The suspension was evenly inoculated on Mueller-Hinton agar (Oxoid, Basingstoke, England) plates [27]. Antimicrobial disks containing amoxicillin (10 µg), azithromycin (15 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), 2 ampicillin (10 µg), imipenem (10 µg), and meropenem (10µg) [28] were placed on the plates using sterile forceps, and were separated by 15 mm from the edge and 24 mm from each other. The plates were inverted and let to sit at room temperature for 5 minutes in order to allow the antimicrobials to permeate into the agar medium. After that, they were incubated for 24 hours at 37 °C. The CLSI criteria were used to classify the disks as resistant or sensitive, based on the diameters of the zone of bacterial growth inhibition surrounding them, which were measured using a digital caliper to the closest millimeter [26,27].

Minimum inhibitory concentration (MIC) calculation

The agar-dilution technique was employed to calculate the minimum inhibitory concentration for azithromycin and ciprofloxacin, in accordance with CLSI standards [2]. This approach involved preparing Muller-Hinton Agar (MHA) plates with different concentrations of azithromycin and ciprofloxacin, and inoculating the test organisms on the agar surface with varying concentration of the antibiotics (from 0.06 µg/mL to 32 µg/mL). The inoculated plates were incubated for 18 to 20 hours at 37 °C [23]. The lowest concentration at which an antibiotic stopped the development of bacterial growth, after adequate incubation, was the MIC [25]. The findings were classified as "sensitive" or "resistant" based on CLSI-approved breakpoints [23,26]. The *Escherichia coli* (*E. coli*) strain ATCC 25922 was used as control [25].

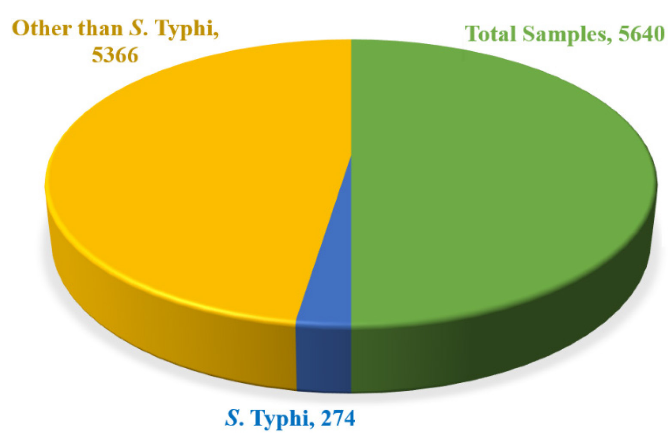
Quality assurance

The primary investigator instructed each data collector prior to data collection. Daily checks were conducted to ensure the accuracy and dependability of the data collected. Standard operating procedures (SOPs), were strictly adhered to throughout the sample collection, storage, and analysis. Reference strains from the American Type Culture Collection (ATCC), such as *E. coli* (ATCC-25922), *Staphylococcus aureus* (*S. aureus*) (ATCC-25923), and *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC-27853), were used to evaluate the quality of the culture media and antimicrobial disks [27].

Data processing and analysis

The data were imported into Microsoft Office Excel 2016 and analyzed using the Statistical Package for Social Sciences (SPSS) version 24.0 (IBM Corp,

Figure 1. Distribution of *Salmonella enterica Typhi* in the cultured blood specimens.



Armonk, NY, USA) [23]. Prevalence of the pathogen was calculated by dividing the frequency of positive samples by the total number of samples examined [25]. The Chi squared (χ^2) test was used to predict the connection between the variables. A *p*-value of less than 0.05 was considered statistically significant [23].

Results

Among the tested isolates, 4.85% (274/5640) tested positive for *S. Typhi*; while 95.14% (5366/5640) had growth of microorganisms other than *S. Typhi*, including *S. aureus*, *E. coli*, *Klebsiella pneumonia*, *P. aeruginosa*, *Enterococcus* species, *Enterobacter* species etc (Figure 1).

Monthly distribution of S. Typhi positive cases

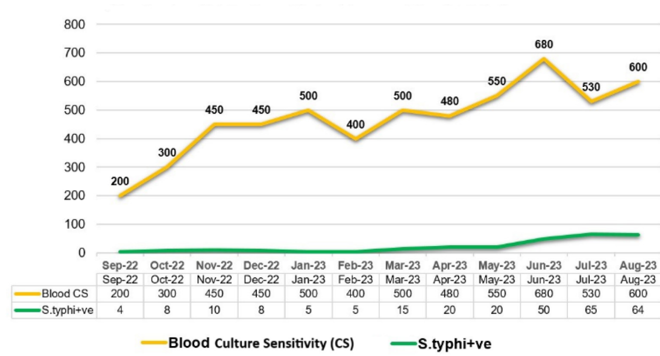
Among the *S. Typhi* positive cases, 22/5640 were isolated between September and November 2022; the maximum number (219/5640) were isolated between April and August 2023, and the lowest number (33/5640) between December 2022 and March 2023 (Figure 2).

Distribution of samples based on age group and gender

Male and female individuals accounted for 56.73% (3200/5640) and 43.26% (2440/5640) of the total samples collected, respectively. The growth positive rates—the proportion of male and female patients whose samples tested positive for bacterial infection after incubation—were 66.78% (183/274) and 33.21% (91/274), for the male and female patients, respectively. The burden of isolates and cases was significantly correlated with gender.

With regard to age-group distribution, 155 (56.56%) samples were isolated from patients who were 0–14 years old, 86 (31.38%) from patients who were 15–30 years old, and 33 (12.04%) from patients who were 31–60 years old. The total prevalence of enteric fever with culture confirmation was 4.85%. Pediatric patients (< 14 years) had a higher prevalence of enteric fever. The burden of cases and isolates was also significantly correlated with the age groups of the patients from whom the samples were collected (Table 1).

Figure 2. Isolation of *Salmonella enterica Typhi* from blood specimens by month.



Antibiotic susceptibility pattern of Salmonella isolates

The results of the antimicrobial susceptibility tests showed that all 274 (100%) isolates of *S. Typhi* were susceptible to azithromycin, meropenem, and imepenem. 100% (274) of the isolates showed resistance to cefotaxime, ciprofloxacin, ampicillin, and amoxicillin; however, ceftriaxone resistance was identified in 95.8% isolates, and only 4.2 % isolates were sensitive to ceftriaxone. The antibiotics susceptibility and resistance ranged from 0 to 100% (Figure 3).

Figure 3. Antibiotic susceptibility profile of *Salmonella enterica Typhi* isolated from blood specimens (n = 274).

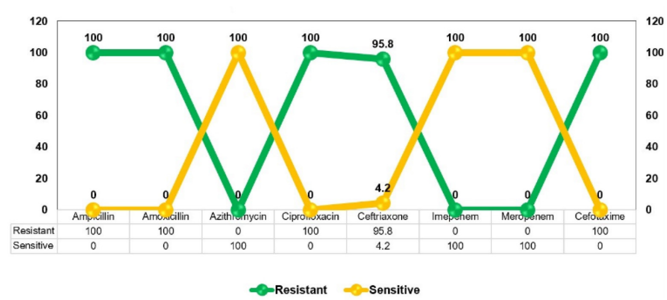


Table 1. Distribution of *Salmonella enterica Typhi* isolates according to age and gender of patients.

	Number of blood cultures n (%)	*Salmonella Typhi* isolated n (%)	*p* value
Gender			
Male	3200 (56.73)	183 (66.78)	0.0010*
Female	2440 (43.26)	91 (33.21)	
Total	5640	274	
Age group (years)			
0–14	2970 (52.65)	155 (56.56)	0.0093*
15–30	1574 (27.90)	86 (31.38)	
31–60	1096 (19.43)	33 (12.04)	

*Statistically significant.

Table 2. Comparison of susceptibility to ciprofloxacin using the disc diffusion method and ciprofloxacin minimum inhibitory concentration (MIC).

Test performed	Total n (%)	Sensitivity to ciprofloxacin n (%)		
		Sensitive	Resistant	*p* value
Disc diffusion method	274 (100)	0	274 (100)	0.08
MIC	274 (100)	3 (1.094)	271 (98.90%)	

Minimum inhibitory concentrations for azithromycin and ciprofloxacin

The MIC values for azithromycin and ciprofloxacin ranged from 0.06 µg/mL to 16 µg/mL. The tests indicated that 1.094% (3/274) of the isolates with MIC of < 0.06 µg/mL for ciprofloxacin were susceptible, while 98.90% (271/274) of the isolates with MIC of ≥ 1 µg/mL were resistant. None of the isolates in the disc diffusion method susceptibility assay were susceptible to ciprofloxacin (Table 2). On the other hand, all the isolates with MICs of ≤ 8 µg/mL for azithromycin were susceptible.

Discussion

Enteric fever is one of the primary endemic illnesses in LMICs such as Pakistan [3]. Pakistan faces multiple challenges in managing the disease burden and increasing drug resistance as a result of inadequate surveillance on the occurrence of enteric fever and the lack of reliable data on AMR. This investigation was carried out to address a research void in the country, and to quantify the occurrence of enteric fever and the drug-resistant nature of the bacteria isolated from suspected patients [23] who spent a year in a tertiary care facility in Swabi City.

The frequency of *S. Typhi* in febrile patients in our study was 4.85% [95% confidence interval]. This frequency was less than that in a study carried out in Karachi, Pakistan, in which the prevalence was 22% [29]. The prevalence in this study was also lower when compared to previous research conducted in various locations such as Ethiopia (5%) [30] and Indonesia (15.5%) [31]; but higher than previous findings in Lalitpur (4.1%) [32], India (2.5%) [33], and Nepal (1.2%) [34]. This variation may be attributed to factors such as the geographical location, differences in the study population, timing of the studies, and variations in laboratory investigation techniques; all of which can influence the outcomes [1].

In our study, we found the highest prevalence of typhoid among children below 14 years of age, which is consistent with the other studies [2,35–37]. However, this finding was not in agreement with research carried out in Ethiopia and Nigeria [38,39]. This may be due to the age distribution of the research participants, study duration, study region, and other factors [27]. However, our study indicated that age groups were a significant factor [23].

In our research, we observed a higher prevalence of typhoid fever during the period from April to August 2023. Enteric fever typically peaks both in the summer and in the wet season. This seasonal variation is linked to events such as floods, and the possibility of food and water contamination from seepage from sewers and water treatment facilities, which increases the frequency of typhoid cases. This result is consistent with a previous report from Nepal [23].

Furthermore, our study indicated a significant relationship between gender and disease prevalence, similar to the study in Nepal [23]. We found higher prevalence of cases in males (66.78%) compared to females (33.21%). Males tend to have enteric fever more commonly than females, which makes sense given that they spend more time outside and are therefore more likely to come in contact with potential infection sources. Studies from Nepal [23] and Pakistan [40] have reported similar findings.

In this investigation, we found that *S. Typhi* was resistant to several tested antibiotic classes. Given the rising incidence of AMR *Salmonella* isolates, use of a single antibiotic is no longer an effective strategy to combat the problem [23]. The majority of the *S. Typhi* isolates in our investigation displayed increased resistance to ciprofloxacin, ampicillin, amoxicillin, and cefotaxime; in accordance with several other studies [1,27,41]. Moreover, our research findings were also comparable to that of earlier studies carried out in Ethiopia [42] and Kenya [43]. This increase in antibiotic resistance among the isolates may be brought on by the accessibility and handling of these medications from pharmacies and drug stores, as well as a lack of knowledge on the proper use of antimicrobials [1]. In our study, the antibiotic profile of *S. Typhi* isolates showed maximum sensitivity to azithromycin, meropenem, and imipenem; similar to some other studies [2,23,28,41,44].

Our MIC analyses showed that 3 isolates were susceptible and 271 isolates were resistant to ciprofloxacin. However, no isolate was susceptible to ciprofloxacin in the disc diffusion test; although 3 isolates showed MIC breakpoint susceptibility. Our findings are supported by a previously published study, which was carried out between 2002 and 2014 [45]. Multiple research studies have reported a progressive rise in ciprofloxacin resistance. Ciprofloxacin susceptibility in *S. Typhi* has been reported to vary from 5% in 1993–2003 [46] to 0% in 2011–2012 [47], indicating complete resistance by that period. Furthermore, between 2012 and 2016, 93.7% of isolates were classified as non-susceptible (including both resistant and intermediate strains), leaving only 6.3% fully susceptible [48]. Therefore, the increasing resistance to ciprofloxacin negates its empirical use among the study age groups. On the other hand, MIC analyses indicated that all the isolates were susceptible to azithromycin, which is similar to the findings of Khanal *et al.* [49] and Khadka *et al.* [2]. Therefore, azithromycin can be used as an empirical treatment for salmonellosis.

Azithromycin susceptibility has been reported in multiple studies [23,48,50]. Nevertheless, all the isolates examined in this investigation exhibited azithromycin sensitivity. Thus, azithromycin appears to be a suitable empirical therapy choice in South and Southeast Asia based on current AMR profiles. Oral azithromycin may be more practical and affordable for outpatient care, than parenteral ceftriaxone; nonetheless, the drug's widespread usage in many regions of South Asia may soon result in the development of resistance, underscoring the significance of effective microbiological surveillance [51]. In addition to this, we found that the isolates were sensitive to carbapenem (imipenem and meropenem), similar to the findings from other studies [41,44,52]. However, the isolates showed high level of resistance to conventional first-line antibiotics (ampicillin, amoxicillin), and third-generation cephalosporin antibiotics (ceftriaxone, cefotaxime), that are considered to be the key components of typhoid treatment [53].

Limitations

Our investigation had some limitations. First, it was a single-center study. Clinical assessments of a sizable research population within multicentral healthcare facilities would have been more comprehensive. Second, even though this was a hospital-based study, we were unable to assess the treatment outcomes and risk variables in our particular contexts. Third, the bacterial isolates were neither genotyped nor serotyped by us. More molecular research is advised in subsequent investigations in order to clarify the potential mechanisms.

Conclusions

In our study, the total incidence of enteric fever with culture confirmation was 4.85%. Age groups and gender were significant factors related to enteric fever. Enteric fever was more prevalent in the age group of 0–14 years. There was a seasonal variation with the summer and monsoon months showing the highest prevalence, and a higher proportion of men being affected by the disease. Azithromycin and carbapenem were identified as suitable empirical therapy choices. However, conventional first-line antibiotics (ampicillin, amoxicillin), second generation fluoroquinolone (ciprofloxacin), and third-generation cephalosporin antibiotics (ceftriaxone, cefotaxime) that are considered as vital in typhoid treatment, were found to be highly resistant.

Therefore, it is important to reevaluate these antibiotics (azithromycin and carbapenem) as implicated medicines in clinical care. MIC analysis indicated that ciprofloxacin showed more resistance than sensitivity, while azithromycin showed more sensitivity to all the tested isolates. No MDR strains were identified. From a diagnostic perspective, utilizing the MIC to identify drug resistance can be more cost-effective than relying solely on disk diffusion. When available, MIC results should be taken into consideration for making judgments on oral antibiotic follow-up.

We hope that this report will inspire more epidemiologic research and investigations into the antimicrobial susceptibility of infections to lessen the impact of *Salmonella* infections. Additionally, we hope that this information will help develop more effective strategies that can be used by public health organizations, the veterinary industry, and the food industry. Continuous surveillance of antibiotic use is required in Pakistan. This surveillance with help track alterations in susceptibility patterns, inform empirical treatment decisions, fortify antibiotic stewardship initiatives, and thwart the emergence of bacteria resistant to antibiotics. It is advised to conduct more research, which should involve genotyping, serotyping, and antimicrobial susceptibility testing utilizing MIC and various salmonellosis risk variables with a sizable sample size from various regions of the country.

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Authors' contributions

Conceptualization: ZK; methodology: ZK, NS, RoA, SAS, SAK, SK, RuA, MR; investigation: NS, RoA; data curation: ZK, NS, RoA; formal analysis: ZK, NS, RoA, RuA; visualization: ZK; validation: ZK, NS, RoA, SAS; writing–original draft: ZK; writing–review and editing: ZK, SAS, SAK, SK, RuA, HA; project administration: ZK, SAS; resources: SAS; supervision: ZK.

Ethical considerations

This study was approved by Ethical Review Board of GKMC/ BKMC Swabi (File No. 2251/ Ethical Board/ GKMC). Informed consent was not required because this was a retrospective study using routine patient examination data and patient privacy was not at risk.

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Conflict of interests

No conflict of interests is declared.

References

1. Awol RN, Reda DY, Gidebo DD (2021) Prevalence of *Salmonella enterica* serovar Typhi infection, its associated factors and antimicrobial susceptibility patterns among febrile patients at Adare general hospital, Hawassa, southern Ethiopia. BMC Infect Dis 21: 30. doi: 10.1186/s12879-020-05726-9.
2. Khadka P, Thapaliya J, Thapa S (2021) Susceptibility pattern of *Salmonella enterica* against commonly prescribed antibiotics, to febrile-pediatric cases, in low-income countries. BMC Pediatr 21: 38. doi: 10.1186/s12887-021-02497-3.
3. Gibani MM, Britto C, Pollard AJ (2018) Typhoid and paratyphoid fever: a call to action. Curr Opin Infect Dis 31: 440–448. doi: 10.1097/QCO.0000000000000479.
4. Qamar MU, Lopes BS, Hassan B, Khurshid M, Shafique M, Atif Nisar M, Nisar MA, Mohsin M, Nawaz Z, Muzammil S, Aslam B, Ejaz H, Toleman MA (2020) The present danger of New Delhi metallo-β-lactamase: a threat to public health. Future Microbiol 15: 1759–1778. doi: 10.2217/fmb-2020-0069.
5. Browne AJ, Hamadani BHK, Kumaran EAP, Rao P, Longbottom J, Harriss E, Moore CE, Dunachie S, Basnyat B, Baker S, Lopez AD, Day NPJ, Hay SI, Dolecek C (2020) Drug-resistant enteric fever worldwide, 1990 to 2018: a systematic review and meta-analysis. BMC Med 18: 1–22. doi: 10.1186/s12916-019-1443-1.
6. John J, Bavdekar A, Rongsen-Chandola T, Dutta S, NSSEFI Collaborators, Kang G (2018) Estimating the incidence of enteric fever in children in India: a multi-site, active fever surveillance of pediatric cohorts. BMC Public Health 18: 594. doi: 10.1186/s12889-018-5498-2.
7. Holt KE, Phan MD, Baker S, Duy PT, Nga TVT, Nair S, Turner AK, Walsh C, Fanning S, Farrell-Ward S, Dutta S, Kariuki S, Weill FX, Parkhill J, Dougan G, Wain J (2011) Emergence of a globally dominant inchi1 plasmid type associated with multiple drug-resistant typhoid. PLoS Negl Trop Dis 5: e1245. doi: 10.1371/journal.pntd.0001245.
8. Crump JA, Karlsson MS, Gordon MA, Parry CM (2015) Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. Clin Microbiol Rev 28: 901–937. doi: 10.1128/CMR.00002-15.
9. Mogasale V, Maskery B, Ochiai RL, Lee JS, Mogasale VV, Ramani E, Kim YE, Park JK, Wierzba TF (2014) Burden of typhoid fever in low-income and middle-income countries: a systematic, literature-based update with risk-factor adjustment. Lancet Glob Health 2: e570–e80. doi: 10.1016/S2214-109X(14)70301-8.
10. Hosoglu S, Aldemir M, Akalin S, Geyik MF, Tacyildiz IH, Loeb M (2004) Risk factors for enteric perforation in patients with typhoid fever. Am J Epidemiol 160: 46–50. doi: 10.1093/aje/kwh172.
11. World Health Organization (2003) Background document: the diagnosis, treatment and prevention of typhoid fever: Available: https://apps.who.int/iris/handle/10665/68122. Accessed: 12 April 2024.
12. Steele AD, Hay Burgess DC, Diaz Z, Carey ME, Zaidi AKM (2016) Challenges and opportunities for typhoid fever control: a call for coordinated action. Clin Infect Dis 62 Suppl 1: S4–S8. doi: 10.1093/cid/civ976.
13. Batool A, Yunus N, Yaqoob A, Lone DS, Khalid A, Ejaz H, Qamar MU, Ahmed Q (2021) Prevalence of multi-drug resistant and extensively drug-resistant *Salmonella enterica* serovar Typhi recovered from pediatrics' septicemia patients in Lahore. Pak J Med Health Sci 15: 843–845.
14. Dyson ZA, Klemm EJ, Palmer S, Dougan G (2019) Antibiotic resistance and typhoid. Clin Infect Dis 68 Suppl 2: S165–S70. doi: 10.1093/cid/ciy1111.
15. Msemo OA, Mbwana J, Mahende C, Malabeja A, Gesase S, Crump JA, Dekker D, Lusingu JPA (2019) Epidemiology and antimicrobial susceptibility of *Salmonella enterica* bloodstream isolates among febrile children in a rural district in northeastern Tanzania: a cross-sectional study. Clin Infect Dis 68 Suppl 2: S177–S182. doi: 10.1093/cid/ciy1126.
16. Britto CD, Dyson ZA, Duchene S, Carter MJ, Gurung M, Kelly DF, Murdoch DR, Ansari I, Thorson S, Shrestha S, Adhikari N, Dougan G, Holt KE, Pollard AJ (2018) Laboratory and molecular surveillance of paediatric typhoidal *Salmonella* in Nepal: antimicrobial resistance and implications for vaccine policy. Plos Negl Trop Dis 12: 1–19. doi: 10.1371/journal.pntd.0006408.
17. Kelly DF, Thorson S, Maskey M, Mahat S, Shrestha U, Hamaluba M, Williams E, Dongol S, Werno AM, Portess H, Yadav BK, Adhikari N, Guiver M, Thomas K, Murdoch DR, Pollard AJ (2011) The burden of vaccine-preventable invasive bacterial infections and pneumonia in children admitted to hospital in urban Nepal. Int J Infect Dis 15: e17–23. doi: 10.1016/j.ijid.2010.05.021.
18. Pradhan R, Shrestha U, Gautam SC, Thorson S, Shrestha K, Yadav BK, Kelly DF, Adhikari N, Pollard AJ, Murdoch DR (2012) Bloodstream infection among children presenting to a general hospital outpatient Clinic in Urban Nepal. Plos One 7: e47531. doi: 10.1371/journal.pone.0047531.
19. Fida S, Mansoor H, Saif S, Iqbal J, Khan AQ (2021) Clinical perspectives of multiple and extensively drug-resistant typhoid; result from a tertiary care hospital from Pakistan. J Infect Dev Ctries 15: 530–537. doi: 10.3855/jidc.13539.

20. Bacha Khan Medical Complex (nd) Bacha Khan Medical Complex, Swabi. Available: https://bkmcs.edu.pk/home/. Accessed: 3 March 2024.
21. Wang C, Li X, Zhang C, Xiao L, Xian J (2024) Prevalence and influential factors of isolated hepatitis B core antibody positivity in a Chinese adult population. Sci Rep 14: 693. doi: 10.1038/s41598-023-50907-6.
22. Cheesbrough M (2005) District laboratory practice in tropical countries. 2nd ed. Cambridge: Cambridge University Press. 297–298. doi: 10.1017/CBO9780511581304.
23. Maharjan A, Dhungel B, Bastola A, Shrestha UT, Adhikari N, Banjara MR, Lekhak B, Ghimire P, Rijal KR (2021) Antimicrobial susceptibility pattern of *Salmonella* spp. isolated from enteric fever patients in Nepal. Infect. Dis Rep 13: 388–400. doi: 10.3390/idr13020037.
24. Isenberg, H.D (2004) Clinical microbiology procedures handbook, 2nd ed. Washington, DC: ASM Press 2840 p.
25. Anvarinejad M, Pouladfar GR, Pourabbas B, Shahidi MA, Rafaatpour N, Dehyadegari MA, Abbasi P, Mardaneh J (2016) Detection of *Salmonella* spp. with the BACTEC 9240 Automated Blood Culture System in 2008– 2014 in Southern Iran (Shiraz): biogrouping, MIC, and antimicrobial susceptibility profiles of isolates. Jundishapur J Microbiol 9: e26505. doi: 10.5812/jjm.26505.
26. Clinical Laboratory Standards Institute (CLSI) (2014) Performance standards for antimicrobial susceptibility testing (Twenty-Fourth Informational Supplement. CLSI document M100-S24). Wayne, PA: Clinical and Laboratory Standards Institute. 50–57.
27. Admassu D, Egata G, Teklemariam Z (2019) Prevalence and antimicrobial susceptibility pattern of *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi among febrile patients at Karamara Hospital, Jigjiga, eastern Ethiopia. SAGE Open Med 7: 1–7. doi: 10.1177/2050312119837854.
28. Shah SAA, Nadeem M, Syed SA, Abidi STF, Khan N, Bano N (2020) Antimicrobial sensitivity pattern of *Salmonella* Typhi: emergence of resistant strains. Cureus 12: e11778. doi: 10.7759/cureus.11778.
29. Yousafzai MT, Irfan S, Thobani RS, Kazi AM, Hotwani A, Memon AM, Iqbal K, Qazi SH, Saddal NS, Rahman N, Dehraj IF, Hunzai MJ, Mehmood J, Garrett DO, Saha SK, Andrews JR, Luby SP, Qamar FN (2020) Burden of culture confirmed enteric fever cases in Karachi, Pakistan: surveillance for enteric fever in Asia Project (SEAP), 2016–2019. Clin Infect Dis 71 Suppl 3: S214–S221. doi: 10.1093/cid/ciaa1308.
30. Habte L, Tadesse E, Ferede G, Amsalu A (2018) Typhoid fever: clinical presentation and associated factors in febrile patients visiting Shashemene Referral Hospital, southern Ethiopia. BMC Res Notes 11: 605. doi: 10.1186/s13104-018-3713-y.
31. Judio MP, Karyanti M, Waslia L, Subekti D, Supriyatno B, Baird K (2017) Antimicrobial susceptibility among circulating *Salmonella* Typhi serotypes in children in Jakarta, Indonesia. Journal of Microbiology and Infectious Diseases 7: 29–35. doi: 10.5799/jmid.328842.
32. Pandey K, Sharma VK, Maharjan R (2015) Prevalence and antibiotic sensitivity test of *Salmonella* serovars from enteric fever suspected patients visiting Alka Hospital, Lalitpur. Current Research in Microbiology 6: 40–43. doi: 10.3844/ajmsp.2015.40.43.
33. Kumar S, Rizvi M, Berry N (2008) Rising prevalence of enteric fever due to multi-drug-resistant *Salmonella*: an epidemiological study. J Med Microbiol 57: 1247–1250. doi: 10.1099/jmm.0.2008/001719-0.
34. Raza S, Tamrakar R, Bhatt CP, Joshi SK (2012) Antimicrobial susceptibility patterns of *S.* Typhi and *S.* paratyphi A in a tertiary care hospital. Journal of Nepal Health Research Council 10: 214–217. doi: 10.33314/jnhrc.v0i0.335.
35. Britto CD, Wong VK, Dougan G, Pollard AJ (2018) A systematic review of antimicrobial resistance in *Salmonella enterica* serovar Typhi, the etiological agent of typhoid. PLoS Negl Trop Dis 12: e0006779. doi: 10.1371/journal.pntd.0006779.
36. Marks F, Kalckreuth VV, Aaby P, Adu-Sarkodie Y, Tayeb MAE, Ali M, Aseffa A, Baker S, Biggs HM, Bjerregaard-Andersen M, Breiman RF, Campbell JI, Cosmas L, Crump JA, Espinoza LMC, Deerin JF, Dekker DM, Fields BS, Gasmelseed N, Hertz JT, Hoang NVM, Im J, Jaeger A, Jeon HJ, Kabore LP, Keddy KH, Konings F, Krumkamp R, Ley B, Løfberg SV, May J, Meyer CG, Mintz ED, Montgomery JM, Niang AA, Nichols C, Olack B, Pak GD, Panzner U, Park JK, Park SE, Rabezanahary H, Rakotozandrindrainy R, Raminosoa TM, Razafindrabe TJL, Sampo E, Schütt-Gerowitt H, Sow AG, Sarpong N, Seo HJ, Sooka A, Soura AB, Tall A, Teferi M, Thriemer K, Warren MR, Yeshitela B, Clemens JD, Wierzba TF (2017) Incidence of invasive *Salmonella* disease in sub-Saharan Africa: a multicentre population-based surveillance study. Lancet Glob Health 5: e310–e323. doi: 10.1016/S2214-109X(17)30022-0.
37. Antillón M, Warren JL, Crawford FW, Weinberger DM, Kürüm E, Pak GD, Marks F, Pitzer VE (2017) The burden of typhoid fever in low- and middle-income countries: a meta-regression approach. PLoS Negl Trop Dis 11: e0005376. doi: 10.1371/journal.pntd.0005376.
38. Andualem G, Abebe T, Kebede N, Gebre-Selassie S, Mihret A, Alemayehu H (2014) A comparative study of Widal test with blood culture in the diagnosis of typhoid fever in febrile patients. BMC Res Notes 7: 653. doi: 10.1186/1756-0500-7-653.
39. Akinyemi K, Smith S, Oyefolu A, Coker A (2005) Multidrug resistance in *Salmonella enterica* serovar Typhi isolated from patients with typhoid fever complications in Lagos, Nigeria. Public Health 119: 321–327. doi: 10.1016/j.puhe.2004.04.009.
40. Qamar FN, Yousafzai MT, Khalid M, Kazi AM, Lohana H, Karim S, Khan A, Hotwani A, Qureshi S, Kabir F, Aziz F, Memon NM, Domki MH, Hasanet R (2018) Outbreak investigation of ceftriaxone-resistant *Salmonella enterica* serotype Typhi and its risk factors among the general population in Hyderabad, Pakistan: a matched case-control study. Lancet Infect Dis 18: 1368–1376. doi: 10.1016/S1473-3099(18)30483-3.
41. Umair M, Siddiqui SA (2020) Antibiotic susceptibility patterns of *Salmonella* Typhi and *Salmonella* Paratyphi in a tertiary care hospital in Islamabad. Cureus 12: e10228. doi: 10.7759/cureus.10228.
42. Dagnew M, Yismaw G, Gizachew M, Gadisa A, Abebe T, Tadesse T (2013) Bacterial profile and antimicrobial susceptibility pattern in septicemia suspected patients attending Gondar University Hospital, Northwest Ethiopia. BMC Res Notes 6: 283. doi: 10.1186/1756-0500-6-283.
43. Mengo D, Kariuki S, Muigai A, Revathi G (2010) Trends in *Salmonella enterica* serovar Typhi in Nairobi, Kenya from 2004 to 2006. J Infect Dev Ctries 4: 393–396. doi: 10.3855/jidc.503.

44. Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, Wong VK, Dallman TJ, Nair S, Baker S, Shaheen G, Qureshi S, Yousafzai MT, Saleem MK, Hasan Z, Dougan G, Hasan R (2018) Emergence of an extensively drug-resistant *Salmonella enterica* serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. mBio 9: e00105-18. doi: 10.1128/mBio.00105-18.
45. Zellweger RM, Basnyat B, Shrestha P, Prajapati KG, Dongol S, Sharma PK, Koirala S, Darton TC, Dolecek C, Thompson CN, Thwaites GE, Baker SG, Karkey A (2017) A 23-year retrospective investigation of *Salmonella* Typhi and *Salmonella* Paratyphi isolated in a tertiary Kathmandu hospital. PLoS Negl Trop Dis 11: e0006051. doi: 10.1371/journal.pntd.0006051.
46. Maskey AP, Basnyat B, Thwaites GE, Campbell JI, Farrar JJ, Zimmerman MD (2008) Emerging trends in enteric fever in Nepal: 9124 cases confirmed by blood culture 1993–2003. Trans R Soc Trop Med Hyg 102: 91–95. doi: 10.1016/j.trstmh.2007.10.003.
47. Chand HJ, Rijal KR, Neupane B, Sharma VK, Jha B (2014) Re-emergence of susceptibility to conventional first line drugs in *Salmonella* isolates from enteric fever patients in Nepal. J. Infect. Dev. Ctries 8: 1483–1487. doi: 10.3855/jidc.4228.
48. Joshi RD, Khadka S, Joshi DM, Shrestha B, Dangal G, Acharya KP, Shrestha S, Dongol Y (2018) Antimicrobial sensitivity trend in blood culture positive enteric fever. J. Nepal. Health Res. Counc 16: 228–232. doi: 10.33314/jnhrc.v16i2.1377.
49. Khanal PR, Satyal D, Bhetwal A, Maharjan A, Shakya S, Tandukar S, Parajuli NP (2017) Renaissance of conventional first-line antibiotics in *Salmonella enterica* clinical isolates: assessment of MICs for therapeutic antimicrobials in enteric fever cases from Nepal. Biomed Res Int 2017: 2868143. doi: 10.1155/2017/2868143.
50. Bhetwal A, Maharjan A, Khanal PR, Parajuli NP (2017) Enteric fever caused by *Salmonella enterica* serovars with reduced susceptibility of fluoroquinolones at a community-based teaching hospital of Nepal. Int J Microbiol 2017: 2869458. doi: 10.1155/2017/2869458.
51. Gibani MM, Britto C, Pollard AJ (2018) Typhoid and paratyphoid fever: a call to action. Curr Opin Infect Dis 31: 440–448. doi: 10.1097/QCO.0000000000000479.
52. Chatham-Stephens K, Medalla F, Hughes M, Appiah GD, Aubert RD, Caidi H, Angelo KM, Walker AT, Hatley N, Masani S, Nash J, Belko J, Ryan ET, Mintz E, Friedman CR (2019) Emergence of extensively drug-resistant *Salmonella* Typhi infections among travelers to or from Pakistan-United States, 2016-2018. Morb Mortal Wkly Rep 68: 11–13. doi: 10.15585/mmwr.mm6801a3.
53. Lin-Hui S, Cheng-Hsun C, Chishih C, Jonathan TO (2004) Antimicrobial resistance in nontyphoid *Salmonella* serotypes: a global challenge. Clin Infect Dis 39: 546–551. doi: 10.1086/422726.