ORIGINAL ARTICLE

FREQUENCY AND ANTIMICROBIAL RESISTANCE PROFILE OF SALMONELLA TYPHI ISOLATED FROM DISTRICT BUNER

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ABSTRACT

Objective: The objective of this study was to investigate the frequency and drug resistance of Salmonella typhi in district Buner, Khyber Pakhtunkhwa.

Material & Methods: This cross-section study was conducted at District Head Quarter Hospital Buner and private diagnostic centers from Jan 2020 to May 2021. A total of 460 blood samples were collected from OPD patients reported with signs and symptoms of Typhoid fever. Samples were cultured on blood and MacConkey agar, incubated at 37^{O} for 24 hours. Bacterial identification was done on colony morphology, gram staining, and polyvalent antisera for strain identification. Finally, antibiotic susceptibility testing was done for the isolated bacteria. All commonly prescribed first-line and second option antibiotics were tested on Mueller Hinton agar by Kirby Bauer disk diffusion method following CLSI guidelines 2020.

Result: A total of 240 out of 460 samples showed bacterial growth where Salmonella typhi was found in 98 (40.8%) cases. Among them, 28(28.6%) were reported as MDR while 1 (17.4%) were XDR. Out of the XDR, 4 strains were found resistant to Vancomycin and clarithromycin as well. The isolated S. typhi was 100% sensitive to Meropenem, and Azithromycin, however showed 100% resistance to Aztreonam, Ampicillin, and Cotrimoxazole. Prevalence of S. typhi was more (50%) in females while resistance was reported more in age group 20-40 years.

Conclusion: The frequency of Salmonella typhi at district Buner was 41% in patients reported with high grade fever where 28% of the strains reported were multidrug resistant. Some extended spectrum drug-resistant S. typhi and Vancomycin and Clarithromycin resistant strains were also reported which is alarming. Meropenem, and Azithromycin were the only drugs effective against most of the strains. However, resistance to these drugs may develop soon if we do not stop empirical excessive use of antibiotics.

Keywords: Multidrug resistant, Salmonella typhi, Typhoid fever, Extended spectrum drug-resistant

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INTRODUCTION

Enteric fever is caused by gram-negative bacteria, Salmonella enterica serovar typhi, and paratyphi. The disease is transmitted primarily by fecal-oral route predominantly via contaminated water and food [1, 2]. Typhoid fever is an acute febrile illness characterized by headache, nausea, anorexia, vomiting, constipation, and sometimes diarrhea [2, 3]. Mortality due to typhoid ranges from 1% to 3%, while global morbidity and mortality is reported more than 27 million per year and 200,000 per year respectively [4]. S. typhi infection is more common in South America, Asia, and Africa. According to a recent research, the prevalence of typhoid fever in India is 493.5/100,000 followed by Pakistan 412.5, Indonesia 180.3, Vietnam 24.4 and china 29.3 [4, 5].

Antibacterial drugs are recommended for the treatment of enteric fever worldwide. Unfortunately antibiotics resistance of Salmonella typhi has increased significantly during the last decade [1, 6]. Initially, the first-line anti-salmonella drugs were considered highly effective for the eradication of enteric fever which includes Ampicillin, Chloramphenicol, and Co-trimoxazole. However with the emergent of MDR, S. typhi in 1980, the first line drugs became useless and the pathogen developed resistance to all the three antibiotics. Thus treatment options changed to quinolones which was considered highly effective and fluoroquinolones became the drug of choice for salmonellosis. Unfortunately, resistance against fluoroquinolone developed soon which was reported in 1992 [4, 7, 8].

In the prevalence of MDR strain of S. typhi, a

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geographic variation was observed during a surveillance study, a high prevalence of MDR strain was found in India, Pakistan, and Nepal while a decline rate was observed in China and Bangladesh, and Vietnam [1, 9]. Most of the Asian countries reported a decline in the incidence rate of MDR S. typhi, but unfortunately, the trend of drug resistance increases in Pakistan [10]. With the decline of MDR S. typhi in most of the South Asian countries, some researchers are working on the reuse and effectiveness of the first-line antibiotics again. This may require careful evaluation during clinical practice before it is started for the treatment of enteric fever [3, 11]. However, the resistance against fluoroquinolone is still very high, more than 90% in Pakistan, Bangladesh, India, and Nepal for the last several years [2, 12, 13].

With the rise in antibiotic resistance particularly against Ceftriaxone and fluoroquinolones, there are limited treatment options for S. typhi. Furthermore, the emergence of extensive drug resistant strains limited the available treatment options for S. typhi. XDR strains are resistant to fluoroquinolones and ceftriaxone in addition to first-line drugs [1, 9, 10].

These strains not only cause severe infection but also prolong hospital stay, cost of treatment and mortality among the infected individual [14]. The situation is alarming in the developing countries especially in the rural areas where the latest antibiotics are out of public reach (either expensive or not available) [15].

Physicians treat patients empirically based on their clinical diagnosis[16], and never consider culture and sensitivity either due to lack of the facility, or cost. These factors contribute to the underreporting of actual blood culture-positive typhoid cases [17, 18].

Although typhoid fever is endemic in most of the South Asian countries, however population-based antimicrobial resistance surveillance is lacking. The surveillance study conducted by "surveillance for enteric fever in Asia Project" (SEAP) was a large (2016-2019), and multicenter study which included Pakistan to report the actual picture of S. typhi infection.

In Pakistan, urban areas of Sindh and Punjab got some attention [19] however, data from rural areas of Khyber Pakhtunkhwa and Baluchistan is limited regarding the prevalence and antimicrobial susceptibility profile S. typhi. Therefore, the current study was designed to determine the frequency of enteric fever and antibiotic resistance patterns of S. typhi at a rural district Buner of Khyber Pakhtunkhwa.

MATERIALS & METHOD

This cross-sectional study was conducted at district hospital and some private labs of district Buner Khyber Pakhtunkhwa from Jan 2020 to May 2021. Patients of all age groups visiting hospital for fever at OPD and those admitted were included in the study. For indoor patient suspected or blood culture-confirmed case of enteric fever was included.

For the outdoor patient, those who advise blood culture and had a febrile illness fever for 3-7 days. Patients with known other cause of fever and those already on antibiotics were excluded. Ethical approval was obtained from the institutional ethical committee and an informed written consent was obtained from patients.

Blood samples for culture were collected using aseptic technique to avoid contamination. The blood samples were transferred to a blood culture media bottle (Oxoid) and incubated for 24 hours at 37°C.

After incubation, the blood samples were processed on BACTEC automated system. After that, gram staining was done for the positive samples and then cultured on blood agar, chocolate agar and MacConkey agar for colonial morphology and strain identification, Later the S. typhi was confirmed by serology testing where a multivalent antiserum (BD Difco TM Salmonella) was used against the H, O, and Vi antigens.

All positive isolates were tested for their antimicrobial susceptibility as per Clinical and Laboratory Standard Institute Guideline M100-ED-31, 2021. All those isolates were considered MDR if they show resistance to first-line drugs (Ampicillin/Amoxicillin, Cotrimoxazole, and Chloramphenicol) but susceptible to Fluoroquinolone. On the other hand, if the isolate shows resistance to first-line drugs plus third-generation Cephalosporin and Fluoroquinolone then they were considered extensive drugs resistant (XDR).

All data including socio-demographic information were entered in to a structure proforma. Descriptive analyses such as antimicrobial resistance by age, gender were calculated.

RESULTS

A total of 240 suspected blood samples were collected for S. Typhi isolation from Jan 2020 to May 2021. Out of 240 samples, 98(40.8%) showed growth of S. Typhi on culture media. Staph aureus was 6 (0.025%), Acinetobacter 3(0.012%), and E.coli 2(0.008%). A high prevalence of enteric fever was reported during the month of May to July.

Infection frequency of the study population was assessed over different age groups. The most common age group was between 31-40 years of age, however, age group 21-30 years also showed a high prevalence. The age and gender distribution of S. typhi infection is shown in table 1. Antimicrobial susceptibility profile of S. typhi; was assessed for all positive samples. Samples were streaked on Muller Hinton agar and nine commonly used

antibiotics were tested according to the CLSI guidelines 2020. The isolated S. typhi strains showed 100% resistance to Aztreonam and Ampicillin while they were 100% sensitive to Meropenem and Azithromycin as shown in the figure 1.

Furthermore, sensitivity of 10 samples from the XDR strains was tested against Clarithromycin, where 5 samples showed resistance. Similarly 7 samples from the XDR strains were tested against Vancomycin where 4 samples showed resistance. The Overall the frequency of MDR, XDR and non-MDR strains is shown in table 2

Table 1: Age and gender-wise frequency of S. typhi infection.

Age group/ Gender	≤ 10 Years	11 - 20 Years	21 - 30 Years	31 - 40 Years	41 - 50 Years	51 - 60 Years	61 - 70 Years	70 - 80 Years	Total
Males	5	12	12	9	3	3	2	2	48
Females	1	10	13	14	7	2	2	1	50
Total	6	22	25	23	10	5	4	3	95

Table 2: Frequency of Multi Drugs Resistant (MDR) and Extended Drug Resistant (XDR) strains (n=98).

Resistance pattern	Frequency	Percentage
Multi-drug Resistant	28	%28.6
Extended-drug Resistant	17	%17.4
Non-MDR*/XDR*	53	%54.0
Total	98	100

*MDR- multidrug resistant

*XDR- extended drug resistant

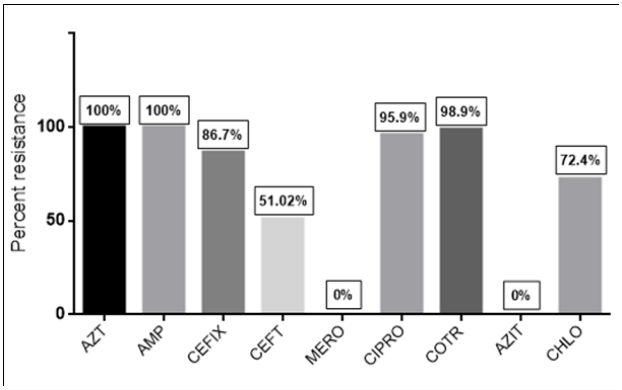

Fig 1: Percent antibiotic resistance of S.typhi

AZT=Aztreonam, AMP=Ampicillin, CEFIX=Cefixime, CEFT=Ceftriaxone, MERO=Meropenem, CIPRO=Ciprofloxacin, COTR=Cotrimoxazole, AZIT=Azithromycin, CHLO=Chloramphenicol

DISCUSSION

Typhoid fever is endemic in some parts of Khyber Pakhtunkhwa, however the resistant strains have increased recently due to the overuse of antibiotics. This study was carried out in a remote area of the Khyber Pakhtunkhwa, district Buner. If the resistant strains are increasing with such high rate, we would have no antibiotics to treat S. typhi in the near future.

In this study we found that S. typhi infection was seen in all age groups however, a high prevalence of enteric fever was reported in middle-aged people between 31-40 years and 21-30 years respectively. Similarly, cases were reported more in females as compared to males. A study conducted by Karkey et al. showed that the prevalence was more between 15-30 years of age which supported our findings, however there were more male patients which is against our findings[20]. This may be because some cultural differences as well as difference in hand hygiene practices of our rural females. Our study found that cases are reported more in May to July of the year due to monsoon rainy season when the sewage water contaminates drinking water, the main source of the enteric pathogen. A similar study conducted at Ghana Hoho Municipality, where the incident rate of enteric fever was higher in June to august while most reported age was found 20 to 35 years, females were found more affected than male [21]. Similarly, another study conducted in Karachi, Pakistan showed that cases occur more during May to August, however they reported higher rate of infection in children [22].

In our study most of the isolates were found resistant to first-line drugs. There was 100% resistance against Aztreonam and Ampicillin, followed by 98.9%, 97%, 72%, and 52% against Cotrimoxazole, Ciprofloxacin, Chloramphenicol, and Ceftriaxone respectively while there was 100% sensitivity to Meropenem and Azithromycin. A study conducted by Shah et al. in 2020 showed nearly the same results and support our findings of the present study [23].

Similarly, some strains of S. typhi were tested against Vancomycin and Clarithromycin and resistance was reported. These drugs are not commonly prescribed in Pakistan, however, resistance is shown against these drugs also which is alarming. The available treatment options for endemic resistant strain of S. typhi are Meropenem and Azithromycin which showed 100% sensitivity.

Studies conducted in Karachi and Hyderabad, Pakistan also showed similar findings of antimicrobial resistance where S. typhi was resistant to most of the antibiotics while there was around 100 % sensitivity to Azithromycin and Meropenem [10, 19, 24] Now-a days the drug of choice is Azithromycin oral or Meropenem intravenous in the hospitalized patients [1, 24], these findings also support our study.

We reported a high prevalence (18%) of XDR along with 28% MDR strains, and our finding was similar to another study conducted in Pakistan [25]. The high prevalence of XDR and MDR is due to misuse of antibiotic either because of self-medication, lack of relevant knowledge, or the influence of medicine companies that facilitate physician by a different mean to sell their product which directly affects the poor community.

CONCLUSION

Our study concludes that resistance to antimicrobial agents is increasing against Salmonella typhi which makes the situation more alarming keeping treatment option limited. The only treatment option left for the endemic strain of S. typhi is Azithromycin and Meropenem. Physicians need to prescribe medication carefully after culture sensitivity to overcome the growing resistance. This study highlights the importance of surveillance study of antimicrobial susceptibilities against S. typhi which would help to set an effective preventive and control measure. Furthermore, the use of the typhoid vaccine must be made available to the local community to lower the burden of antibiotics.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Ali A:	Principal investigator, Concept and data analysis
Rahman N:	Data handling and critical analysis
Adeeb H:	Manuscript drafting
Ullah I:	Bibliography, Project design, Critical review, analysis of data

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