ONLINE ISSN :3007-309X
PRINT ISSN :3007-3081

Journal of Medical & Health Sciences Review

ANTIMICROBIAL RESISTANT PATTERN OF *SALMONELLA* TYPHI ISOLATED FROM CHILDREN

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ARTICLE INFO

Keywords:
Salmonella Typhi, MDR, XDR, Antimicrobial Susceptibility testing

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ABSTRACT

The aim of this research was to investigate the prevalence of multidrug-resistance and extensively drug-resistance *Salmonella (S.)* Typhi isolated from the blood samples from children having typhoid. *S.* Typhi has developed resistance against first line of drugs (MDR), in addition with fluoroquinolones and 3rd generation of drugs cephalosporins (XDR). Aseptically a total of 130 samples were collected from different hospitals and clinics. Typhoid positive samples were inoculated in blood culture broth. Antimicrobial susceptibility testing was performed by using modified method of Kirby-Bauer disc diffusion and results were compared according to CLSI manual. The bacteria were investigated against different antibiotics. Majority of the *S.* Typhi were extensively drug-resistant but some samples showed unusual antibiogram pattern where resistant to all tested antibiotics except trimethoprim-sulfamethoxazole and tetracycline was observed. Data was analyzed statistical analysis and percentage positivity was recorded for culture positive and resistant serovar of *S. enterica* (*S.* Typhi). Out of 50 samples 10% (14 samples) were positive for *S.* Typhi isolates. Kirby Bauer's disc diffusion assay of these 14 samples was performed to evaluate antibiogram pattern and observed that 28.57% of *S.* Typhi isolates were highly resistant to first line of medication on the basis of which they were regarded as MDR *S.* Typhi. 50% of the samples were resistant to first and second line of drugs and were regarded as XDR *S.* Typhi. 21.42% samples showed resistant to all tested antibiotics conferring a very unusual antibiogram pattern. This study showed the increased prevalence of resistant *S.* Typhi in children.

INTRODUCTION

Salmonella Typhi serovar of *S. enterica* (species of *Enterobacteriaceae* family), are Gram-negative, morphologically rods, and unable to form spores. Typhoidal fever, which is an endemic illness in developing nations, is caused by *S.* Typhi. S. Typhi spreads by way of the fecal-oral pathway (Muturi et al., 2024). The World Health Organization has published data showing that there are over 21 million cases of typhoid fever worldwide, with 200,000 deaths. 200–300 new infections are reported yearly in the US, most of which are caused by travelers returning from endemic regions (Gouda, 2024).

It has become a very serious public health concern because the serovar *S.* Typhi is developing antibiotic resistance. Drug-resistant *S.* Typhi was first identified in Mexico in 1972 and has since spread to Thailand, Vietnam, Bangladesh, Korea, Pakistan, Peru, and India (Walker et al., 2023). Until the mid-1980s, the conventional therapy for enteric fever was chloramphenicol, ampicillin, and cotrimoxazole (ACC) (Paul, 2024). Resistance to three different classes of antibiotics or more than three of them simultaneously is known as MDR *S.* Typhi. MDR in *S.* Typhi may occur for a variety of reasons, including medicine addiction, drug overuse, and poor physician prescription

techniques. MDR *S.* Typhi has been found all over the world, most notably in South America, Africa, the Indian subcontinent, and Southeast Asia (da Silva et al., 2022). Extensively drug-resistant individuals include those who are resistant to first-line medications, fluoroquinolones (ciprofloxacin), and third-generation cephalosporins (including ceftriaxone, ceftazidime, and cefuroxime) (Imran et al., 2024). Appropriate approaches for promoting typhoid vaccination before to travel, monitoring with early detection of XDR-Typhi infections, employing various empiric treatments whenever predicted infection rate is high, and essential for avoiding and treating more travel-related diseases (Bisola Bello et al., 2024). There are two typhoid fever vaccines available for tourists: an injectable Vi-capsular polysaccharide vaccine and an oral live attenuated vaccine. 50-80 % of people are somewhat protected by both vaccines (Jones et al., 2024). The Objectives of this study are to investigate the prevalence of *S.* Typhi from blood samples of children with typhoid and to observe the anti-biogram pattern of *S.* Typhi isolated from typhoid patients.

Materials and Methods

Ethical consent

The present study was approved by Institutional BioSafety/ BioEthics Committee, University of Agriculture, Faisalabad (IBC, UAF) and ethical committee of Allied hospital Faisalabad, Children Hospital Faisalabad, DHQ hospital Faisalabad, Faisal hospital Faisalabad and the privet clinics. Informed consent was obtained from all individuals or their caregivers prior to sampling and use of samples for additional laboratory analysis.

Sampling and Data Collection

During the period of six months, a total of 130 blood samples of typhoid-affected children with ages above 5-15 years were collected from different hospitals of Faisalabad. From each hospital and clinic, about 20 samples were collected. All Typhoid positive samples were inoculated in blood culture broth for growth enrichment. To study the colony characteristics and fermenting properties *S.* Typhi, the samples were grown on different agar media including MacConkey, and *Salmonella-Shigella* agar. Following culturing the colonies were processed for Gram staining to study morphology. Further, serovar was confirmed by performing biochemical test involving; catalase test, indole test, methyl red, citrate utilization, Voges-Proskauer, urease, triple sugar iron test, and nitrate reduction test.

To check the prevalence of multidrug-resistant and extensively drug-resistant *S.* Typhi utilizing a modified Kirby-Bauer disc diffusion method, an antibiotic susceptibility test was conducted to determine the antibiogram pattern. Isolate was tested against 8 antibiotics of various classes and the results were interpreted according to Clinical and Laboratory Standard Institute's guidelines (CLSI, 2022).

Statistical analysis

The data was analyzed by using specific statistical tools. To investigate the prevalence of *S.* Typhi Graph-pad prism software was used and percentage positivity was compared.

RESULTS

The objective of the current investigation was to separate, identify, and assess the prevalence of *S.* Typhi from blood samples. A total of 130 samples were collected from the patients suspected for typhoid fever in which 63 were male and 67 females. Gender and Age based distribution of blood samples collected for the isolation of *S.* Typhi is shown in Table 1 and Figure 1.

Table 1. Gender based distribution of blood samples collected for the isolation of *S.* Typhi

Hospital/Clinics	No. of samples	Distribution of samples on the basis of gender	
		Male	**Female**
Allied Hospital, Faisalabad	20	8	12
Children Hospital Faisalabad	20	5	15
DHQ Hospital, Faisalabad	20	11	9
Faisal Hospital Faisalabad	30	16	14
Chughtai Lab Satiana road, Faisalabad	20	17	3
Hameed clinic Jaranwala road, Faisalabad	20	6	14
Total	**130**	**63**	**67**

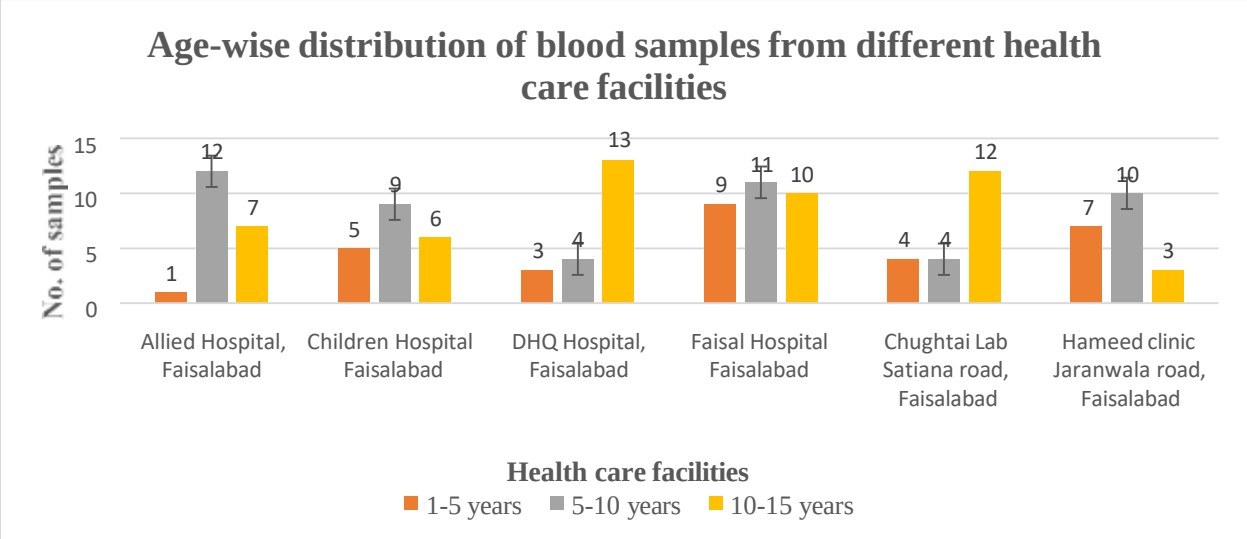

Figure 1. Age wise distribution of blood samples collected from different hospitals and clinics for the isolation of *S.* Typhi from typhoidal patients

Growth in blood culture broth

Typhoid positive and negative samples were inoculated in the blood culture broth to enrich the bacterial growth. Blood culture broth was used to enhance and enrich the growth blood bacteria. The samples were incubated at 37°C for 24 hours. Out of 130 samples, 76 samples showed turbidity and these are typhoid positive samples and the %age positivity is 58% described in Table 2.

Table 2. Percentage positivity of blood samples based on the total Typhoid positive samples

Areas	No. of samples	Typhoid positive samples	%age positivity
DHQ Faisalabad	20	13	65%
Children hospital Faisalabad	20	10	50%
Hameed clinic road, Jaranwala Faisalabad	20	10	50%
Chughtai Lab, Faisalabad	20	7	35%
Allied hospital Faisalabad	20	16	80%
Faisal hospital Faisalabad	30	20	66%
Total	**130**	**76**	**58%**

Antimicrobial susceptibility testing for *S.* Typhi

The samples (n=14) were tested against eight different antibiotics and zone of inhibition was observed as shown in Table 4. Zone of inhibition was measured and data was compared according to the CLSI guidelines as shown in table 3. Samples (n=4) showed high resistance to first line of drugs including trimethoprim-sulfamethoxazole, tetracycline and ampicillin. A total of n=7 samples showed resistance to first as well as second line of drugs include fluoroquinolones and third generation of cephalosporins categorized as XDR. Data was distributed on the basis of sensitivity and resistance pattern and percentage positivity was calculated as shown in table5. Some of *S.* Typhi's samples showed very unusual antibiogram pattern, resistance to all tested antibiotics as shown in table 5.

Standards of antibiogram pattern of *S.* Typhi

According to the CLSI guidelines, the zone of inhibition standards as shown in Table 3 were compared with the observed zones of tested antibiotics.

Tabl3 3. Zone of Inhibition (mm) according to CLSI standards

Antibiotics	Conc.	Zone of Inhibition (mm) according to CLSI standards		
		Sensitive	**Intermediate**	**Resistant**
Imipenem	10µg	≥23	20-22	≤19
Azithromycin	15µg	≥13	-	≤12
Amikacin	30µg	≥17	15–16	≤14
Ampicillin	25µg	≥17	14-16	≤13
Ciprofloxacin	5µg	≥31	21-30	≤20
Chloramphenicol	20µg	≥18	13-17	≤12
Tetracycline	10µg	≥15	12-14	≤11
Trimethoprim-Sulfamethoxazole	25µg	≥16	11-15	≤10

Table 4. Observed antibiogram Pattern of *S.* Typhi, n=14

antibiotics	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14
Imipenem	R	S	R	S	S	S	R	S	R	S	S	I	S	S
Azithromycin	R	R	R	S	S	S	S	S	S	S	S	S	S	R
Amikacin	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Ciprofloxacin	R	R	R	R	S	S	R	R	I	R	R	R	S	S
Chloramphenicol	R	R	S	R	R	R	S	R	R	S	S	R	S	S
Tetracycline	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Trimethoprim-sulfamethoxazole	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Ampicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Table 5. Antibiogram pattern of sample of *S.* Typhi n=14

Antibiotics	Conc.	Zone of Inhibition (mm) CLSI standards			Observed Zone (mm)	Sensitive (S), Resistant (R), Intermediate (I)
		Sensitive	Intermediate	Resistant		
Imipenem	10µg	≥23	20-22	≤19	12	R
Azithromycin	15µg	≥13	-	≤12	6	R
Amikacin	30µg	≥17	15–16	≤14	12	R
Ampicillin	25µg	≥17	14-16	≤13	6	R
Ciprofloxacin	5µg	≥31	21-30	≤20	7	R
Chloramphenicol	20µg	≥18	13-17	≤12	10	R
Tetracycline	10µg	≥15	12-14	≤11	12	I
Trimethoprim-Sulfamethoxazole	25µg	≥16	11-15	≤10	14	I

Table 6. Percentage positivity of *S.* Typhi on the basis of antibiogram pattern

Antibiotics	Conc.	Antibiogram pattern of samples according to CLSI guidelines		
		Sensitive	**Intermediate**	**Resistant**
Imipenem	10µg	9	1	4
Azithromycin	15µg	10	0	4
Chloramphenicol	10µg	6	0	8
Amikacin	30µg	0	0	14
Ampicillin	25µg	0	0	14
Ciprofloxacin	5µg	4	1	9
Tetracycline	10µg	0	0	14
Trimethoprim-Sulfamethoxazole	25µg	0	0	14

Drug resistance pattern of *S.* Typhi

The drug resistance pattern as shown in Figure 2 showed that *S.* Typhi is resistant to first line and second line of drugs leading to the emergence of MDR and XDR serovar respectively. Percentage positivity on the basis of sensitivity pattern is shown in Figure 3. The highest percentage of extensively drug resistance *S.* Typhi was observed compared with multidrug resistance as shown in table 7 and 8. While unusual pattern was also observed with (21.42%) as shown in table 9. It was Observed that MDR are most common among people with age 1-10 years as shown in Fig 4 while XDR are common among patients with age fall under 10-15 years as shown in Fig 5. The percentage positivity of MDR, XDR and those sample unusual pattern are shown in Fig. 6.

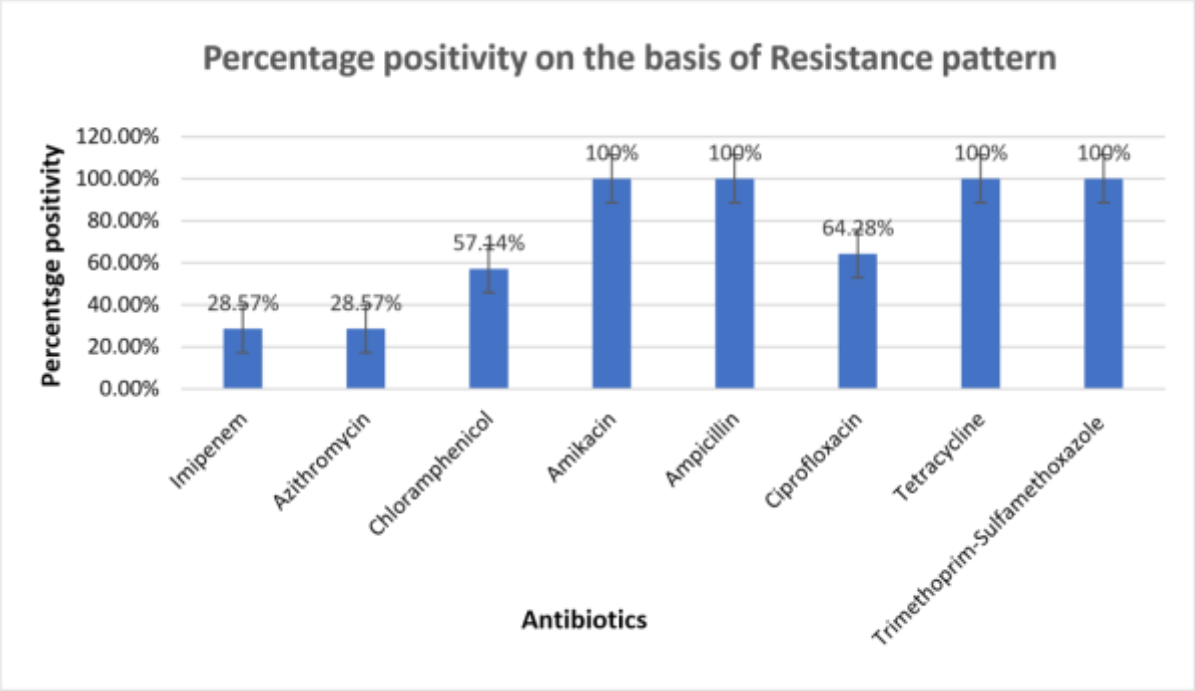

Figure 2. Percentage positivity of *S.* Typhi isolated from children blood on the basis of drug resistance pattern

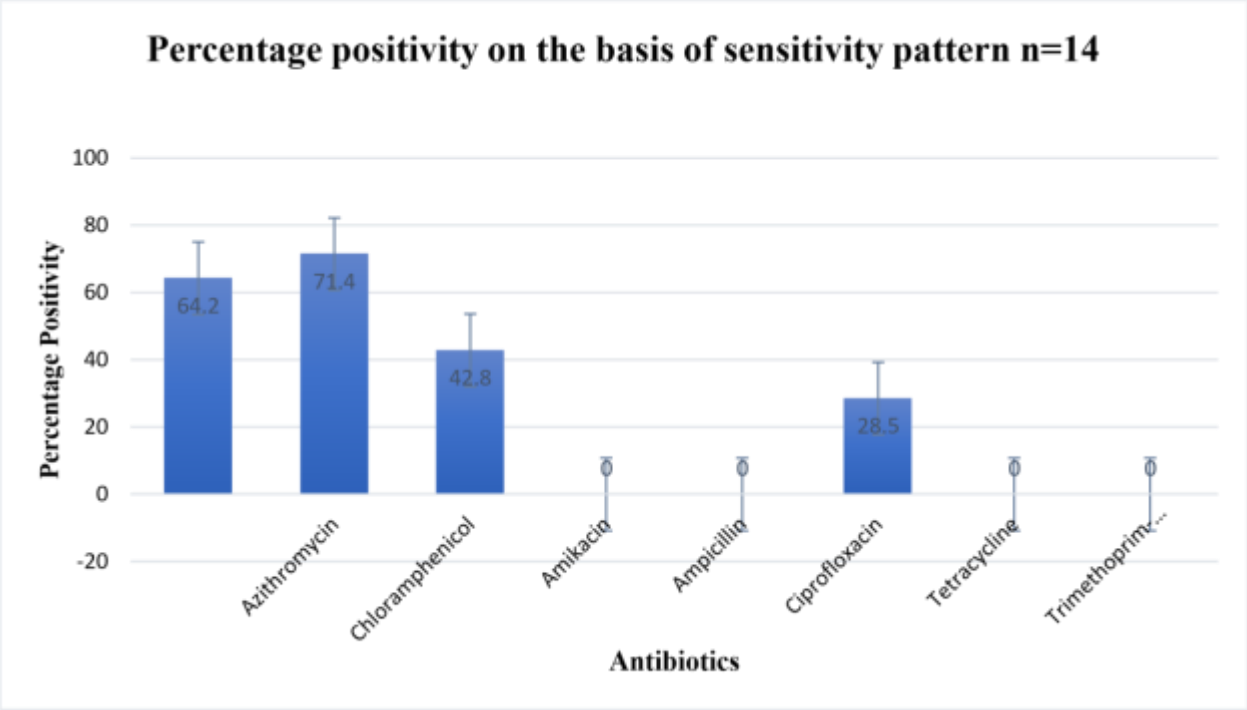

Figure 3. Percentage positivity of *S.* Typhi isolated from Children blood on the basis of sensitivity pattern

Table 7. Percentage positivity of MDR *S.* Typhi

Total number of *S.* Typhi resistant to the first line of drugs n=14	Percentage positivity
4	28.57%

Table 8. Percentage positivity of XDR *S.* Typhi

Total number of *S.* Typhi resistant to the second line of drugs n=14	Percentage positivity
7	50%

Table 9. Percentage positivity of *S.* Typhi unusual antibiogram pattern

Total number of *S.* Typhi showed unusual antibiogram pattern n=14	Percentage positivity
3	21.42%

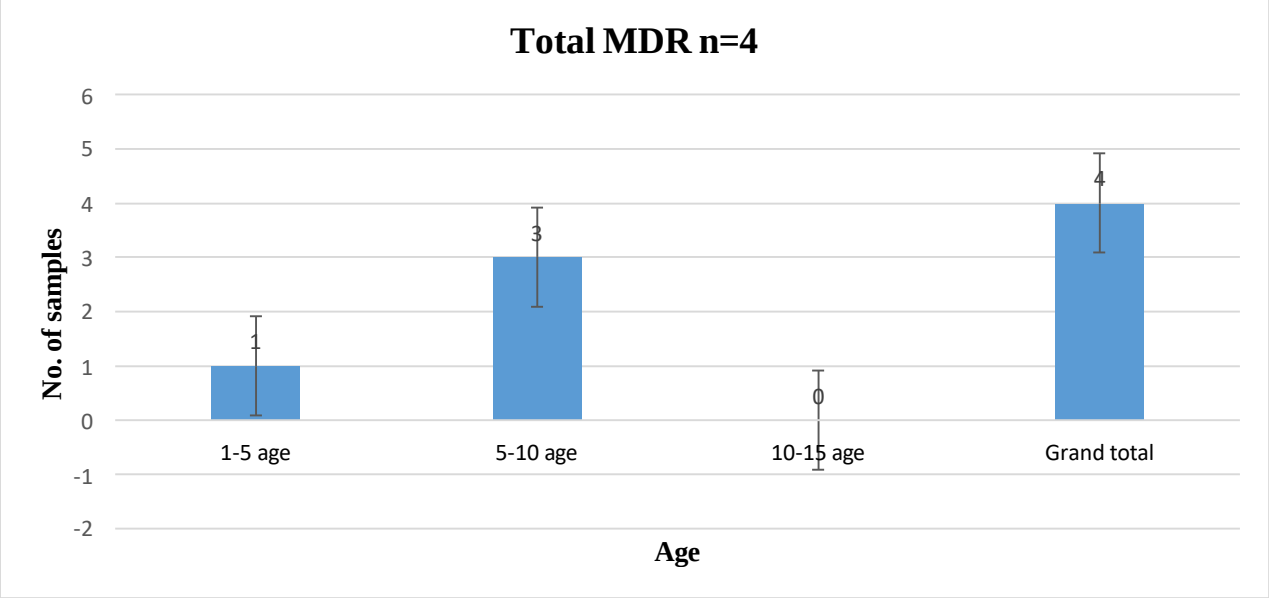

Figure 4. Gender-wise distribution and percentage positivity of multidrug-resistant distribution and percentage positivity of MDR

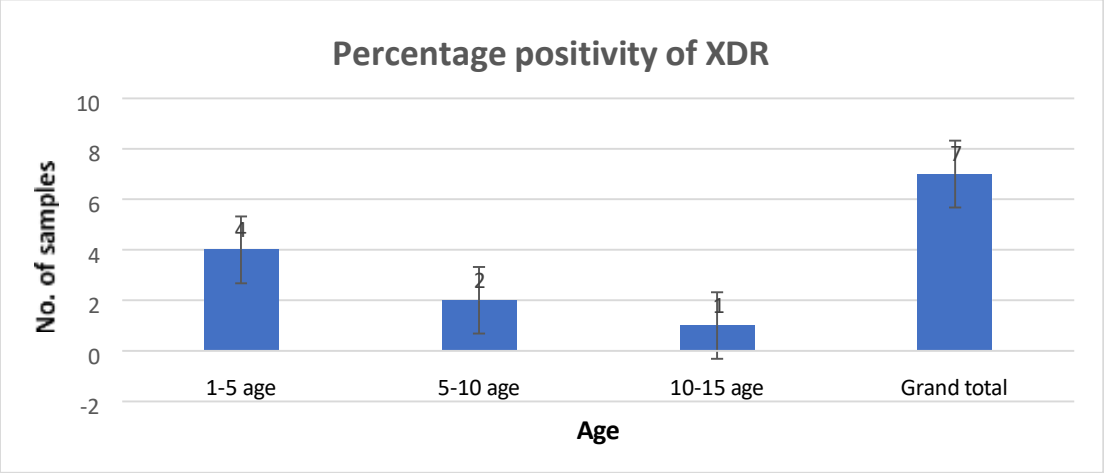

Figure 5. Age-wise distribution and percentage positivity of *S.* Typhi isolated from Children blood

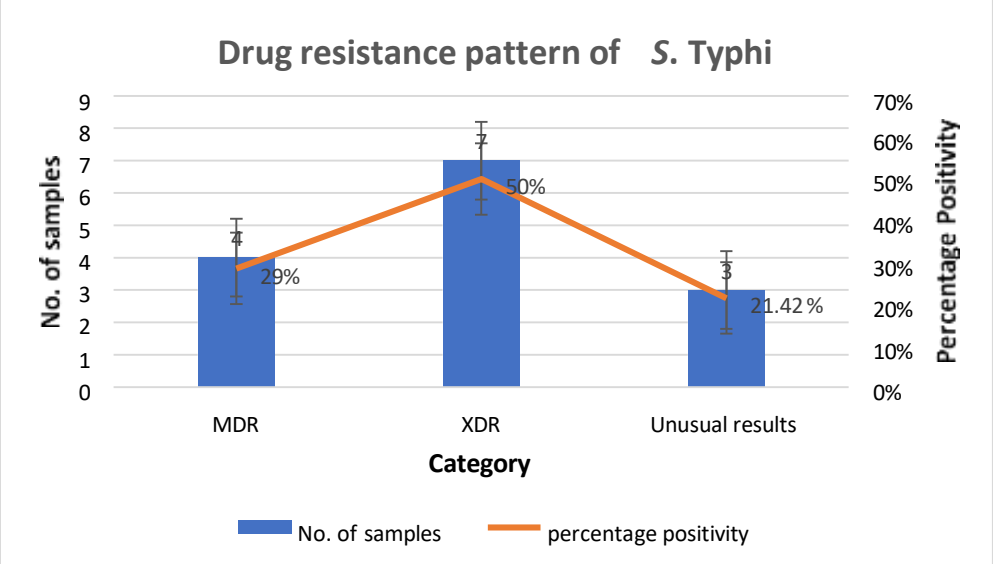

Figure 6. Percentage positivity of *S.* Typhi isolated from children's blood on the basis of drug resistance pattern

DISCUSSION

Salmonella Typhi is a member of the *Enterobacteriaceae* family. Typhoid is the most prevalent disease in underdeveloped countries. Typhoid, a multi-systemic disease whose causative agent is *S.* Typhi infection, is still a substantial public health issue in developing countries, where a majority of the population has low socioeconomic status and lives in areas with limited resources and constraints on sanitation infrastructure (Muturi et al., 2024).

In a hospital based study on children, blood samples were collected and complete blood count and urine analysis were also done to ensure the typhoid. Further, the samples were cultured on selective

agar, and culture positivity was counted. According to the results culture positivity of CBC positive was 82% and 80.5% of samples after urine analysis showed culture positivity for *S.* Typhi.
In current study Blood samples (130 total) were collected from four different tertiary care hospitals and private clinics from different areas of Faisalabad. The samples with typhoid positive results were processed further for culturing. Among total collected samples 58% positivity was found for Typhoid. From these 55% Typhoid positive samples 76% were culture positive after culturing on different selective and differential agar medium including MacConkey and *Salmonella-Shigella* agar. Following culturing and phenotypic confirmation, the colonies were processed for Gram staining to study morphology. Further, Biochemical testing was used to confirm the serovar, involving; the catalase test, indole test, methyl red, citrate utilization, Voges-Proskauer, urease, triple sugar iron test, sugar fermentation test, and nitrate reduction test.

Antimicrobial resistance explained the ability of an organism to withstand an environment containing different antibiotics. In Asian countries specifically in Pakistan after the endemic of *S.* Typhi, various resistant serovar was discovered. Several cases were recorded including those resistant to the first line of drugs i.e. trimethoprim-sulfamethoxazole, ampicillin, and chloramphenicol. These bacteria are classified as multidrug-resistant (MDR) (Fatima et al., 2023). In this study, the prevalence of MDR and XDR *S.* Typhi antibiogram pattern was measured by performing an antibiotic susceptibility testing by the modified Kirby-Bauer disc diffusion method (Mina et al., 2023). The isolate was tested against 8 antibiotics of various classes and the results were interpreted according to Clinical and Laboratory Standard Institute's guidelines. The percentage positivity of MDR and XDR were found 28.57% and 50% in the blood samples.

Typhoid fever is usually treated with first line of antibiotics but an increase in antibiotic resistance in bacteria is a major issue in treatment. Currently in some parts of the world Cephalosporins are considered sufficient for the treatment of typhoid fever but recently this bacterium has gained resistance against this drug too. So, in the areas where highly resistant *S.* Typhi strains are reported, treatment can be done with effective doses of Ceftriaxone (Marchello et al., 2020).

This study has shown that antimicrobial resistance among *S.* Typhi strains is a serious issue in Asian nations. Typhi that is resistant to several drugs is still common in Asia, and as more antimicrobial classes show resistance, the threat posed by XDR S. Typhi is growing. It's not the issue of a country as typhoid is spreading all around the world through travelers and due to the development of resistance, this may lead to a huge disaster worldwide. In this study, the percentage positivity of MDR and XDR was found 10% in the blood samples. Out of total collected blood samples (n=130), 14 were positive for *S.* Typhi where 4 were classified as MDR and 7 were XDR.

It was studied that antibiotic is the main issue in *S.* Typhi, the main serovars causing typhoid fever with resistance positivity 14%. MDR isolates are common in Africa as well as Asia and are frequently linked with the prevailing H58 haplotype. The emergence of antibiotic resistance is facilitated by haplotype H58 (Mashe et al., 2021). Resistance against fluoroquinolones is common as well, and isolated incidences of prevention from 3rd-generation cephalosporins and azithromycin have been demonstrated. Three first-line antibiotics (trimethoprim- sulfamethoxazole, chloramphenicol, and ampicillin), as well as fluoroquinolones and third- generation cephalosporins (XDR), were all ineffective against the new S. Typhi replicate, which

made its first notable appearance and began to multiply in the Sindh province of Pakistan. In the current study, 75% samples showed resistance against fluoroquinolones (Ahmad et al., 2024).

About 28.57% of the samples that showed resistance against first line of drugs (sulfamethoxazole-trimethoprim, ampicillin, and tetracycline) categorized as MDR, 50% showed resistance against fluoroquinolones, 3rd generation cephalosporins, and first line of drugs categorized as XDR. 21.42% approximate (3 samples) showed very unusual antibiogram pattern showing resistance against all used antibiotics containing both first- and second-line medications azithromycin and macrolides (meropenem and imipenem). The prevalence of *S.* Typhi is 8.7% in Pakistan (Butt et al., 2022). In this study, the prevalence observed was 10%. It was concluded that impact of COVID-19, convergent evolution of mutation in the drug-resistant genes (azithromycin) and misuse of antibiotics lead to the occurrence of unusual antibiogram patterns.

Conclusion

The purpose of this study was to determine the prevalence of MDR and XDR *S.* Typhi at various hospitals in Faisalabad. The results revealed that S. Typhi has become resistant to fluoroquinolones, third-generation cephalosporins (XDR), and first-line medications (MDR). When the zone of inhibition was assessed, the samples revealed high resistance to first and second line medications, such as ampicillin, tetracycline, trimethoprim-sulfamethoxazole, fluoroquinolones, and third-generation cephalosporins classified as XDR. Azithromycin was found to be the most effective antibiotic that shows the highest sensitivity. Although this study presents a comprehensive view of occurrence the MDR and XDR *S.* Typhi.

Conflicts of Interest:

The authors declare no conflict of interest regarding the publication of this article.

Funding: No funding was received for conducting this study

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