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The characteristic of antibiotic drug resistance of *Salmonella* Typhi isolated from tertiary care hospital in Faisalabad

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Abstract: *Salmonella* Typhi, a human-restricted pathogen, is demonstrating multi-drug resistance (MDR) due to widespread and inappropriate antibiotic use. This study aims to molecular identify the pattern of antibiotic resistance. Blood samples from 2456 suspected patients were assessed. Molecular identification of *Salmonella* Typhi was performed by amplifying the fliC gene. The Disc diffusion method was used to measure the susceptibility of antibiotics. 2456 patient samples, bacterial growth and *Salmonella* Typhi were 152 (6.2 %) positive. PCR analysis confirmed that all 152 isolated strains were *Salmonella* Typhi (100%) through the amplification of the *fliC* gene. *Salmonella* Typhi isolates showed resistance to trimethoprim (58%), ampicillin (63%), ciprofloxacin (79%) and chloramphenicol (58%). Fifty-eight percent of the isolates showed multi-drug resistance, whereas 26 percent had extensive drug resistance. Antibiotic resistance gene of quinolones was isolated as 44 (36.4%), whereas 88 (57.9 %) were positive for $bla_{\text{CTX-M}}$ gene were detected among cephalosporin-resistance bacteria 56 (36.8 %) resistance bla_{IMP} *and* $bla_{\text{OXA-48}}$ were detected among carbapenem-resistance bacteria. For the azithromycin resistance, *more* genes were detected as a percentage 03 (50 %) from isolates. It concludes that several multidrug resistance and extensive drug-resistance *Salmonella* Typhi were found. The majority of isolates were sensitive to meropenem, Imipenem and *Azithromycin*.

Keywords: Multidrug resistance, extensive drug resistance, polymerase chain reaction.

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INTRODUCTION

Typhoid fever, caused by *Salmonella* Typhi, is a critical infectious disease, particularly impacting children and young adults studies by (Goay *et al*., 2016). Symptoms encompass diarrhea, enlarged spleen, meningitis, systemic temperature elevation, nausea, loss of appetite and severe headaches (Baker *et al*., 2020). *Salmonella* Typhi, a Gram-negative, rod-shaped, motile bacterium with flagella, poses a mortality risk of 12-30% if untreated, contrasting with a 99% survival rate with timely treatment (Osuntokun and Olajubu, 2015).

One of the nations with the highest rates of typhoid is Pakistan. 451.7 cases per 100,000 people is the annual incidence rate. It is uncertain what molecular mechanism *S.* Typhi uses to cause enteric fever. Clinical signs and symptoms that connect with other bacterial infections (Yasin e*t al*., 2022). From 2016 to 2018, the Health Authority of Pakistan reported that out of 8188 cases in Sindh, 5274 cases were extensively drug-resistant (XDR) typhoid. XDR resistance often arises when multidrug-resistant (MDR) bacteria acquire plasmids. In densely populated places with inadequate sanitation, tainted water and compromised immune systems, the prevalence of typhoid fever is higher. MDR strains of Salmonella are resistant to co-trimoxazole, ampicillin and chloramphenicol (Hooda *et al*., 2019). Resistance to ampicillin, trimethoprim-sulfamethoxazole and chloramphenicol is known as an MDR, whereas resistance to fluoroquinolones, ampicillin, co-trimoxazole and chloramphenicol is also known the XDR (Akram *et al*., 2020). First identified between the late 1970s to early 1980s. MDR *Salmonella* Typhi is resistant to these two groups (ceftriaxone, a third-generation cephalosporin, or quinolones) of antibiotics are known as extensively drug-resistant (XDR). In areas with limited resources, azithromycin is a cost-effective and effective oral first-line antibiotic for treating XDR typhoid (khan *et al*., 2024). Carbapenem antibiotics are the final opetion for treating infections caused by MDR and XDR bacteria. MBL is produced by bacteria that are sensitive to colistin but resistant to β-lactam antibiotics (carbapenems and other antibiotics) (Sawa *et al*., 2020).

MATERIALS AND METHODS

Study design

Before starting the research project ethical permission was obtained from the Ethics Review Committee at Faisalabad University, Government College with code CUF/ERC/4173, dated December 21 and also obtained the informed consent forms to collect clinical samples.

Collection of clinical samples

A total of 2456 blood samples were taken from patients exhibiting symptoms such as fever, drowsiness and stomach discomfort in accordance with the doctor's prescription for the blood culture test. The samples were taken from a tertiary care center in Faisalabad.

Isolation, biochemical identification

The blood samples were grown using Tryptic Soy Broth Medium (Oxoid, United Kingdom). Two milliliters of

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blood were collected immediately after and 16 milliliters of TSB (1:8) were added without any anticoagulant. Mixture incubated at 37°C for 48-72 hours.

The Tryptic Soy Broth Medium (Oxoid, United Kingdom) was used to cultivate the blood samples. Following the collection of two millilitres of blood, 16 millilitres of TSB (1:8) were added without the use of an anticoagulant. mixture incubated for 48-72 hours at 37°C.

Then mixture was used to create a loopful culture that was streaked on both *Salmonella* Shigella and MacConkey's agar (Oxoid) (Ain *et al*., 2022).

Primary identification of the isolate used methyl red, citrate test, Gramme staining and triple sugar iron agar (Oxoid) for colony morphology, culture and biochemical test.

***Molecular confirmation of Salmonella* Typhi**

The molecular identification of *Salmonella* Typhi involved the utilization of a specific primer set known as *flic*. Thermo Fisher Scientific's Gene JET genomic DNA extraction kit was used for the extraction and purification of genomic DNA. The isolated DNA was then immediately refrigerated at -20°C. According to the study by Kumar *et al*. 2005), 36 cycles made up the reaction amplification conditions: 30 seconds of denaturation at 95°C, 30 seconds of annealing at 96°C, 1 minute of extension at 72°C and 5 minutes of final extension at 72°C. After being separated by gel electrophoresis, the PCR product was deposited onto a 2% agarose gel with ethidium bromide and examined under gel documentation. (Fermantis, USA,). table 1

Antibiotic susceptibility test

Salmonella Typhi resistance to antibiotics, such as ciprofloxacin (CIP) (10 µg), ampicillin (AMC) (20 µg), cefixime (CFM) (5 µg), ceftriaxone (CFT) (30 µg), trimethoprim (TRI) (23.75 µg), chloramphenicol (C) (30 µg), azithromycin (AZT) (15 µg) and imipenem (IMP) (10 µg) were evaluated using Kirby- Bauer disc diffusion testing, using Oxoid, UK. Every host strain was uniformly distributed on a Mueller-Hinton agar plate after being diluted to a turbidity equal to the 0.5 McFarland standard. To determine antibiotic susceptibility, the clear zone surrounding the antibiotic discs was measured. The CLSI standards were used to classify strains as susceptible (S) or resistant (R) based on the size of the zone.

Molecular identification antimicrobial resistance genes

Molecular identification of the genes responsible for Cephalosporin antibiotic resistance (*blaCTX-M*) and Carbapenemase resistance (*blaIMP*, *blaOXA-48* and *blavim*) (Qamar *et al*., 2020). genes for plasmid resistance The *qnr*A, *qnr*B and *qnr*S quinole-resistant genes were amplified (Herrera-Sánchez *et al*., 2021). Using a PCR assay, the azithromycin *msr*A, *era*A, *ere* A and *mef*A genes were identified. (Yasin *et al*., 2022). The details of primers and annealing temperature is provided below table 2.

RESULTS

Bacterial isolation and identification

Out of the 2456 samples collected and examined, 152 (6.2%) isolates of *Salmonella* Typhi were identified from individuals suspected of typhoid. The data reveals more infections in males (57.9 %) as compared to females (42.1 %) (table 3). Within the age group of 1 to 61 years. Notably, typhoid was more prevalent among age groups between 1 to 10 years (8.66 %) and 21-30 years (20%) (table 4). The pure *Salmonella* Typhi samples were preserved as stock in 20% (v/v) glycerol at -80°C.

From our data, it looks like we are comparing proportions (percentage of infected) between two groups (Male and Female), which suggests that a chi-squared test for independence would be appropriate. The P-value from the chi-squared test is approximately 0.467. This P-value suggests that there is no significant difference in the infection rates between males and females in your data because the P-value exceeds the usual thresholds for significance (e.g., 0.05). Therefore, we cannot reject the null hypothesis that the proportion of infected individuals is the same for both genders.

The degree of freedom for this test is 4. The expected frequencies, based on the assumption that there's no effect of age group on infection rates. Since the p-value is much less than 0.05, we reject the null hypothesis, suggesting a significant in infection rates among the age groups. In other words, the age range of individuals with notable bacterial proliferation is not uniform (table 4).

Morphological properties and identification of isolates

Out of the 2456 samples, 152 (6.2%) tested positive for *Salmonella* Typhi. On XLD, all pure isolates were identified by their dark metallic sheen. Results are shown in fig. 1. *Salmonella* Typhi is detected by nitrate, citrate test and methyl red positive. The isolates' TSI test results show a significant blackening of the slant as a result of increased H2S production and the butt's medium is positioned above the bottom side, indicating high acid production.

Molecular identification of bacterial isolated strains

Amplification of the *fliC* gene identified 152 (100%) of the isolates as *Salmonella* Typhi, as indicated in fig. 2.

Antimicrobial susceptibility testing

According to tests for antibiotic susceptibility, *Salmonella* Typhi pure strains were resistant to ciprofloxacin, ampicillin, trimethoprim and chlorphenicol (C) in 79%, 63%, 58% and 58% of cases, respectively. All of the purified isolates, however, showed complete susceptibility to imipenem (IMP) and azithromycin (AZT). Of the

isolates, only 26% showed extensive drug resistance, while 58% showed resistance to multiple drugs (table 5).

Molecular detection of resistance gene

The results of this research proved that 121 of the 152 S. Typhi strain isolates tested positive for ciprofloxacine (CIP) resistance. For quinolones, 44 (36.4%) tested positive for the *QnrS* gene. For *QnrA*, no amplification was seen. Of the isolates, 11 (9.0%) had *QnrB* identified.

The *bla*$_{CTX-M}$ gene has been identified to be present in 88 (57.9%) of the cephalosporin-resistant bacteria. Among the bacteria resistant to carbapenem, 56 (36.8%) had resistance *bla*$_{IMP}$ and 40 (26.3%) had resistance *blaOXA*$_{-48}$. Six isolates were positive for azithromycin resistance. Three genes linked to azithromycin resistance were examined. Three isolates (50%) were positive for the *msr*A gene. Only 02 (33%) had *mef*A gene positivity.

Fig. 1: On the plate, the colonies are smooth and red. And have a diameter of 2-3 mm, with a discernible black center.

DISCUSSION

Salmonella Typhi, responsible for typhoid, presents a serious risk to public health. The study characterizes typhoid patients based on clinical symptoms, age distribution and gender prevalence. In our study total of 152 (6.2%) isolates of *Salmonella* Typhi were identified from individuals suspected of having typhoid. The data reveals a higher prevalence of infections in males (57.9 %) as compared to females (42.1 %). within the age group of 1 to 61 years (Qamar *et al*., 2020) according to the study,

Typhoid was higher in age groups between one and ten years 8.66 % and 21 to 30 years (20%). Sepsis was observed in 66.6% of males compared to 33.3% of females. Because of their lower immune systems, males may be unfavorable in septic conditions, whereas females may have innate advantages due to the sex hormones they produce. It has been demonstrated that male sex hormones inhibit the immune system's response to cellular mediation. (Lin *et al*., 2021). Ten antibiotics from seven groups of antimicrobials were used in antimicrobial susceptibility assays for all 152 isolates. 79%, 63%, 58% and 58% of the pure *Salmonella* Typhi strains tested positive for antimicrobial sensitivity to trimethoprim, ciprofloxacin, ampicillin and chlorphenicol (C), respectively. An alarming issue is the resistance to ciprofloxacin and ampicillin.

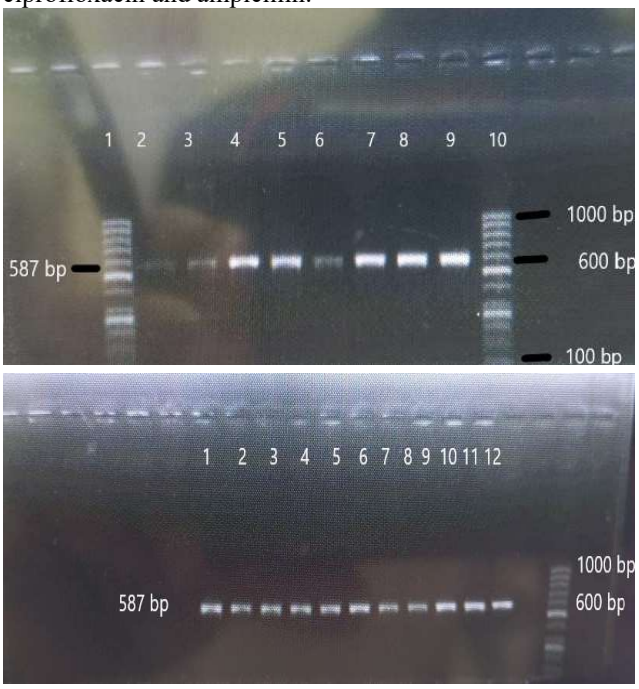

Fig. 2: (A) The Gene ruler with bands of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bps displayed in lanes 01 and 10; Lanes 2–09: Isolated *Salmonella* Typhi was identified with the use of the *fliC* gene (587 bps) amplification. (B): Lane 12: Bands of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bps are displayed by the gene ruler; Lanes 01-12: Isolated *Salmonella* Typhi was identified with the use of the *fliC* gene (587 bps) amplification.

All of the purified isolates, however, showed complete susceptibility to imipenem (IMP) and azithromycin (AZT).

With resistance levels of 45%, 31% and 35% for ciprofloxacin, levofloxacin and ofloxacin, respectively, the fluoroquinolones performed better (Afzal *et al*., 2012).

According to another study, 30.4% of adolescents are infected. The impact was greater on men. While men are outside for work and may consume more unhygienic food, women tend to stay at home for a variety of reasons (Hasan *et al*., 2023). One of the main causes of antibiotic-resistant bacterial infections in people is *Salmonella* Typhi, which poses a serious threat to public health. The development of antibiotic resistance as a result of improper antibiotic use has given rise to MDR and XDR strains of S. Typhi. Many factors contribute to the prevalence of resistant pathogens,

including the overuse of antibiotics, bed-sharing, careless behavior, crowded wards with insufficient medical staff and pathogen colonization on hands and medical equipment. (Ullah *et al*., 2025).

According to a study, *S.* Typhi (n = 415) shown a significant level of resistance to ciprofloxacin and cefotaxime (33%) distinct from meropenem and colistin. (Das *et al*., 2021). Additional findings included the discovery that a sizable portion of isolates had resistance to Cefoperazone (11%) Ceftazidime (25%), Ceffixime (20%), Cefotaxime (16%) and Cefepime (13%). 75 % of the isolates were azithromycin-resistant (Mahmood *et al*., 2025). In our study, 26 % of isolates had extensive drug resistance displayed and 58% of the isolates showed resistance to multiple drugs. Data from Aga Khan University in Pakistan shows that between 2001 and 2006, the rate of ciprofloxacin resistance rose from 1.6% to 64.1%, while the prevalence of MDR S Typhi remarkably increased from 34.2% to 48.5% (Huss, & Raman, 2020).

In the Pakistani province of Sindh, the first reports of XDR S. Typhi were received in 2016. While local instances of XDR S. Typhi dominate, certain tragic cases have been reported from other countries, including Italy, which has a history of sending people to Pakistan (Procaccianti *et al*., 2020). *Salmonella* Typhi may be correctly identified by amplifying the flagellin (fliC) gene locus. Of 152 (100%) isolates were confirmed to belong to *Salmonella* Typhi this was confirmed. Kumar *et al*., 2010 Study has shown that the fliC genes are particular genes that are used to identify S. typhi in enteric fever patients. This gene acts as a diagnostic for quickly identifying isolates of S. typhi from various samples. No other species of bacterium have it.

The molecular analysis of antimicrobial resistance genes revealed that 48 (31.6%) isolates had resistance genes against quinolones and among bacteria resistant to cephalosporins, 88 (57.9%) were positive for bla_{CTM} genes, while among bacteria resistant to carbapenems, 56 (36.1%) had resistance genes for bla_{IMP} *and* $bla_{\text{OXA-48}}$. There were found to be 40 (26.3%) more genes related to azithromycin resistance in isolates. Sixty-two (34%) of the *Salmonella* Typhi isolates in Lahore research were XDR.

The common resistance gene was ampC, which had a 47% prevalence. Genes to be examined were isolates with 45, 40, 21, 6, 18, 3, 11, 6 and 2% of gyrA, catA1, tet(A), aac (3)-la, qnrS, blaNDM-1 and blaCTX-M-15, respectively (Zahid *et al*., 2022).Ciprofloxacin (CIP) resistance was detected in the S. Typhi strain 121 isolates used in our study. For quinolones, 44 (36.4%) tested positive for the *QnrS* gene. For *QnrA*, no gene was found. Of the isolates, 11 (9.0%) had *QnrB* identified. Three genes linked to azithromycin resistance were examined. Three isolates (50%) were positive for the msrA gene. The mefA gene was positive in just 02 (33%) cases.

Ain *et al*., 2022 studied an analogous investigation was carried out on 40 isolates that produced ESBL; *bla*TEM (n = 37, 92.5%), *bla*CTX15 (n = 11; 27.5%) and *bla*SHV1 (n = 3; 7.5%) were found. *Salmonella* Typhi was shown to contain genes resistant to fluoroquinolones, such as 164 for gyrA, 160 for gyrB, 164 for parC and 160 for parE, qnrS (n = 15) and qnrB (n = 3). Furthermore, dfrA7 was found in 145 isolates, the cat gene in 147 isolates and the ac(6)-ib-cr gene in 163 isolates; no isolate had the resistance genes qnrA, qnrC, blaOXA, or drfA14. Seven genes were examined in 37 isolates resistant to azithromycin; only one of the genes tested positive for *mefA*, whereas three tested positive for *msrA* (Yasin *et al*., 2022).

The overall occurrence of S. typhi from blood samples attending tertiary care hospitals in Faisalabad, was 152 (6.2%). Patients aged 21-30 years harbored more bacteria. Gender influence on occurrence 88 (57.9%) in males. The antibiotics Azithromycin (AZT), Imipenem (IMP) and Meropenum (MEM) were the most effective against the isolates.

It concludes that a rise in the dosage of ciprofloxacin is causing clinical isolates of *Salmonella* Typhi from the Faisalabad district to exhibit growing fluoroquinolone resistance.

Significant research has revealed that *Salmonella* Typhi is developing a resistance to carbapenem. This means that physicians must carefully consider their alternatives when using antibiotics, as the bacteria may have been exposed to this most effective class of medications. Azithromycin, Imipenem (IMP) and Meropenum (MEM) were recommended as the best medications to treat XDR *Salmonella* Typhi (Mahmood *et al*., 2025).

The oral medicine azithromycin is used to treat XDR typhoid. Additional therapeutic selections include Intravenous carbapenems like meropenem and imipenem. Although all the extensive drug resistance isolates were not resistant to carbapenems, carbapenem resistance is not common globally. A new study published from Punjab, Pakistan, 48% of isolates had carbapenem resistance (Shah *et al*., 2020).

CONCLUSION

In conclusion, this study supports the hypothesis that patients from various geographic regions may have different host variations and transmission protocols. These results also indicate that almost all locally circulating S. Typhi will probably eventually acquire molecular markers for resistance to azithromycin and carbapenem. This may soon become a worldwide issue. Further research would have helped discover mechanisms of resistant and potential antibiotic-resistant connections

Table 1: Specific primer sequences to identify *S.* Typhi

Target	Primer name	Gene	Sequence (5'-3')	Product size	References
Salmonella Typhi	*fliC*	*fliC*	GCTTAATGTCCAAGATGCCTAC(F) GAGCAACGCCAGTACCATCTG(R)	587bp	(Kumar *et al*., 2005)

Table 2: The details of primers and annealing temperatures

Target	Primer name	Gene	Sequence (5'-3')	Product size	Annealing Temperature
Quinolone resistant gene	*QnrS*	*QnrS*	ACGACATTCGTCAACTGCAA-F TAAATTGGCACCCTGTAGGC- R	417bp	55◦C
	qnrA	*qnrA*	F- CCGCTTTTATCAGTGTGACT R- ACTCTATGCCAAAGCAGTTG	188 bp	55◦C
	qnrB	*qnrB*	F-GATCGTGAAAGCCAGAAAGG R-ACGATGCCTGGTAGTTGTCC	469 bp	54◦C
Carbapenemase gene	bla_{IMP}	bla_{IMP}	F 5-GGAATAGAGTGGCTTAATTCTC-3 R 5 -CCAAACCAC TACGTTATC-3	232bp	52◦C
	bla_{OXA-48}	bla_{OXA-48}	F- GCGTGGTTAAGGATGAACAC R- CATCAAGTTCAACCCAACCG	438bp	52◦C
	bla_{vim}	bla_{vim}	F 5 -GATGGTGTTTGGTCGCATA-3 R 5 -CGAATGCGCAGCACCAG-3	390 bp	52◦C
Cephalosporin gene	bla_{CTX-M}	bla_{CTX-M}	F 5-ATGTGCAGYACCAGTAARGT-3 R 5-TGGGTRAARTARGTSACCAGA-3	593 bp	52◦C
	msrA	*msrA*	F-TCCAATCATTGCACAAAATC R- AATTCCCTCTATTTGGTGGT	163bp	58◦C
Azithromycin	*ere A*	*ereA*	F- GCCGGTGCTCATGAACTTGAG R-CGACTATTCGATCAGAGGC	420 bp	60 ◦C
	mefA	*mefA*	F-AGTATCATTAATCACTAGTGC R:TTCTTCTGGTACTAAAAGTGG	345bp	54◦C

Table 3: Sex distribution of bacterial isolates from patient

SEX	No. of Infected	Percentage of infected	No. of Un-Infected	Percentage of Un-Infected	Total No. of samples
Male	88	57.9 %	1256	54.5 %	1344
Female	64	42.1%	1048	45.5 %	1112
Total	152	100 %	2304	100 %	2456

Table 4: The Age distribution of patients with notable bacterial growth

Age Group	Infected No.	Percentage of infection (%)	Total Number	Chi-square	p-value
Children 0-10	60	39 %	1016		
Adolescent 11- 19	41	27 %	108		
20-30	24	16 %	223		
31-59	20	13 %	625		< 0.001
Above 61	7	4.6 %	484	54.8	
Total	152	100 %	2456		

Table 5: Antibiotic susceptibility pattern of bacterial isolates from Typhoid Patients.

Sr.no	Antibiotic class	Antibiotics	Susceptibility Pattern		
			Sensitive	Intermediate	Resistant
1	Penicillin	Amicilllin (AMP)	46 (30 %)	10 (7%)	96 (63%)
		Ammoxillin	30 (20 %)	15(10%)	106(70 %)
2	Cephalosporins	Ceftriaxone	38 (25 %)	41 (27%)	71 (47%)
		Cefixime (CEF)	46 (30%)	46 (30 %)	60 (40%)
3	Diaminopyrimidins	Trimethoprim (TRI)	30 (20 %)	33 (22%)	88(58%)
4	Quinolones	Ciprofloxacine (CIP)	18 (12 %)	14 (9%)	120 (79%)
5	Cholorophenicol inhibit peptide bond form	Cholorophenicol(C)	38 (25 %)	26 (17%)	88 (58%)
6	Macrolides	Azithromycin (AZT)	173 (90 %)	15 (10%)	0 (0%)
7	Carbapenems	Imipenem (IMP)	129 (85 %)	22 (15%)	0 (0%)
		Meropenum (MEM)	124 (82 %)	27 (18%)	0 (0%)
8	Multidruge resistance	MDR: Resistance to AMPICILLIN, TRIMETHOPRIM (Third generation cephalosporin), CHLORAMPHENICOL	49 (32%)	15 (10%)	88 (58%)
9	Extensive drug resistance	Resistance to AMPICILLIN, TRIMETHOPRIM (Third generation cephalosporin), CHLORAMPHENICOL, Ciprofloxacin (fluoroquinolones), and a ceftriaxone third-generation cephalosporin.	91 (60%)	21 (14%)	40 (26%)

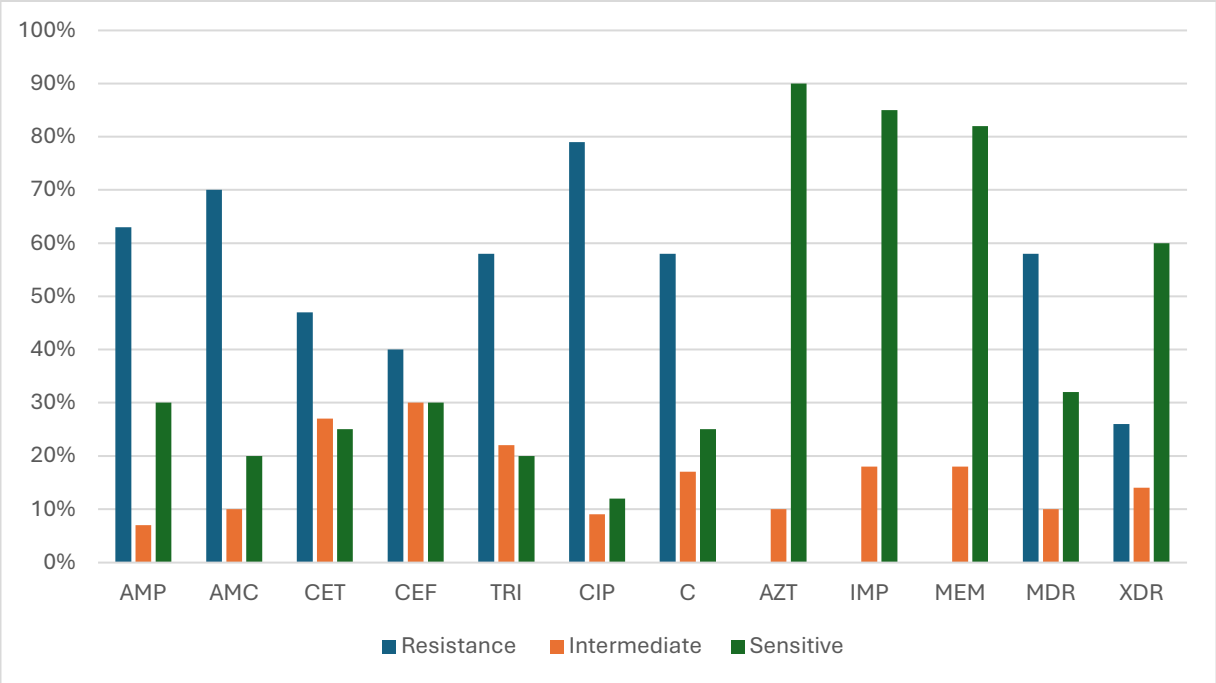

Fig. 3: The comparison of *Salmonella* Typhi antibiotic resistance profile is shown in this graph.

S-Sensitive, R-Resistant, AP-Amicilllin, AM-Ammoxillin, SXT: Ceftriaxone, TRI: Trimethoprim, MEM: Meropenum, CIP: Ciprofloxacine, C: Cholorophenicol, AZT: Azithromycine, IMP: Imipenem, CEF: Cefixime**.**

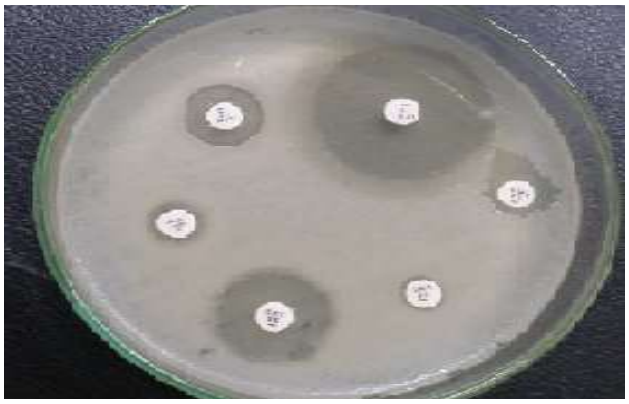

Fig. 4: *S.* Typhi resistance to antibiotics was determined by the Kirby Bauer method on Mueller Hinton Agar.

Ethical approval and consent to participate

The procedures for taking blood samples from human subjects were closely adhered to in this investigation. The Institutional Ethics Committee granted ethical clearance under code GCUF/ERC/4173 on December 20, 2021, ensuring compliance with ethical standards for research involving human participants. Furthermore, informed consent was provided by every participant in research involving human subjects.

Conflict of interest

The authors declare that the research was no conflict of interest.

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REFERENCES

Abd Al Mayahi FS and Jaber SM (2020). A preliminary study of multiple antibiotic resistance (MAR) and extensively drug-resistant (XDR) of bacterial causing typhoid fever isolated from stool specimens in Al-Diwaniya, Iraq. *Eur. Asian J. Bio. Sci*., **14**(1): 2369-2378.

Ain Q, Tahir M, Sadaqat A, Ayub A, Awan AB, Wajid M and Sarwar Y (2022). First detection of extensively drug-resistant *Salmonella* Typhi isolates harboring VIM and GES genes for carbapenem resistance from Faisalabad, Pakistan. *Microb. Drug Resist*., **28**(12): 1087-1098.

Akram J, Khan AS, Khan HA, Gilani SA, Akram SJ, Ahmad FJ and Mehboob R (2020). Extensively drug-resistant (XDR) typhoid: Evolution, prevention and its management. *Biomed. Res. Int*., **2**: 6432580.

Baker S, Blohmke CJ, Maes M, Johnston PI and Darton TC (2020). The current status of enteric fever diagnostics and implications for disease control. *Clin. Infect Dis*., **71**(2): 64-70.

Das R, Haque MA, Chisti MJ, Faruque ASG and Ahmed T (2021). Association between non-typhoidal Salmonella infection and growth in children under 5 years of age: analyzing data from the Global Enteric Multicenter Study. *Nutrients*., **13**(2): 392.

Goay YX, Chin KL, Tan CLL, Yeoh CY, Ja'afar JAN, Zaidah AR and Phua KK (2016). Identification of Five Novel Salmonella Typhi-Specific Genes as Markers for Diagnosis of Typhoid Fever Using Single-Gene Target PCR Assays. *BioMed research international*, **1**: 8905675.

Hasan M, Wang J and Ahn J (2023). Ciprofloxacin and tetracycline resistance cause collateral sensitivity to aminoglycosides in *Salmonella* Typhimurium. *Antibiotics*., **12**(8): 1335.

Herrera-Sánchez MP, Castro-Vargas RE, Fandiño-de-Rubio LC, Rodríguez-Hernández R and Rondón-Barragán IS (2021). Molecular identification of fluoroquinolone resistance in Salmonella spp. isolated from broiler farms and human samples obtained from two regions in Colombia. *Vet World*., **14**(7): 1767-1773.

Hooda Y, Sajib MS, Rahman H, Luby SP, Bondy-Denomy J, Santosham M and Saha S (2019). Molecular mechanism of azithromycin resistance among typhoidal Salmonella strains in Bangladesh identified through passive pediatric surveillance. *PLoS Neglected Tropical Diseases*, **13**(11): 0007868.

Huss P and Raman S (2020). Engineered bacteriophages as programmable biocontrol agents. *Curr. Opin. Biotechnol*., **61**: 116-121.

Irfan Z, Afridi FI, Fatima A, Zafar H, Pervaiz YM and Farooq L (2024). Prevalence of extensively drug resistant *Salmonella* Typhi and its susceptibility against meropenem, tigecycline, fosfomycin and azithromycin among clinical isolates from a tertiary care hospital laboratory. *Pak. J. Health Sci*., **5**(8): 49-54.

Khan A, Khan I, Babar AN, Khan Y, Shah G and Khan MI (2024). Effectiveness of Oral Azithromycin in Treating Enteric Fever: A Hospital-Based Study on Pediatric Patients. *Cureus*, **16**(8): e67024.

Kumar S, Balakrishna K and Batra HV (2005). Detection of Salmonella enterica serovar Typhi (*S.* Typhi) by selective amplification of invA, viaB, fliC-d and prt genes by polymerase chain reaction in multiplex format. *Lett Appl. Microbiol*., **42**(2): 149-154.

Lin FH, Chen BC, Chou YC, Hsieh CJ and Yu CP (2021). Incidence and risk factors for notifiable typhoid and paratyphoid in Taiwan during the period 2011-2020. In *Healthcare*, **9**(10): 1316.

Mahmood S, Rasool MH, Khurshid M and Aslam B (2025). Genetic outlook of Colistin resistant Salmonella enterica Serovar Typhimurium recovered from Poultry-Environment Interface: A One Health Standpoint. *Pak. Vet. J.*, **45**(1): 246-256

Muleta A and Meseret N (2025). Seroprevalence of typhoid fever and its associated risk factors among clinically diagnosed febrile patients visiting the outpatient department at Debark Hospital and drug susceptibility patterns of isolates. *Biomed. Res. Int*., **2025**(1): 1717780.

Mylona E, Voong Vinh P, Qureshi S, Karkey A, Dongol S, Ha Thanh T and Baker S (2021). Tebipenem as an oral alternative for the treatment of typhoid caused by XDR *Salmonella* Typhi. *J. Antimicrob. Chemother*., **76**(12): 3197-3200.

Procaccianti M, Motta A, Giordani S, Riscassi S, Guidi B, Ruffini M, Maffini V, Esposito S and Dodi I (2020). First case of typhoid fever due to extensively drug-resistant *Salmonella* enterica serovar Typhi in Italy. *Pathogens*. **9**(2): 151.

Osuntokun OT and Olajubu FA (2015). Antibacterial and phytochemical properties of some Nigerian medicinal plant on Salmonella typhi and Salmonella paratyphi isolated from Infected Human Stool in Owo local Government. *J. Sci. Res. Rep.,* **4**(5): 441-449.

Qamar MU, Ambreen A, Batool A, Rasool MH, Shafique M, Khan A and Ejaz H (2020). Molecular detection of extensively drug-resistant *Salmonella* Typhi and carbapenem-resistant pathogens in pediatric septicemia patients in Pakistan-a public health concern. *Future Microbiol*., **16**(10):731-739.

Sawa T, Kooguchi K and Moriyama K (2020). Molecular diversity of extended-spectrum β-lactamases and carbapenemases and antimicrobial resistance. *J Intensive Care*. **8**(1): 13.

Shah SAA, Nadeem M, Syed SA, Abidi STF, Khan N and Bano N (2020). Antimicrobial sensitivity pattern of *Salmonella* Typhi: emergence of resistant strains. *Cureus*. **12**(11):11778.

Shakir M.Z., Rizvi F, Masoud F, Rafique A, Usmani MW, Naseem MN and Usman M (2024). Seroprevalence, molecular confirmation and application of bacteriophage as a potential prophylactic therapy against fowl typhoid disease in commercial poultry birds. *Pak Vet J*., **44**(4): 1185-1192.

Ullah A, Shabil M, Abdulsamad SA, Jan A, Naeem AA, Ullah H and Zakiullah (2025). Prevalence of the Antibiotic Resistance of *Salmonella* Typhi and Salmonella paratyphi in Pakistan: A systematic review and meta-analysis. In *Open Forum Infectious Diseases*, **12**(4): 131.

Yasin N, Rahman H, Sarwar Y, Qasim M, Nisa I, Ikram A and Alzahrani KJ (2022). *Salmonella* Typhi from Northwest Pakistan: Molecular strain typing and drug resistance signature. *Microb Drug Resist*., **28**(1):120-126.

Zahid I, Sarwar A, Hussain A, Sohail M and Amin A (2022). Antibiotyping and genotyping of extensively drug-resistant (XDR) Salmonella sp. isolated from clinical samples of Lahore, Pakistan. *J Appl Microbiol*., **132**(1): 633-641.