

Pattern of Drug Resistance of Salmonella Typhi, Clinical Profile and Complications Developed Among Children Admitted in A Tertiary Care Set Up

Sumaira Siddique Mughal¹, Shaista Bakhat², Naseem Khalid Baloch³, Sehrish Shafique⁴, Luqman Satti⁵, Hina Wasti⁶, Yasmeen Taj⁷, Faisal Faheem⁸

- 1 Post Graduate Trainee, Department of Microbiology, PNS Shifa (Pakistan Naval), Karachi Pakistan
Main idea, Samples collection, Methodology, Writeup
- 2 Assistant Professor, Department of Pathology, Bahria University Health Sciences, Karachi Pakistan
Whole manuscript writeup, Review, Layout & Correspondence
- 3 Post Graduate Trainee, Department of Pediatrics, PNS Shifa (Pakistan Naval), Karachi Pakistan
Provides patients detail history, Clinical presentation, Complications & lab reports
- 4 Associate Professor, Department of Medicine, Bahria University Health Sciences, Karachi Pakistan
Provide medical details of patients
- 5 Professor, Department of Microbiology, PNS Shifa (Pakistan Naval), Karachi Pakistan
Supervision, Facilitate in bench work
- 6 Assistant Professor, Department of Pathology, Bahria University Health Sciences, Karachi Pakistan
Layout & Editing
- 7 Professor, Department of Pathology, Bahria University Health Sciences, Karachi Pakistan
Review & make corrections
- 8 Lecturer, Department of Physiotherapy, PNS Shifa (Pakistan Naval), Karachi Pakistan
Statistical analysis

CORRESPONDING AUTHOR

Dr. Shaista Bakhat
Assistant Professor, Department of Pathology,
Bahria University Health Sciences, Karachi Pakistan
Email: shaistakhurram2@gmail.com

Submitted for Publication: 27-09-2022
Accepted for Publication 31-01-2023

How to Cite: Mughal SS, Bakhat S, Baloch NK, Shafique S, Satti L, Wasti H, Taj Y, Faheem F. Pattern of Drug Resistance of Salmonella Typhi, Clinical Profile and Complications Developed Among Children Admitted in A Tertiary Care Set Up. APMC 2023;17(1):6-10. DOI: 10.29054/APMC/2023.1402

ABSTRACT

Background: Typhoid fever is an alarming threat all over the world in general and in particular in our geographical area like Pakistan. This situation is worsened by extensively drug resistant strain of *Salmonella species* particularly in province of Sindh. **Objective:** The aim of study to highlight the drug resistance pattern in isolates of *Salmonella typhi* and *Salmonella Para typhi* in order to overcome the complications developed in children. **Study Design:** Observational cross sectional study. **Settings:** Microbiology laboratory of PNS Shifa with collaboration of Medicine Department, Karachi Pakistan. **Duration:** August 2021 till August 2022. **Methods:** This study was conducted by taking isolates of XDR *Salmonella Typhi* from samples of blood using a BD BACTEC™ automated blood culture system. Clinical and diagnostic summary of the patients including gender, age, duration of illness, laboratory parameters and antibiotic susceptibility pattern of the pathogen were monitored by logistic regression to recognize the factors associated with complications. **Results:** Total of 1200 patients were admitted to PNS Shifa hospital as clinically diagnosed cases of typhoid fever. Out of these, 106 were found as XDR (extensively drug resistant). Median (IQR) age of the patients was 4.5 years; 52% were male. Laboratory findings revealed C-reactive protein was 8.1 with confidence interval 6.51-7.5. Out of 106 two patients (1.9%) were expired as a result of intestinal perforation. 45 patients (42.5%) patients were cured properly. Amongst culture-screened cases (n=106), isolates resistant to ampicillin, chloramphenicol and trimethoprim, 3rd generation cephalosporins, fluoroquinolons, and sulphamethoxazole was 106 /106 (100%) respectively, were extensively drug-resistant. **Conclusion:** Our study explains that enteric fever in under-five year children poses a chief public health crisis in Pakistan. We need to take measures and make policies to control this endemic; we are standing on the edge of pan-drug resistance in isolates of *Salmonella typhi*. We conclude from our findings that the implementation of screening tests is effective for detection of resistant strain.

Keywords: Typhoid fever, *Salmonella typhi*, Antibiotic susceptibility pattern, XDR.

INTRODUCTION

Typhoid fever is systemic disease majorly caused by *Salmonella enterica serovar Typhi* (*S. Typhi*) and less

commonly with *Salmonella para typhi* A, B, C, Gram-negative bacteria.¹ Enteric fever is the most ubiquitous all over the world especially in third world countries, with estimated range from 11.9 million to 27.1 million cases

per year of global burden,² with highest morbidity in the south and Southeast Asia.³ Unluckily Pakistan is one of those five countries which have been declared endemic for typhoid with increased cases.⁴ The rate of typhoid fever is reported to be 451.7/100,000 persons in age group of 2-15 years in Pakistan.⁵ The frequency rate was elevated in group of children aged 1-15 years, and children below 5 years were considered a defenseless group in the extremely endemic area.⁶

The typhoid fever presents classical symptoms like slow onset of fever, abdominal pain and hepatosplenomegaly. Infuriating symptoms are vomiting, diarrhea, toxicity; consequences like disseminated intravascular coagulation and encephalopathy are rare but still common. Besides these complications intestinal perforation and peritonitis are common during early onset of disease.⁷ Complications are associated with age, antibiotic resistance, period of illness before admission, and blood pressure less than 100mm Hg.⁸ Antibiotic like chloramphenicol have played important role in reducing the fatality rate. Though, rampant and inappropriate use of chloramphenicol and attainment of plasmid-mediated R factor accelerated the emergence of resistance towards amoxicillin, chloramphenicol, and cotrimoxazole, resulting in multidrug resistance (MDR) of typhoid.⁹ This issue was overcome by fluoroquinolones such as ciprofloxacin.¹⁰ This was followed by emergence of ciprofloxacin resistance due to altered DNA gyrase.¹¹ Nowadays, we are dealing with extensively drug resistance (XDR with Penicillin,^{3rd} generation of Cephalosporin, chloramphenicol, cotrimoxazole, and fluoroquinolones.

Pakistan is situated in the area where enteric fever is highly endemic; this study will facilitate to characterize the age, clinical and diagnostic profile, antibiotic susceptibility pattern and consequences of admitted patients with pathogen *Salmonella*.

METHODS

This observational cross sectional study was conducted at Microbiology laboratory of PNS Shifa with collaboration of medicine department, Karachi Pakistan from August 2021 till August 2022. Prior ethical approval was taken from ERC (ethical review committee) of PNS Shifa hospital. This study was conducted by taking isolates of XDR *Salmonella Typhi* and *Salmonella para-typhi* from samples of blood using a BD BACTEC™ automated blood culture system. The majority of clinically considerable organisms grew within 3 days of incubation. BACTEC™ bottles were checked until the next seven days of incubation. BACTEC system signaled when there was a positive culture. Afterwards, positive cultures were inoculated on 5% Sheep Blood agar and MacConkey agar. Plates were incubated for 24 hours at 37°C±2°C and then

checked for bacterial growth. All GNR (Gram negative rods) from positive bottles were identified by their growth characteristics, colony morphology, staining characters, and motility and biochemical profiles. Antibiotic sensitivity and resistance pattern was determined by using disc diffusion method as recommended by Clinical Laboratory Standard Institute (CLSI) guideline.¹² A standardized inoculum was prepared by using freshly obtained (overnight) bacterial colonies grown on Mueller-Hinton plates in sterile NaCL to obtain a 0.5 McFarland optical density (105 CFU/mL), which corresponds to a 10-µL full loop of bacterial colonies. Positive blood cultures were followed and admitted cases among them were kept for observation. Enteric fever patients with confirmed culture report for *S.Typhi* and *Para-typhi*, exhibiting resistance towards five classes of antimicrobial drugs (ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, fluoroquinolone, and cephalosporin (ceftriaxone or cefixime) were added in this study. Patients with incomplete medical records especially missing information on antibiotic therapy, treatment failure, patients who left against medical advice (LAMA) within 2 days of admission, those who had a positive blood culture but did not seek treatment at either hospital or those who did not return for follow-up visit were excluded from the study. All patients' daily record of physical examination, laboratory reports, improvement in clinical conditions and medical outcome on day to day basis were recorded.

This study was statistically analyzed by using SPSS version 23.0. All continuous parametric data was presented as mean and standard deviation. All continuous non parametric data were shown as Median and interquartile range (IQR) with 95% confidence interval. All categorical variables were presented as frequency and percentages. Statistical significance differences were checked through Binary Logistics Regression Analysis at P value less than or equal to 0.05.

RESULTS

Total of 1200 patients were admitted to PNS shifa hospital as clinically diagnosed cases of typhoid fever were admitted to PNS shifa hospital as clinically diagnosed cases of typhoid fever. Respondents with age group of 1-7 years observed with Intestinal Perforation, Acute Renal Failure, Thrombocytopenia, Sepsis, DIC, Abdominal distension and Hepatitis were found more at-risk Odds=1.4 with 95% confidence interval [0.436 - 4.496] with no statistical differences observed. Female gender was found to have risk at different complications of typhoid fever. Odds ratio was 1.091 with 95% confidence interval [1.091-0.350] in female diagnosed with Intestinal Perforation, Acute Renal Failure, Thrombocytopenia, Sepsis, DIC, Abdominal distension and Hepatitis with no

statistical differences observed. While in admitted patients, Hepatitis diagnosis was found at some risk with odds ratio=1.111 with 95% confidence interval [0.262-4.719]. Similarly, Thrombocytopenia, Abdominal Distension and Hepatitis diagnosis was found more at risk with odds ratio=1.548 with 95% confidence interval [0.438-5.470] with no statistical differences observed. (Table 4)

Out of these, 106 were found as XDR (extensively drug resistant). Median (IQR) age of the patients was 4.5 years; 52% were male.

Table 1: Demographic, clinical, mode of admission, and complications with typhoid fever

Age		Median (IQR)	95% Confidence Interval
		4.5 (6)	5.12-6.48
		Frequency	Percent %
Gender	Female	54	50.9%
	Male	52	49.1%
	Total	106	100.0%
Mode of Admission	ER	66	62.3%
	OPD	40	37.7%
Clinical Presentation	Fever	106	100.0%
	Abdominal Pain	97	91.5%
	Bleeding per rectum	3	2.8%
	Blood in stool	10	9.4%
	Diarrhea	20	18.9%
	Hepatomegaly	22	20.8%
	Rash	20	18.9%
	Splenomegaly	18	17.0%
	Vomiting	20	18.9%
Complications Developed	Abdominal Distension, Hepatitis	15	14.2%
	Hepatitis	46	43.4%
	Intestinal Perforation, Acute Renal Failure, Thrombocytopenia, Sepsis, DIC, Abdominal distension	2	1.9%
	Thrombocytopenia	27	25.5%
	Thrombocytopenia, Abdominal Distension, Hepatitis	16	15.1%
Outcome	Cured	45	42.5%
	Discharged	44	41.5%
	Expired	2	1.9%
	Lama	10	9.4%
	lost to follow up	5	4.7%

Table 2: Laboratory Features with Typhoid patients

Laboratory investigations	Median (IQR)	95% Confidence Interval
TLC (Per Cubic Milli Meter of Blood)	8000 (3625)	7339 - 8334
CRP (mg/ dl)	8.1 (3.8)	6.51 - 7.5
Neutrophil Count (%)	38 (23.25)	41.35 - 48.04
Serum Potassium (mmol/L)	3.3 (1.23)	3.10 - 3.44
Creatinine (mg/ dl)	1 (2)	1.06 - 3.35
Platelets (x10 ³ / mL)	167.5 (40)	152.81 - 182.02
HB (g/ dl)	13 (2)	12.54 - 13.24
LFTs (IU/L)	49 (31.25)	87.46 - 244.14

Table 3: Antibiotic susceptibility pattern of strains isolated from patients with *Salmonella typhi* and *Salmonella paratyphi*

Treated With		Frequency	Percent
Azithromycin + Meropenem	Sensitive	90	84.9%
	Resistant	16	15.1%
Ampicillin	Sensitive	0	0.0%
	Resistant	106	100.0%
Chloramphenicol	Sensitive	0	0.0%
	Resistant	106	100.0%
Trimethoprim	Sensitive	0	0.0%
	Resistant	106	100.0%
Sulfamethoxazole	Sensitive	0	0.0%
	Resistant	106	100.0%
Fluoroquinolone	Sensitive	0	0.0%
	Resistant	106	100.0%
3rd generation of cephalosporin	Sensitive	0	0.0%
	Resistant	106	100.0%

Among culture-positive cases (n = 106), the number of isolates resistant to ampicillin, chloramphenicol and trimethoprim, 3rd generation cephalosporins, fluoroquinolones, and sulphamethoxazole was 106 /106 (100%) respectively, were extensively drug-resistant. Azithromycin and meropenem were mostly 84.9% sensitive.

Table 4: Factors associated with complications in typhoid fever: results of logistic regression

Complications of typhoid Fever with Age, gender & Mode of Admission		Odds Ratio	95% C.I. for EXP(B)		P-value
			Lower	Upper	
Age					
1-7 years	Abdominal Distension, Hepatitis	1			
	Hepatitis	0.256	0.042	1.551	0.138
	Intestinal Perforation, Acute Renal Failure, Thrombocytopenia, Sepsis, DIC, Abdominal distension, Hepatitis	1.400	0.436	4.496	0.572
	Thrombocytopenia	0.000	0.000	0.000	0.999
	Thrombocytopenia, Abdominal Distension, Hepatitis	0.476	0.122	1.854	0.285
8-15 years		1			
Gender					
Female	Abdominal Distension, Hepatitis	1			
	Hepatitis	0.667	0.161	2.769	0.577
	Intestinal Perforation, Acute Renal Failure, Thrombocytopenia, Sepsis, DIC, Abdominal distension, Hepatitis	1.091	0.350	3.404	0.881
	Thrombocytopenia	1.000	0.053	18.915	1.000
	Thrombocytopenia, Abdominal Distension, Hepatitis	0.929	0.270	3.199	0.907
Male		1			
Mode of Admission					
ER	Abdominal Distension, Hepatitis	1			
	Hepatitis	1.111	0.262	4.719	0.886
	Intestinal Perforation, Acute Renal Failure, Thrombocytopenia, Sepsis, DIC, Abdominal distension, Hepatitis	0.806	0.247	2.638	0.722
	Thrombocytopenia	0.000	0.000	0.000	0.999
	Thrombocytopenia, Abdominal Distension, Hepatitis	1.548	0.438	5.470	0.498
OPD		1			

*Binary Logistics Regression Analysis was applied at P-value < 0.05 statistically significant.

DISCUSSION

Enteric fever exists as a foremost source of morbidity and mortality globally, majorly in central and south-east Asia.¹³ This study noticed the clinical presentation of patients diagnosed with enteric fever with confirmed *Salmonella typhi* and *Para typhi* detected from blood and its antibiotic susceptibility and resistance during one year. We also designed a regression model to find the associating factors for complication in typhoid fever.

Children less than 5 years of age are the majority affected by enteric fever in Pakistan; under-five years were 12 times more often affected by enteric fever as compare to adults.¹⁴ Enteric fever leads to complications and mortality rate (1.9%) in children under five years of age in Pakistan as compare to 0.2% in India.¹⁵ The two fatal cases in this study were two children with intestinal perforation, which are recognized risk factors of case fatality.¹⁶ Njarekkattuvalappil SK *et al*, revealed that non-traumatic ileal-perforation can occur as a outcome of typhoid fever.¹⁷ Consequences or complications can be overcome by early detection of pathogen in the laboratory, and then physicians will be able to select appropriate treatment. Laboratory findings revealed that children had presented with anaemia, leucopenia, electrolyte imbalance and thrombocytopenia. Thrombocytopenia, a recognized analyst of complications with typhoid fever, was found to be an

independent factor associated with complications in accordance with case report.¹⁸ This study also showed a rare cases of typhoid fever with complication such as renal failure in association of case report of Thailand.¹⁹ Hepatitis is not only caused by hepatotropic viruses but it can happen as a consequence of enteric fever by deranging liver function tests, as reported by study conducted in Colombia.²⁰ Extensively drug resistant *Salmonella typhi* is common in Asia and specifically in our part of world, also reported by another study conducted by Aga Khan at Pakistan.²¹ The emergence of extensively drug resistance of *Salmonella Typhi* is becoming a great threat in support of our study.²² Our neighbor country India is also facing the same situation of extensively drug resistant *Salmonella typhi* in accordance with our study.²³ Azithromycin might be an another choice for low socioeconomic countries, though resistance towards azithromycin were also experienced in some patients of India in association of our study.²⁴ Meropenem is last resort that is hope in this situation not only for patients but also for physicians in order to overcome this infection.^{25,26}

CONCLUSION

Our study explains that typhoid fever in under-five children poses a major public health crisis in Pakistan. We need to take measures and make policies to control this endemic; we are standing on the edge of pandrug

resistance in isolates of *Salmonella typhi*. We conclude from our findings that the implementation of screening tests are effective for detection of resistant strain and should be done as a first stage of treatment strategy. We can also avoid complications by early detection and management of disease.

LIMITATIONS

This study can't be generalized as a result of single center.

SUGGESTIONS / RECOMMENDATIONS

Vaccination and screening of cases in community are recommended.

CONFLICT OF INTEREST / DISCLOSURE

No conflict of interest is involved.

ACKNOWLEDGEMENTS

The role of faculty members and PNS Shifa hospital is highly appreciated in this study.

REFERENCES

- Umair M, Siddiqui SA. Antibiotic Susceptibility Patterns of *Salmonella Typhi* and *Salmonella Paratyphi* in a Tertiary Care Hospital in Islamabad. *Cureus*. 2020 Sep 3;12(9):e10228.
- Antillón M, Warren JL, Crawford FW, Weinberger DM, Kürüm E, Pak GD, et al. The burden of typhoid fever in low- and middle-income countries: A meta-regression approach. *PLoS Negl Trop Dis*. 2017 Feb 27;11(2):e0005376.
- Buckle GC, Walker CL, Black RE. Typhoid fever and paratyphoid fever: Systematic review to estimate global morbidity and mortality for 2010. *J Glob Health*. 2012 Jun;2(1):010401.
- Crump JA, Mintz ED. Global trends in typhoid and paratyphoid fever. *Clin Infect Dis*. 2010 Jan 15;50(2):241-6.
- Ochiai RL, Acosta CJ, Danovaro-Holliday MC, Baiqing D, Bhattacharya SK, Agtini MD, et al. A study of typhoid fever in five Asian countries: disease burden and implications for controls. *Bulletin of the world health organization*. 2008 Apr;86(4):260-8.
- Buckle GC, Walker CL, Black RE. Typhoid fever and paratyphoid fever: Systematic review to estimate global morbidity and mortality for 2010. *J Glob Health*. 2012 Jun;2(1):010401.
- Bhandari J, Thada PK, DeVos E. Typhoid Fever. 2022 Aug 10. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. PMID: 32491445.
- Khatun H, Islam SB, Naila NN, Islam SA, Nahar B, Alam NH, Ahmed T. Clinical profile, antibiotic susceptibility pattern of bacterial isolates and factors associated with complications in culture-proven typhoid patients admitted to an urban hospital in Bangladesh. *Trop Med Int Health*. 2018 Apr;23(4):359-366.
- Rahman M, Ahmad A, Shoma S. Decline in epidemic of multidrug resistant *Salmonella typhi* is not associated with increased incidence of antibiotic-susceptible strain in Bangladesh. *Epidemiol Infect*. 2002 Aug;129(1):29-34.
- Adhikari D, Acharya D, Shrestha P, Amatya R. Ciprofloxacin susceptibility of *Salmonella enteric* serovar *Typhi* and *Paratyphi A* from blood samples of suspected enteric fever patients. *International Journal of Infection and Microbiology*. 2012 Oct 8;1(1):9-13.
- Eibach D, Al-Emran HM, Dekker DM, Krumkamp R, Adu-Sarkodie Y, Cruz Espinoza LM, et al. The emergence of reduced ciprofloxacin susceptibility in *Salmonella enterica* causing bloodstream infections in rural Ghana. *Clinical Infectious Diseases*. 2016 Mar 15;62(suppl_1):S32-6.
- Ali Shah SA, Nadeem M, Syed SA, Fatima Abidi ST, Khan N, Bano N. Antimicrobial Sensitivity Pattern of *Salmonella Typhi*: Emergence of Resistant Strains. *Cureus*. 2020 Nov 29;12(11):e11778.
- Fatima M, Kumar S, Hussain M, Memon NM, Vighio A, Syed MA, et al. Morbidity and Mortality Associated with Typhoid Fever Among Hospitalized Patients in Hyderabad District, Pakistan, 2017-2018: Retrospective Record Review. *JMIR Public Health Surveill*. 2021 May 17;7(5):e27268.
- Saha S, Sayeed KMI, Saha S, Islam MS, Rahaman A, Islam M, et al. Hospitalization of Pediatric Enteric Fever Cases, Dhaka, Bangladesh, 2017-2019: Incidence and Risk Factors. *Clin Infect Dis*. 2020 Dec 1;71(Suppl 3):S196-S204.
- Cao Y, Karthikeyan AS, Ramanujam K, Raju R, Krishna S, Kumar D, et al. Geographic Pattern of Typhoid Fever in India: A Model-Based Estimate of Cohort and Surveillance Data. *J Infect Dis*. 2021 Nov 23;224(224 Suppl 5):S475-S483.
- Contini S. Typhoid intestinal perforation in developing countries: Still unavoidable deaths? *World J Gastroenterol*. 2017 Mar 21;23(11):1925-1931.
- Njarekkattuvalappil SK, Thomas M, Kapil A, Saigal K, Ray P, Anandan S, et al. Ileal Perforation and Enteric Fever: Implications for Burden of Disease Estimation. *J Infect Dis*. 2021 Nov 23;224(Suppl 5):S522-S528.
- Al Reesi M, Stephens G, McMullan B. Severe thrombocytopenia in a child with typhoid fever: a case report. *J Med Case Rep*. 2016 Nov 30;10(1):333.
- Pancharoen C, Wongsawat J, Pancharoen S, Thisyakorn U. Typhoid glomerulonephritis in a child: a rare complication of typhoid fever. *Southeast Asian J Trop Med Public Health*. 2001 Dec;32(4):869-71.
- León S LP, Otero W, Gómez M. Fever, jaundice and hepatitis: it is not always a viral infection. *Revista colombiana de Gastroenterología*. 2015 Sep;30(3):292-7.
- Qureshi S, Naveed AB, Yousafzai MT, Ahmad K, Ansari S, Lohana H, et al. Response of extensively drug resistant *Salmonella Typhi* to treatment with meropenem and azithromycin, in Pakistan. *PLoS Negl Trop Dis*. 2020 Oct 15;14(10):e0008682.
- Ghurnee O, Ghosh AK, Abony M, Aurin SA, Fatema AN, Banik A, et al. Isolation of multi-drug resistant (MDR) and extensively drug resistant (XDR) *Salmonella typhi* from blood samples of patients attending tertiary medical centre in Dhaka city, Bangladesh. *Advances in Microbiology*. 2021 Sep 2;11(9):488-98.
- Pustake M, Giri P, Tambolkar S, Nayak S. Extensively drug-resistant typhoid fever: A call to action. *Indian Journal of Community Medicine: Official Publication of Indian Association of Preventive & Social Medicine*. 2022 Jan;47(1):153.
- Carey ME, Jain R, Yousuf M, Maes M, Dyson ZA, Thu TN, et al. spontaneous emergence of azithromycin resistance in independent lineages of *Salmonella Typhi* in northern India. *Clinical Infectious Diseases*. 2021 Mar 1;72(5):e120-7.
- Islam K, Mahmud R, Chowdhury MK, Hossain FS, Biswas PK, Sarker S. Recent Sensitivity Pattern of *Salmonella Typhi* in a Private Hospital. *Journal of Medicine*. 2018;19(1):15-7.
- Wong W, Al Rawahi H, Patel S, Yau Y, Eshaghi A, Zittermann S, et al. The first Canadian pediatric case of extensively drug-resistant *Salmonella Typhi* originating from an outbreak in Pakistan and its implication for empiric antimicrobial choices. *IDCases*. 2019 Jan 1;15:e00492.