Original article

Frequency of the multi and extensively drug-resistant *Salmonella* Typhi in a health care facility

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ABSTRACT

Background: Antibiotic resistance is increasing against *Salmonella* Typhi *(S.* Typhi*)*, causative agent of enteric fever, at a very high pace in Pakistan. The objective of this study was to evaluate the emergence of multi and extensively drug resistant *Salmonella* Typhi among suspected patients in a public health care facility in Lahore.

Patients and methods: 5cc blood samples from the patients with high grade fever (>38°C), vomiting and bradycardia (heart rate 84 bpm) were collected from laboratory of Arif Memorial Teaching Hospital, Lahore over six months (August 2020-January 2021), After informed consent, questionnaire were filled and total 97 samples were taken from suspected patients and screened for *Salmonella* Typhi by using Typhidot IgG/IgM by ELISA kit (one step typhoid fever) for *S.* Typhi. Gram staining, culture and biochemical testing was performed for confirmation. Antibiotic susceptibility testing was used to evaluate the emergence of antibiotic resistance. Statistical analysis was performed by using SPSS 20.0, software. Chi square test was used,

Results: Total 55 (56.7%) samples out of 97 (100%) were reported positive for *Salmonella* Typhi. Antimicrobial susceptibility testing results indicated that 6 samples were resistant to more than one antibiotic commonly called as multidrug resistant, 44 samples were extensively drug resistant and 5 samples were nonresistant/sensitive to selected antibiotics. Highest resistance was reported against Ampicillin (51 samples (92.7%) and Co-trimoxazole (50 samples (90%)). Resistance against Moxifloxacin and Tobramycin, Ciprofloxacin, Ceftriaxone Cefuroxime and Cefotoxime, Cefxime, Levofloxacin, Salbactum, Imepenum and Amikain was 47 (85.4%), 46 (83.6%), 45 (81.81%), 44 (80%), 37 (67.2%), 32 (58.18%), 14 (45%) and 9 (16.3%) respectively. Lowest resistance was reported against Meropenum 5 (9%) and Azithromycin 1 (1%).

Conclusion: It is concluded that the monitoring of MDR and XDR must be performed by all clinical microbiology/pathology laboratories to implement effective measures to reduce the emergence of antimicrobial resistance.

Keywords:

Multidrug resistant (MDR), Extensively drug resistance, Enteric fever, *Salmonella* Typhi.

INTRODUCTION

Antibiotic resistance is an alarming phenomenon in developing countries with an increasing prevalence of pathogenic multidrug-resistant bacteria. In Asia, the expected percentage of antibiotic resistance is 70%, meanwhile Pakistan is among the top listed countries facing threat of both multi-drug resistant (MDR) and extensively drug resistant (XDR) bacteria. MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories whereas XDR is defined as nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two antimicrobial categories). [1] A rod shape gram negative bacteria, *Salmonella* Typhi*,* causative agent of typhoid fever is changing from MDR to XDR through plasmid at a very high pace.[2] In 2017, almost >300 cases of XDR *Salmonella* Typhi strain were reported in Pakistan. Ceftriaxone (CRO) and Fluroquinolones (FQ) resistant *Salmonella* Typhi strains were reported for the first time in Hyderabad, Sindh, Pakistan in 2016 and overall $\approx 5.01\%$ XDR emergence in *Salmonella* Typhi cases has been documented since 2015 in Sindh.[3,4] *Salmonella* Typhi), is transmitted by fecal-oral route in human due to the consumption of food and water contaminated with human feces.[5] WHO has declared highest incidence rate of typhoid infection in south-central and south-eastern region of Asia (> 100/100 000

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persons/year). According to world health organization (WHO) the incidence rate of tyhphoid infection in Pakistan is 412 per 100,000 person/years.[6]. Studies showed that the consumption of contaminated water and travelling are among the main source of transmission of XDR strains of *S.* Typhi in Pakistan.[7] In low to middle income countries (LMIC) typhoid fever is screened by serological testing and diagnosed by blood and bone marrow culture followed by using stool or urine culture in first and second week of infection respectively.[88] The first line drugs for the treatment of typhoid infection are Ampicillin (Amp 10), Cotrimoxazole (Cot 25), Ciprofloxacin (Cip)/Oflaxacin (Ofl 5)/ Enoxacin (Eno 30) and 2nd line drugs are Cefixime (CFM 30), Ceftriaxone (CRO)/ Cefotaxime (CTX 30), Aztreonam (ATM 30), Cefpodoxime (CPD 30) and Chloramphanicol (CAP 30).[1, 5, 9] Now a days, 2nd line drugs i.e. fluoroquinolones and 3rd generation cephalosporins and macrolides are being used to treat MDR Salmonella infections but continuously increasing resistance against antibiotic requires significant attention.[10, 11, 12] In the continuationthe objective of this study was to evaluate the frequency of MDR and XDR *S.* Typhi from Arif Memorial Teaching Hospital, Lahore, Punjab, Pakistan. The patterns of antibiotic sensitivity were also observed to study the resistance behavior of XDR and MDR *S.* Typhi isolates, respectively.

PATIENTS AND METHODS

This cross sectional study was reviewed and approved by ethical review committee of Rashid Latif Medical College, Lahore. Total 97 blood samples (5cc) were collected from suspected patients of typhoid fever (>38°C), vomiting and bradycardia (heart rate 84 bpm) who visited Arif Memorial Teaching Hospital, Lahore. Both male and female patients with the symptoms (especially fever 100-103° F) were included. An informed consent was obtained from the patients before sample collection. Demographic and clinical parameters of each patient were recorded in the questionnaire developed for the study. Blood samples were collected in vacutainer before processing. Laboratory investigations were performed at Pathology Laboratory of Arif Memorial Teaching Hospital Lahore. Initial screening was done using Typhidot IgG/IgM by ELISA kit (one step typhoid fever) for *Salmonella* Typhi that separately identified Immunoglobulin M and Immunoglobulin G, representing initial and chronic infection respectively. Screened samples were processed for culture and biochemical testing. For culture, 5ml blood sample was taken in the brain heart infusion (BHI) taken from each suspected patients and incubated at 37°C and growth was observed for 2-3 days. Gram staining technique was used to examine morphological features under light microscopy. Colonies were sub-cultured on blood agar and MacConkey agar. After that, biochemical tests including API 20E (Biomerieux France), Oxidase, Indole, Urease, Methyl Red, TSI (Triple sugar iron), Cytochrome oxidase and Catalase were performed. After confirmation isolates were streaked on Mueller-Hinton agar by using Kirby Bauer method for antibiotic susceptibility testing by using fifteen drugs i.e. Cotrimoxazole (TMP-SMX), Ampicillin (Amp), Ciprofloxacin (Cip), Levofloxacin (Lev), Moxifloxacin (Mxf), Cefixime (CFM), ceftriaxone (CRO), Meropenum (Mem), Imipenum (Imp), Azithromycin (Azt), Amikacine (AK/Ami), Tobramycine (Tob/NN), Cefuroxime (CXM), cefotaxime (Ctx) and Sulbactum (SCF) to distinguish sensitive/non-resistant, MDR and XDR S. Typhi.[5] The inhibition zones were interpreted and graded as MDR and XDR according to the resistance behavior towards 3 to 4 classes of antibiotics and 5 to 6 antibacterial groups, respectively according to the Clinical Laboratory Standard Institute (CLSI) guidelines.[13] Statistical analysis was performed by using SPSS version 20.0, software (Chicago, IL, USA).. Chi square test was used .The value of $p \leq 0.05$ was considered statistically significant.

RESULTS

In this study, total 97 suspected patients of typhoid fever were selected from Arif Memorial Teaching Hospital Lahore within the duration of six months (august 2020 to January 2021). In this study, commonly observed signs and symptoms in confirmed patients were fever (99.7%), diarrhea (98.42%) and abdominal discomfort (96.85%). Out of 97 blood samples including 73 (75.3%) male patients and 24 (24.7%) female, 55 (56.7%) were reported positive for *Salmonella* Typhi and 42 (43.29%) were negative after performing ELISA dot kits, culture, biochemical and serological testing. After confirmation, antimicrobial susceptibility testing was performed on 55 positive samples out of which, 4 (66.7%) males and 2 (33.3%) females were reported multidrug resistant (MDR) and 35 (79.5%) male and 9 (20.5%) female was reported as extensively drug resistant (XDR) whereas 3 (60%) male and 2 (40%) female was reported as nonresistant / sensitive as shown in Figure 1.

We had taken total 55 samples out of which 51(92.7%) showed resistance to first line drug ampicillin (Amp) showing highest resistant against S. Typhi. Then, 50 (90%) samples were resistant to co-trimoxazole that made it 2nd highest resistant antibiotic followed by 47 (85.4%) samples resistant to moxifloxacin and tobramycine and 46 (83.6%) samples resistant to ciprofloxacin. Moreover 45 (81.81%) samples showed resistance against ceftriaxone, cefuroxime and cefotaxime, 44 (80%) were resistant against cefixime and 37 (67.2%) were resistant to levofloxacin. Resistance was observed against salbactum, imepenum and amikain in 32 (58.18%), 14 (45%) and 9 (16.3%) samples respectively. Lowest resistance was shown against meropenum and azithromycin. Only 5 (9%) samples showed resistance against meropenum and 1 (1.8%) sample was resistant to azithromycin (Table 1).

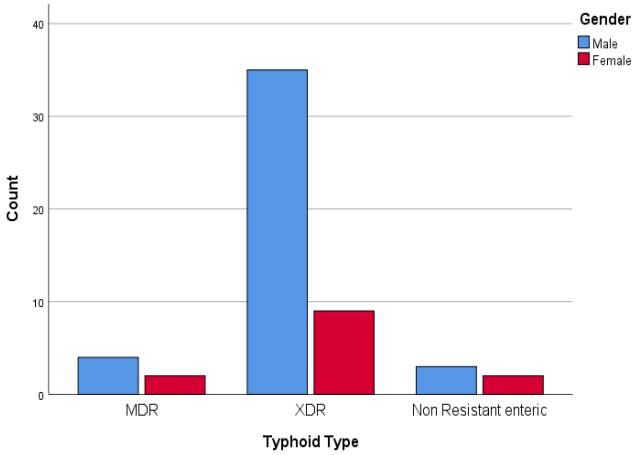

Figure 1: Gender-based drug resistance of samples

Table 1: Frequency of drug resistance in MDR and XDR patients

Drugs	MDR	XDR	Non-resistant/Sensitive	n=55	
				Resistant	Sensitive
TMP-SMX	6 (100%)	44 (100%)	0(0.0%)	50 (90%)	5 (10%)
AMP	6 (100%)	44 (100%)	1 (20%)	51 (92.7%)	4 (7.3%)
CIP	4 (67.7%)	42 (95.5%)	1 (20%)	46 (83.6%)	9 (16.4%)
LEV	3 (50%)	34 (85%)	0(0.0%)	37 (67.2%)	18 (32.8%)
MXF	4 (66.7%)	43 (97.71%)	0(0.0%)	47 (85.4%)	8 (14.6%)
CFM	0 (0.0%)	44 (100%)	0(0.0%)	44 (80%)	11 (20%)
CRO	1 (16.7%)	44 (100%)	0(0.0%)	45 (81.81%)	10 (18.19%)
MEM	0(0.0%)	5 (11.4%)	0(0.0%)	5 (9%)	50 (91%)
IMP	0(0.0%)	14 (31.8%)	0(0.0%)	14 (25.45%)	41 (74.55%)
AZT	0(0.0%)	1 (2.3%)	0(0.0%)	1 (1%)	54 (99%)
AK/AMI	0(0.0%)	9 (20.5%)	0(0.0%)	9 (16.3%)	46 (83.7%)
TOB/NN	6 (100%)	40(90.97%)	1 (20%)	47 (85.45%)	8 (14.55%)
CXM	1 (16.7%)	44 (100%)	0(0.0%)	45 (81.81%)	10 (18.19%)
CTX	1(16.7%)	44 (100%)	0(0.0%)	45 (81.81%)	10 (18.19%)
SCF	1(16.7%)	31 (73.8%)	0(0.0%)	32 (58.18%)	23 (41.82%)

Abbreviations: Cotrimoxazole (TMP-SMX), Ampicillin (Amp), Ciprofloxacin (Cip), Levofloxacin (Lev), Moxifloxacin (Mxf), Cefixime (CFM), ceftriaxone (CRO), Meropenum (Mem), Imipenum (Imp), Azithromycin (Azt), Amikacine (AK/Ami), Tobramycine (Tob/NN), Cefuroxime (CXM), cefotaxime (Ctx) and Sulbactum (SCF).

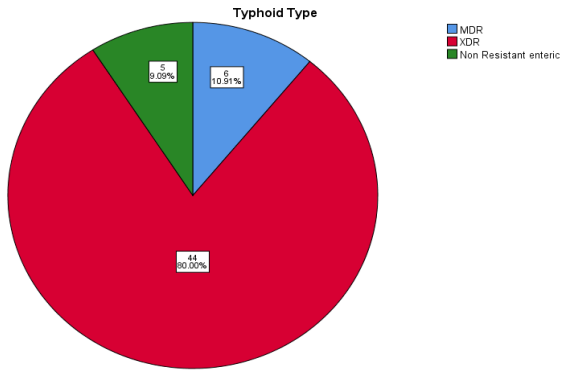

Figure 2: Percentage of nonresistant, MDR and XDR

In multidrug resistant (MDR) S. Typhi samples, resistant drugs were cortimoxazole, ampicillin, and tobramicine. Ciprofloxacin, levofloxacin and moxifloxacin were found resistant in 4, 3 and 4 MDR samples respectively. Carbapenums, cefixime, amikacin and azithromycin were sensitive to all MDR. All XDR samples were resistant to cortimoxazole, ampicillin, cefixime, cefuroxime and cfeotoxime. Except one, rest of XDR samples were resistant to moxifloxacin. Total 42 samples were resistant to ciprofloxacine. Levofloxacin and salbactum were resistant in 34 and 31 XDR samples respectively. Total 5 and 14 XDR samples were resistant to meropenum and imepenum respectively. Azithromycin was the only drug that showed resistance against one sample and was sensitive to all other antibiotics. This study showed that out of 55 typhoid positive samples, 6 (10.9%) samples were multidrug resistant, 44 (80.0%) samples were

extensively drug resistant and 5 (9.1%) samples showed no drug resistance/sensitive to drugs as shown in Figure 2.

DISCUSSION

Enteric fever is among major health threats in Pakistan with high rate of morbidity and mortality due to insufficient sanitary practice, unsafe and inadequate supply of clean drinking water, lack of health care facilities and most importantly lack of antimicrobial susceptibility evaluation criteria at basic level. Salmonella *typi,* causative agent of enteric fever is among top bacteria that have become resistance against antibiotics.

A related study on the increasing resistance against nucleic acid inhibitors fluoroquinolones and cell wall synthesis inhibitors cephalosporins in *Salmonella* Typhi in Lahore city showed susceptibility to Azithromycin (AZT), Carbapenems (CRE) and piperacillin, while all samples were resistant to the standard treatment of enteric fever including ceftriaxone (CRO). After performing AST, the authors performed phenotypic and genotypic characterization of 27 XDR *Salmonella* Typhi and reported the first ever emergence of XDR *Salmonella* Typhi in the province Punjab by performing whole genome sequencing (WGS) concluding that the extensively drug resistant isolates were indistinguishable from the strains that were reported in outbreak in southern Pakistan, indicating the cases of Lahore as a part of said outbreak and its spread due to travelling.[14]

Another related study from Lahore in 2020 by Iqra and colleagues on the antibiotyping and genotyping of XDR *Salmonella* Typhi from the suspected patients of typhoid fever evaluated the prevalence of XDR *Salmonella* Typhi in Lahore and along with genotyping of isolates for antibiotic resistant genes. Total 200 suspected samples were investigated for S. Typhi by performing the same pattern of methodology as of our study i.e. serological testing, cultural identification, biochemical testing and antibiotic susceptibility testing with the additional last step of genotyping of positive isolates for antibiotic resistance genes. Out of these 200 sample, 157 were positive for *Salmonella* Typhi and 121 (67.2%) and 62 (34.4%) were reported as MDR and XDR respectively. A predominance resistance of XDR *Salmonella* Typhi was reported against third generation cephalosporins.[8]

In 2020, a study was conducted that included 81 confirmed XDR typhoid patients and there was male predominance of XDR S. Typhi with n=45 (56%). Common symptom so the patients at the time of presentation were fever and vomiting. In this study, treatment profile was assessed for XDR *Salmonella* Typhi by using selected antibiotics and in the result, azithromycin, meropenem and combination of azithromycin and meropenem were reported as some preferable treatment options against XDR *S.* Typhi.[15]

Another study on 52 suspected samples identified 47 (90.4%) positive samples for *Salmonella* Typhi and 5 (9.6%) samples were positive for *Salmonella* Paratyphi, whereas 37(71.2%) XDR, 11(21.2%) MDR and 4(7.7%) nonresistant *Salmonella* Typhi samples were reported.[16] These results are comparable to present study and the overall emergence of this resistance is alarming. There are a lot of reports and studies that shows even higher resistance profiles against 2nd line and third line drugs mainly associated with travelling histories and unsafe drinking water facilities among developing and rural areas of Pakistan especially in Sindh southern Punjab. This trend is increasing day by day.[17,18] However, the sample data used in this study was not enough to reach the statistical significance due to low sample size and no genotyping was performed. More epidemiological and functional genomics studies at larger scale in Pakistan to inquire the overall prevalence of resistance to implement best strategies against this emerging health threat.

CONCLUSION

All these findings led us to the conclusion that the antibiotic resistance is drastically increasing against *Salmonella* Typhi in Pakistan. It becomes necessary to take initiatives for awareness of proper antibiotic usage, improvement in health facilities, implementation of recommended diagnostic profile for specific bacterial species and pre-travelling guidelines/precautions related to vaccines & drugs, research on large scale to complete the gap of missing information from different developing regions, drug re-proposition and development of alternative strategies for effective control of resistant strains. The monitoring of MDR and XDR must be performed by all clinical microbiology/pathology laboratories to implement effective measures to reduce the emergence of antimicrobial resistance.

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REFERENCES

1. Bilal H, Khan MN, Rehman T, Hameed MF, Yang X. Antibiotic resistance in Pakistan: a systematic review of past decade. BMC infectious diseases. 2021 Dec;21(1):1-9. 9.
2. Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, et al. Emergence of an extensively drug-resistant Salmonella enterica serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. MBio. 2018;9(1):e00105-18.
3. Qamar FN, Yousafzai MT, Khalid M, Kazi AM, Lohana H, Karim S, et al. 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. 2018;18(12):1368-76.
4. Saeed N, Usman M, Khan EA. An overview of extensively drug-resistant *Salmonella* Typhi from a tertiary care hospital in Pakistan. Cureus. 2019 Sep;11(9):e5663.
5. Khan FA, Ahmed, Ahmed S, Ahmed TA, Khan DA, Hussain AB, Abbasi SA -Manual of Laboratory Medicine-4th Ed.Rawalpindi Armed Force Institution of Pathology-Pakistan ; 2012 page 151-153.
6. Rasheed MK, Hasan SS, Ahmed SI. Extensively drug-resistant typhoid fever in Pakistan. Lancet Infect. Dis . 2019;19(3):242-3.
7. Latif S, Zia A, Ali SB, Hafeez S. Extensively drug resistant typhoid fever seen at tertiary care hospital in Lahore. J. Infect Dis. 2019;28(3):51.
8. 4.Zahid I, Sarwar A, Hussain A, Sohail M, Amin A. Antibiotyping and genotyping of extensively drug-resistant (XDR) Salmonella sp. isolated from clinical samples of Lahore, Pakistan. J. Appl. Microbiol . 2022;132(1):633-41.
9. Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive Salmonella infections. Clin. Microbiol. Rev.. 2015;28(4):901-37.
10. Stanaway JD, Reiner RC, Blacker BF, Goldberg EM, Khalil IA, Troeger CE, et al. The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect. Dis. 2019;19(4):369-81.
11. Mahindroo J, Thanh DP, Nguyen TN, Mohan B, Thakur S, Baker S, Taneja N. Endemic fluoroquinolone-resistant Salmonella enterica serovar Kentucky ST198 in northern India. Microb. Genom. 2019;5(7): e000275
12. Sjölund-Karlsson M, Joyce K, Blickenstaff K, Ball T, Haro J, Medalla FM, et al. Antimicrobial susceptibility to azithromycin among Salmonella enterica isolates from the United States. Antimicrobial agents and chemotherapy. 2011;55 (9):3985-9.
13. Wayne PA. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing: 20th informational supplement. CLSI document M100-S20. 2010.
14. Parry CM, Threlfall EJ. Antimicrobial resistance in typhoidal and nontyphoidal salmonellae. Curr Opin Infect Dis 2008;21 (5):531-8.
15. Rasheed F, Saeed M, Alikhan NF, Baker D, Khurshid M, Ainsworth EV, et al. Emergence of resistance to fluoroquinolones and third-generation cephalosporins in *Salmonella* Typhi in Lahore, Pakistan. Microorganisms. 2020;8 (9):1336.
16. Qureshi S, Naveed AB, Yousafzai MT, Ahmad K, Ansari S, Lohana H, Mukhtar A, Qamar FN. Response of extensively drug resistant *Salmonella* Typhi to treatment with meropenem and azithromycin, in Pakistan. PLoS Negl Trop Dis . 2020;14(10):e0008682.
17. Fida S, Mansoor H, Saif S, Iqbal J, Khan AQ. Clinical Perspectives of Multiple and Extensively Drug-Resistant Typhoid; result from a tertiary care hospital from Pakistan. J. Infect. Dev. Ctries. . 2021;15(04):530-537
18. Akram J, Khan AS, Khan HA, Gilani SA, Akram SJ, Ahmad FJ, Mehboob R. Extensively drug-resistant (XDR) typhoid: evolution, prevention, and its management. Biomed Res. Int.2020;2020:1-7