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Continuous Threat of Growing Antimicrobial Resistance Among Salmonella Typhi Isolates: A Multicenter Study from Pakistan

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ABSTRACT

Objective: To determine current susceptibility patterns of *Salmonella enterica* serovar Typhi (S. Typhi) in four cities of Pakistan and to deduce the frequency of multidrug resistant, extensively drug-resistant S. Typhi.

Study Design: Cross-sectional study.

Place and Duration of Study: Departments of Microbiology, Pakistan Navy Ship Shifa Hospital, Karachi, Pak Emirates Military Hospital, Rawalpindi, Combined Military Hospital, Sialkot, Combined Military Hospital, Abbottabad, Pakistan, from Apr 2019 to Sep 2020.

Methodology: Blood culture specimens of patients with suspected Enteric fever from four different institutes were received in respective laboratories. Culture and identification of isolates were done and specimens yielding growth of S. Typhi, were included in the study. Antibiotic susceptibility was tested according to Clinical and Laboratory Standards Institute guidelines 2019 and 2020.

Results: A total of 970 blood culture specimens yielded growth of S. Typhi. Maximum isolates were found from Pak Emirates Military Hospital, Rawalpindi, 395(40.7%) followed by Pakistan Navy Ship Shifa, Karachi 366(37.7%). The total number of multidrug resistant isolates were 679 (70%) and extensively drug resistant isolates were 457(47.1%) with maximum number of multidrug resistant and extensively drug resistant S. Typhi from Pakistan Navy Ship Shifa. Ciprofloxacin susceptible isolates were 50(5.2%) and maximum sensitive isolates were from Combined Military Hospital, Abbottabad. Resistance to azithromycin was found in four isolates (0.4%). There were no isolates resistant to meropenem.

Conclusion: Emergence of antibiotic resistance among S. Typhi strains is a threat which emphasizes the importance of surveillance of antimicrobial susceptibility patterns and amendment of current antibiotic prescribing practices.

Key words: Antimicrobial Resistance, Drug Resistance, Multidrug Resistant (MDR), Salmonella enterica Typhi (S. Typhi).

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INTRODUCTION

Enteric fever is a bacterial illness that is caused by *Salmonella enterica* subspecies enterica serovars Typhi (S. Typhi) and Paratyphi (S. Paratyphi) A, B, and C. It is an important cause of diseases globally with the highest burden exerted on middle and low-income countries,[1-2] particularly in South Asia.[3-5] Typhoid fever is particularly dangerous in children with reported incidence of 451/100,000 in Karachi annually.[4-6] Typhoid fever is also worrisome for high-income countries due to travel-related exposure as many of those cases have a travel history to South Asia.[7-9] While chloramphenicol, ampicillin, and co-trimoxazole were considered as first-line therapy for typhoid fever and remained effective until the 1970s, the emergence of multidrug-resistant (MDR) strains, due to H58 haplotype, forced a shift to the use of fluoroquinolones, which became the most-routinely prescribed antimicrobial 2,10 with decreased susceptibility of fluoroquinolones emerging in 2000s.[2] Third-generation cephalosporins then became the first choice for typhoid fever treatment but Ceftriaxone resistance was soon reported with <1% resistant strains in Pakistan during 2009–2014.[1,10] In November 2016, an outbreak of ceftriaxone-resistant S. Typhi was reported from Hyderabad, Pakistan, classified as extensively drug-resistant (XDR) S. Typhi, which was found resistant to first-line antimicrobials along with resistance to fluoroquinolones and third-generation cephalosporins due to acquisition of bla-CTX-M-15 gene.[2,10] With XDR S. Typhi, azithromycin is the last option being used for the treatment of non-admitted patients but excessive use of azithromycin in COVID-19 cases may augment the development of azithro-mycin resistance in XDR S. Typhi,[7] as parenteral treatment options for complicated XDR typhoid cases are only carbapenems.[9] Monitoring antimicrobial

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susceptibility trends of S. Typhi is cardinal for efficient management of typhoid cases.[10] Thus, our study was planned to determine the antibiotic susceptibility profile of S. Typhi and to determine the burden of MDR and XDR S. Typhi in four different geographical regions of Pakistan.

METHODOLOGY

This cross-sectional study was carried out at Departments of Microbiology Laboratories of four different institutes including PNS Shifa, Karachi, PEMH, Rawalpindi, CMH Skardu and CMH Abbottabad from April 2019 to September 2020. These four hospitals are located in three different provinces of Pakistan and receive specimens from various geographic regions. Our study was approved by Institutional Ethics Committee through Ethics Review Board (ERB)/ Institution Review Committee (IRC) certificate number ERC/08/2019. Sample size was estimated by using the World Health Organization (WHO) sample size calculator using prevalence of 43%, as found in literature.[10] Patients were enrolled in the study by using non-probability consecutive sampling technique after obtaining informed consent and their details regarding age, gender and site of sample collection were recorded.

Inclusion Criteria: Patients with suspected enteric fever were included from outpatient and inpatient departments of Pediatric and Medicine specialties of all four institutes, below the age of 70 years and belonging to either gender.

Exclusion Criteria: Repeat specimens from the same patients were excluded.

All blood specimens were inoculated into Brain Heart Infusion (BHI) broth and incubated in a conventional incubator at 35±2 ◦C in CMH Abbottabad and automated BacT/ALERT blood culture system (Biomerieux) in the rest of the three labs. Specimens flagged positive were inoculated on both differential MacConkey agar and enriched solid plating blood agar. In CMH Abbottabad serial subcultures from Brain Heart Infusion (BHI) broth to similar solid media were done. The growth of isolates on these media was identified by colony morphology, biochemical testing using API 20E (Biomerieux, France) and serotyping with specific antisera. Antibiotic susceptibility was done by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines 201913 and 202014. The following antibiotic discs were applied on Muller-Hinton agar: azithromycin (15 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg disk), meropenem (10 µg), ampicillin (10 µg), chloramphenicol (30 µg), ceftriaxone (30 µg), and ciprofloxacin (5 µg). Zone diameters were interpreted as per CLSI guidelines. For interpretation of meropenem susceptibility, general zone diameters for Enterobacteriaceae were used. Quality control of discs was performed by control strains *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 (for meropenem only), *S. aureus* ATCC 25923 (for azithromycin disk diffusion testing in S. Typhi only). The susceptibility of azithromycin was further confirmed by the E-test method in all four institutes. Susceptibility testing of ceftriaxone and meropenem was confirmed by E-test as well as VITEK 2 system (Biomerieux, France) at PNS Shifa and only by E-test at three other institutes. Analysis of data was done on Statistical Package for Social Sciences (SPSS) version 24.0. Frequencies and percentages were calculated for qualitative variables, i.e resistance and susceptibility of antimicrobials.

RESULTS

Blood culture samples of a total of 1935 patients with suspected typhoid fever were collected and analyzed. Only 970 blood culture specimens yielded growth of S. Typhi. These 970 samples from different patients were further studied for antimicrobial susceptibility patterns and patient characteristics. The distribution of S. Typhi positive blood culture specimens from different labs has been listed in Table-I.

Table-I: Distribution of S. Typhi Isolates (n=970)

Location	n(%)
PNS Shifa (Karachi)	366 (37.7)
PEMH (Rawalpindi)	395 (40.7)
CMH Skt (Sialkot)	85 (8.8)
CMH Atd (Abbottabad)	124 (12.8)

Out of a total of 970 patients with positive blood culture, 681(70%) were male whereas 289(30%) were female. The majority of patients were between the ages of 12 to 50 years 478(49.3%) followed by ≤12 years 445(45.9%). Indoor patients admitted in different wards were 522(53.8%) while outdoor patients were 448(46.2%). Detailed characteristics of patients from different labs with respect to gender, age groups, and hospital admission status have been enumerated in Table-II.

DISCUSSION

Enteric fever remains a major health concern in Pakistan with untreated cases resulting in serious

complications and death.[4] Evolving antimicrobial resistance and increasing frequency of XDR typhoid cases predicts that the management of this disease will be very difficult, clinically and financially, in

Table-II: Characteristics of Patients with Positive Blood Culture (n=970)

Characteristic	n(%)			
	CMH Skt (n=85)	PNS Shifa (n=366)	PEMH (n=395)	CMH Atd (n=124)
Gender				
Male	62(73)	260(71)	279(71)	80(65)
Female	23(27)	106(29)	116(29)	44(35)
Hospital Admission status				
Outdoor	23 (27)	200(55)	177(45)	48(39)
Inpatient	62(73)	166(45)	218(55)	76(61)
Age (years)				
Maximum	31	54	62	65
Minimum	01	1.5	1.5	02
Age Groups (years)				
≤12	46(54)	170(46)	211(53)	18(15)
12-50	39(46)	186(51)	168(43)	85(68)
>50	0(0)	10(3)	16(4)	21(17)

Table-III: Susceptibility Pattern of S. Typhi Isolates Against Antimicrobials Across Labs (n=970)

Antimicrobials	Labs	Resistant	Intermediate	Susceptible
Ampicillin	CMH Skt (n=85)	54(63.5%)	-	31(36.5)
	PNS Shifa (n=366)	310(84.7%)	-	56(15.3%)
	PEMH (n=395)	271(68.6%)	-	124(31.4%)
	CMH Atd (n=124)	95(76.6%)	-	29(23.4%)
	Total (n=970)	730(75.3)	-	240(24.7%)
Chloramphenicol	CMH Skt (n=85)	54(63.5%)	-	31(36.5%)
	PNS Shifa (n=366)	297(81.1%)	-	69(18.9)
	PEMH (n=395)	293(74.2%)	-	102(25.8%)
	CMH Atd (n=124)	73(58.9)	-	51(41.1)
	Total (n=970)	717(73.9)	-	253(26.1%)
Trimethoprim-sulfamethoxazole	CMH Skt (n=85)	54(63.5%)	-	31(36.5%)
	PNS Shifa (n=366)	294(80.3%)	-	72(19.7%)
	PEMH (n=395)	281(71.1%)	-	114(28.9)
	CMH Atd (n=124)	80(64.5%)	-	44(35.5%)
	Total (n=970)	709(73.1%)	-	261(26.9)
Ciprofloxacin	CMH Skt (n=85)	65(76.5%)	8(9.4%)	11(12.9%)
	PNS Shifa (n=366)	304(83.1%)	52(14.2%)	10(2.7%)
	PEMH (n=395)	269(68.1%)	119(30.1%)	07(1.8%)
	CMH Atd (n=124)	67(54%)	35(28.2%)	22(17.7%)
	Total (n=970)	705(72.7%)	214(22.1%)	50(5.2%)
Ceftriaxone	CMH Skt (n=85)	37(43.5%)	-	48(56.5%)
	PNS Shifa (n=366)	213(58.2%)	-	153(41.8%)
	PEMH (n=395)	210(53.2%)	-	185(46.8%)
	CMH Atd (n=124)	15(12.1%)	-	109(87.9%)
	Total (n= 970)	475(48.9%)	-	495(51%)
Azithromycin	CMH Skt (n=85)	-	-	85(100%)
	PNS Shifa (n=366)	01(0.3%)	-	365(99.7%)
	PEMH (n=395)	02(0.5%)	-	393(99.5%)
	CMH Atd (n=124)	01(0.8%)	-	123(99.2%)
	Total (n= 970)	04(0.4%)	-	966(99.6%)
Meropenem	CMH Skt (n=85)	-	-	85(100%)
	PNS Shifa (n=366)	-	-	366(100%)
	PEMH (n=395)	-	-	395(100%)
	CMH Atd (n=124)	-	-	124(100%)
	Total (n=970)	-	-	970(100%)

developing nations.[2,10-11,12] Our study highlights the importance of continued surveillance of antimicrobial resistance and significance of antimicrobial preservation. In our study, 681(70%) of patients were male whereas 289(30%) of patients were female but this difference in male to female ratio was most likely because of the fact that male population in Pakistan has more exposure to outdoor activities particularly food consumption with a similar difference seen in all four sites of our study and also reported in other studies.[4,11,15-18] In our study, most patients were between the ages of 12-50 years followed by ≤12 years, however, >50 years patients were least affected in all four institutes. These findings were in agreement with other previously published Pakistani,[4,15] and international studies.[16-18] Resistance to all three first line antimicrobials was higher in our study as compared to studies conducted at various cities of Pakistan,[4,11] even in older studies done in Karachi,[19-20] but comparable to recent studies done there.[15,21] However, resistance to first line drugs was quite less in various international studies, which is cause for concern in Pakistan.[3,16-18] Susceptibility to ciprofloxacin was higher in some studies,[16-18,22] but similar susceptibility trends were reported by local studies done at tertiary care hospitals of Pakistan,[15,19-21] as easy availability of this antibiotic without prescription have contributes to its widespread use in multiple infections. Resistance to ceftriaxone was first reported in November 2016 as a large outbreak from Hyderabad, Pakistan, which expanded throughout Pakistan.[1,11,15,23-25] In our study, XDR cases have been reported in all four sites from three provinces of Pakistan. Ceftriaxone resistance was lesser in certain local studies,[4,11] but our findings are consistent with findings in a previous study from Pakistan.[15] Ceftriaxone resistance was also reported internationally in people who traveled to or from Pakistan.[8,9] As per the findings of our study, ceftriaxone can no longer be used empirically in most parts of the country but can be considered in regions where resistance is lower, such as CMH Abbottabad in our study. Emergence of azithromycin in XDR cases is ominous as it would limit therapy of typhoid fever to just parenteral carbapenems after hospitalization. Over the counter availability along with and prophylactic use in COVID-19 cases[7] have led to azithromycin resistance and reduced susceptibility have led to therapeutic failure in Pakistan,[4,11,15,23,24] and abroad.[2,17,18] Treatment in XDR cases is a dilemma leaving only carbapenems as parenteral options which are quite expensive, limiting their use in developing countries while other treatment options like tigecycline, piperacillin-tazobactam, fosfomycin, ceftazidime-avibactam and colistin are not only costly but have limited clinical application as well.[24] There is a need to conduct more regional studies to ascertain local antimicrobial susceptibility pattern which would help to formulate local guidelines for empirical treatment of typhoid cases.

Table-IV: Frequency of MDR and XDR S. Typhi Across Labs (n=970)

Labs	MDR	XDR
CMH Skt (n=85)	54(63.5%)	37(43.5%)
PNS Shifa (n=366)	286(78.1%)	203(55.5%)
PEMH (n=395)	266(67.3%)	202(51.1%)
CMH Atd (n=124)	73(58.9%)	15(12.1%)
Total (n= 970)	679(70%)	457(47.1%)

CONCLUSION

The rise in antimicrobial resistance among S. Typhi isolates throughout the country with decreased susceptibility of XDR strains to azithromycin is a worrying health concern. Emphasis must be placed on routine surveillance of antimicrobial susceptibility trends in different regions of country and judicious use of antibiotics encouraged.

Conflict of Interest: None.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

AH & MF: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

MLS & FH: Conception, data analysis, drafting the manuscript, approval of the final version to be published.

SS & FS: Data acquisition, critical review, approval of the final version to be published.

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

REFERENCES

1. Yousafzai MT, Qamar FN, Shakoor S,. Ceftriaxone-resistant Salmonella Typhi outbreak in Hyderabad City of Sindh, Pakistan: high time for the introduction of typhoid conjugate vaccine. Clin Infect Dis 2019; 68: S16-21. https://doi.org/10.1093/cid/ciy877

2. Hooda Y, Sajib MS, Rahman H, Luby SP. Molecular mechanism of azithromycin resistance among typhoidal Salmonella strains in Bangladesh identified through passive pediatric surveillance. PLoS Negl Trop Dis 2019; 13: e0007868. https://doi.org/10.1371/journal.pntd.0007868
3. Patil N, Mule P. Sensitivity pattern of Salmonella Typhi and Paratyphi A isolates to chloramphenicol and other anti-typhoid drugs: An in vitro study. Infect Drug Resist 2019; 12: 3217. https://doi.org/10.2147/IDR.S223119
4. Umair M, Siddiqui SA. Antibiotic susceptibility patterns of Salmonella Typhi and Salmonella Paratyphi in a tertiary care hospital in Islamabad. Cureus 2020; 12: e10228. https://doi.org/10.7759/cureus.10228
5. Kim S, Lee KS, Pak GD, Excler JL, Sahastrabuddhe S, Marks F, et al. Spatial and temporal patterns of typhoid and paratyphoid fever outbreaks: a worldwide review, 1990–2018. Clin Infect Dis 2019; 69: S499-509. https://doi.org/10.1093/cid/ciz611
6. Ameya G, Tsalla T, Getu F, Getu E. Antimicrobial susceptibility pattern, and associated factors of Salmonella and Shigella infections among under five children in Arba Minch, South Ethiopia. Ann Clin Microbiol Antimicrob 2018; 17: 1-7. https://doi.org/10.1186/s12941-018-0288-0
7. 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: 1336. https://doi.org/10.3390/microorganisms8091336
8. 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; 15: e00492. https://doi.org/10.1016/j.idcr.2019.e00492
9. Chatham-Stephens K, Medalla F, Hughes M, Appiah GD, Aubert RD, Caidi H, et al. Emergence of extensively drug-resistant Salmonella Typhi infections among travelers to or from Pakistan—United States, 2016–2018. Morb Mortal Wkly Rep 2019; 68: 11-13. https://doi.org/10.15585/mmwr.mm6801a3
10. Saeed M, Rasool MH, Rasheed F, Saqalein M, Nisar MA, Imran AA, et al. Extended-spectrum beta-lactamases producing extensively drug-resistant Salmonella Typhi in Punjab, Pakistan. J Infect Dev Ctries 2020; 14: 169-176. https://doi.org/10.3855/jidc.11706
11. Laghari GS, Hussain Z, Hussain SZ, Kumar H, Uddin SM, Haq A, et al. Antimicrobial susceptibility patterns of Salmonella species in Southern Pakistan. Cureus 2019; 11: e4379. https://doi.org/10.7759/cureus.4379
12. 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: 105-118. https://doi.org/10.1128/mBio.00105-18
13. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 29th ed. Document M100. Wayne, PA: CLSI; 2019.
14. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 30th ed. Document M100. Wayne, PA: CLSI; 2020.
15. Hussain A, Satti L, Hanif F, Zehra NM, Nadeem S, Bangash TM et al. Typhoidal Salmonella strains in Pakistan: an impending threat of extensively drug-resistant Salmonella Typhi. Eur J Clin Microbiol Infect Dis 2019; 38: 2145-2149. https://doi.org/10.1007/s10096-019-03657-0
16. Diaz-Guevara P, Montaño LA, Duarte C, Zabaleta G, Maes M, Martinez Angarita JC et al. Surveillance of Salmonella enterica serovar Typhi in Colombia, 2012–2015. PLoS Negl Trop Dis 2020; 14: e0008040. https://doi.org/10.1371/journal.pntd.0008040
17. Bhetwal A, Maharjan A, Khanal PR, Parajuli NP. Enteric fever caused by Salmonella enterica serovars with reduced susceptibility of fluoroquinolones at a community based teaching hospital of Nepal. Int J Microbiol 2017; 2017: 1-8. https://doi.org/10.1155/2017/9095128
18. Makkar A, Gupta S, Khan ID, Gupta RM, Rajmohan KS, Chopra H et al. Epidemiological profile and antimicrobial resistance pattern of enteric fever in a tertiary care hospital of North India–a seven year ambispective study. Acta Medica 2019; 61: 125-130. https://doi.org/10.14712/18059694.2019.151
19. Das JK, Hasan R, Zafar A, Ahmed I, Ikram A, Nizamuddin S et al. Trends, associations, and antimicrobial resistance of Salmonella Typhi and Paratyphi in Pakistan. Am J Trop Med Hyg 2018; 99: 48-54. https://doi.org/10.4269/ajtmh.18-0193
20. Qamar FN, Yousafzai MT, Sultana S, Baig A, Shakoor S, Hirani F et al. A retrospective study of laboratory-based enteric fever surveillance, Pakistan, 2012–2014. J Infect Dis 2018; 218: S201-205. https://doi.org/10.1093/infdis/jiy210
21. Zehra NM, Irfan F, Mirza IA, Imtiaz A, Nadeem S, Hameed F. Current trends of antimicrobial susceptibility of typhoidal Salmonellae isolated at tertiary care hospital. J Coll Physicians Surg Pak 2017; 27: 690-692.
22. Mutai WC, Muigai AW, Waiyaki P, Kariuki S. Multi-drug resistant Salmonella enterica serovar Typhi isolates with reduced susceptibility to ciprofloxacin in Kenya. BMC Microbiol 2018; 18: 1-5. https://doi.org/10.1186/s12866-018-1340-5
23. Iqbal J, Dehraj IF, Carey ME, Dyson ZA, Garrett D, Seidman JC et al. A race against time: reduced azithromycin susceptibility in Salmonella enterica serovar Typhi in Pakistan. mSphere 2020; 5: e00215-220. https://doi.org/10.1128/mSphere.00215-20
24. Levine MM, Simon R. The gathering storm: is untreatable typhoid fever on the way? mBio 2018; 9: e00482-18. https://doi.org/10.1128/mBio.00482-18
25. Satti ML, Hanif F, Hussain A, Nadeem S, Younis F, Zehra NM. Rapid detection of ceftriaxone resistance in clinical isolates of extensively drug-resistant Salmonella enterica serovar Typhi. J Pak Med Assoc 2021; 71: 1639-43. https://doi.org/10.47391/JPMA.152