ORIGINAL ARTICLE

AN UPDATE ON CURRENT STATUS OF ANTIBIOTIC RESISTANCE IN SALMONELLA TYPHI: A RETROSPECTIVE ANALYSIS OF BLOOD CULTURE REPORTS FROM A TERTIARY CARE HOSPITAL, PAKISTAN

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ABSTRACT

Objective: Pakistan bears most of the burden of drug-resistant typhoid, challenging its healthcare systems with substantial cost. This study's purpose is to investigate resistance in community-acquired salmonella typhi to guide its evidence-based empiric treatment.

Material & Methods: This study used retrospective data from the microbiology laboratory, Khyber Teaching Hospital, Peshawar (September 2022-March 2023). Data of routine diagnostic samples for typhoid was retrieved from the database. Kirby–Bauer disk diffusion method was employed to determine the sensitivity of S. Typhi against a panel of 21 selected antibiotic discs (not exclusively for each isolate).

Results: Among 1742 suspected patients, 239 (13.92%) were positive for S. Typhi with 212/238 (89.1%), 227/233 (97.4%), and 196/239 (82.0%) isolates resistant to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole, respectively. High resistance (88.0% to ciprofloxacin) was detected against fluoroquinolones. Third-generation cephalosporins also showed poor activity with 144/158 (91.1%) and 120/136 (88.2%) resistance towards ceftriaxone and cefixime, respectively, except for cefoperazone/sulbactam (2/123, 1.6%). Among 157 tested isolates, 3 (1.9%), 9 (5.7%), and 123 (78.3%) were labeled as non-resistant, MDR, and XDR strains, respectively. XDRs were less resistant to azithromycin (1/114), piperacillin/tazobactam (1/123), carbapenems (0/123), and tigecycline (0/123).

Conclusion: XDR S. Typhi was observed as the dominant strain in Peshawar regions for which azithromycin is still the drug of choice. With emerging resistance, azithromycin safety should be ensured through antibiotic stewardship principles. An interesting finding was the enhanced activity of cefoperazone/sulbactam, suggesting future studies. Alternative options are expensive antibiotics which can be used as a last resort.

Keywords: Typhoid, Antibiotic resistance, Extensive drug-resistant (XDR) S. Typhi, Pakistan.

This article may be cited as: Khan M, Idrees M, Rehman MU, Osama M, Khan P, Khan HA. An Update On Current Status Of Antibiotic Resistance In Salmonella Typhi: A Retrospective Analysis Of Blood Culture Reports From A Tertiary Care Hospital, Pakistan. J Med Sci 2024 July-September;32(3):260-266

INTRODUCTION

Salmonella enterica serotype Typhi (S. Typhi), a rod-shaped gram-negative bacterium, is the causative organism of enteric fever, commonly known as typhoid fever. It spreads through the fecal-oral route and more frequently affects the pediatric population in the developing world. [1] Nonavailability of clean water and an unhygienic environment have been attributed to its widespread prevalence among poor countries. [2] Lack of adequate diagnostic tools and the spread of resistant strains in such countries make it even more challenging. According to a 2019 report by the Institute for Health Metrics and Evaluation (IHME), each year across the globe approximately 9 million cases and 110,000 deaths occur due to typhoid. [3]

The rapid but obsolete serological tests for typhoid fever diagnosis such as Widal and Typhidot are already banned in Pakistan because of their diagnostic inaccu-

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Date Received: 04/05/2024
Date Revised: 12/07/2024
Date Accepted: 20/08/2024

racy. [4] The gold standard is the blood culture test which makes it even more important to curb inappropriate use of antibiotics. Additionally, current automated blood culture systems are preferred now for their enhanced sensitivity, rapidity, and less contamination over conventional methods. [5, 6] Among the available tests to check in vitro resistance such as disk diffusion, broth microdilution, or agar dilution methods, keeping in view the cost, speed, and convenience of equipment, the disc diffusion method is employed in our setup. [7]

Antibiotics serve as the mainstay treatment for typhoid. The popular approach in the endemic regions is that these cases are dealt with in outpatient settings while only a few needs hospitalization. [8] Traditionally, chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole have been used as the first-line treatment for S. typhi. [9] They were rendered ineffective in the 1990s when multidrug-resistant (MDR) strains emerged in South-East Asia. Fluoroquinolones became the preferred therapeutic agents for MDR. [10] But soon in 1997, such MDR strains were isolated that had acquired resistance to ciprofloxacin as well. Third-generation cephalosporins became widely used as a drug of choice for fluoroquinolones non-susceptible MDRs. [11] In 2016, several years after its usage, Pakistan detected isolates in a typhoid outbreak which were more powerfully resistant even to ceftriaxone named as extensively drug-resistant (XDR) S. typhi. [12] Resistance to third-generation cephalosporins is attributed to salmonella acquisition of ESBLs (CTX-M genes). [13] Although, there are propositions about independent multi-centric origin of the XDR strains in Pakistan, Nepal, and Bangladesh. [14] XDRs have not only been endemic in Pakistan [15] since then but also have spread beyond borders to USA [15, 16], Canada [17], Italy [18,] and other Asian countries. [19] The last resort and remaining options for XDR strains are oral azithromycin or intravenous carbapenem [9] which are already prescribed as empiric treatment in endemic regions. [4]

XDR emergence is marked as a crucial event in the evolution of anti-microbial resistance (AMR). These strains are already challenging healthcare systems across the globe with substantial cost, pushing for more expensive antibiotics and complicated management of a former easily treatable illness. [20] The Strategic Advisory Group of Experts (SAGE) of WHO has endorsed conjugated typhoid vaccines for several population groups. Pakistan has taken the lead in this regard by initiating vaccination campaigns in the endemic parts of the country in 2019. [21]

Our hospital is a sentinel site for AMR surveillance. The purpose of this study is to investigate the current status of antibiotic resistance in community-acquired S. Typhi in the blood cultures of typhoid fever suspected patients in Khyber Teaching Hospital, Peshawar. These results will consequently guide evidence-based empiric treatments for typhoid fever and will further improve typhoid-control practices.

MATERIALS AND METHODS

This study was carried out on the retrospective data in the microbiology laboratory, department of Pathology of Khyber Teaching Hospital (KTH) Peshawar which serves as a tertiary care hospital. Seven months` data was retrieved from the laboratory database which was generated as a result of active surveillance of the resistance of the pathogen from the community it serves between the duration of September 2022 to March 2023. At the time of the commencement of this study, reports of the samples were already completed which were collected for the diagnosis and treatment purpose. The microbiology laboratory of KTH is a biosafety level 2 (BSL 2) public health laboratory and provides clinical services to promote evidence-based medicine in the hospital. It has the facility of an automated blood culture system (Versa Trek, USA). The hospital also serves as a sentinel site for monitoring resistance patterns of different pathogens.

Documented histories of patients` illnesses were recovered from clinical records as taken by their dealing physician. Patients whose culture yielded positive results for pathogens other than S. Typhi were excluded.

The 21 antibiotics checked for their sensitivity towards S. Typhi were; Chloramphenicol (30ug), amoxicillin (10µg), Trimethoprim-Sulfamethoxazole (25ug), Ciprofloxacin (5ug), Moxifloxacin (5ug), Levofloxacin (5ug), Ceftriaxone (30µg), Cefixime (30µg), Cefoperazone/sulbactam (75/30µg), Cefotaxime (30µg), Ceftazidime (30µg), Azithromycin (15µg), meropenem (10µg), Doripenem (10µg), Imipenem (10µg), Ertapenem (10µg), Piperacillin/tazobactam (110µg), Tigecycline (15µg), Gentamicin (10µg), Amoxicillin/clavulanic acid (30µg), and Cefepime (10µg). All of the isolates were not checked exclusively for all the antibiotics mentioned.

The World Health Organization (WHO) criteria [22] were used to interpret non-resistant, MDR, and XDR strains as shown in Table 1. Age and gender data were also obtained from the database. Duration of illness, outcome, and any prior use of antibiotics were not taken into account. The study was approved by the Institutional Research and Ethical Review Board (IREB) of Khyber Medical College Peshawar under reference number 408/DME/KMC dated; 13/7/2023.

After data acquisition from the laboratory database, it was entered into S.P.S.S version 24.0 for Windows (SPSS Inc., Chicago, IL, USA). Cases with only positive cultures for S. Typhi were considered for analysis. After careful examination, subsequently, any missing values were identified and endorsed in the analysis. Quantitative data i.e., age was presented as mean ± SD while qualitative data such as gender and resistance status were presented as frequencies and percentages.

RESULTS

A total blood samples of 1742 typhoid-suspected patients of which 949 (54.5%) were males and 793 (45.5%) females, were received in the microbiology laboratory during the study period. Their mean age was 17.64 ± 15.68 (years). After the isolation process, 239 samples were found positive for S.Typhi with a positivity rate of 13.92%. The majority of isolates were detected in males (147, 61.5%) as compared to females (92, 38.5%). The age distribution of the diagnosed patients was positively skewed with a mean of 12.33 ± 10.32 (years) and a median of 9 years (Range = 0-70 years). Due to the high positivity rate among younger patients, 93.7% (223/239) of the confirmed cases were up to 30 years (Range = 0-70 years) while only 81.68% (1422/1742) of all the combined suspected cases were up to 30 years (Range = 0-100 years).

Among the tested isolates, high resistance was seen against the first-line, second-line, and third-line agents, as shown in Figure 1.

Of the 239 positive isolates, 232 had been tested for all three first-line antibiotics. Only 180/232 (77.6%) were found to be resistant to all three antibiotics.

Further, 157/239 were tested for the three first-line agents plus fluoroquinolones and third-generation cephalosporins. Among them, only 3/157 (1.9%) were susceptible to all the first-line agents plus third-generation cephalosporins and labelled as non-resistant. Additionally, 9/157 (5.7%) were resistant to all first-line antibiotics and susceptible to third-generation cephalosporins and were labeled as multiple drug-resistant (MDR). While 123/157 (78.3%) were resistant to all of the five classes of drugs and labeled as extensively drug-resistant (XDR) (Table 2).

All of the labeled MDRs (n = 9) were found susceptible to fluoroquinolones (9/9 ciprofloxacin, 3/3 moxifloxacin, 4/4 levofloxacin). The susceptibility of XDRs is given in Table 3.

DISCUSSION

The current study yields very concerning data about the newly evolved resistances in S. Typhi from a tertiary care hospital in Pakistan. Over three-quarters of isolates were found resistant to all three first-line agents. Almost 90% were resistant to all fluoroquinolones and the

Table No 1: WHO criteria for Non-resistant, MDR and XDR Salmonella Typhi

Non-resistant	If the isolates are sensitive to first-line antibiotics (ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole) as well as to third-generation cephalosporins (ceftriaxone and cefixime), with or without resistant to second-line antibiotics (quinolones and fluoroquinolones).
Multi drug-resistant	If the isolates are resistant to first-line antibiotics but sensitive to third-line antibiotics (third-generation cephalosporins), with or without resistant to second-line antibiotics.
Extensively drug-resistant	If S. typhi is resistant to all three lines of antibiotics (ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, ceftriaxone, cefixime, quinolones, and fluoroquinolones) but still sensitive to few antibiotics like azithromycin and carbapenems.

Table No 2: Classification of Salmonella Typhi isolates by Antibiotic resistance status (Data from Khyber Teaching Hospital Peshawar, Pakistan during Sep 01, 2022 to Mar 31, 2023, N = 157*)

Status	n (%)
Non-resistant	3 (1.9)
Multi drug-resistant (MDR)	9 (5.7)
Extensively drug-resistant (XDR)	123 (78.3)
Footnote: * Tested for first, second and third-line antibiotics exclusively	

Table No 3: Extensively drug-resistant (XDR) Salmonella Typhi isolates susceptibility to tested antibiotics (N = 123)

Antibiotics	No. of XDRs tested	Sensitive, n (%)	Resistant, n (%)
Cefoperazone/sulbactam	123	121 (98.4)	2 (1.6)
Cefotaxime	27	1 (3.7)	26 (96.3)
Ceftazidime	36	2 (5.6)	34 (94.4)
Azithromycin	114	113 (99.1)	1 (0.9)
Meropenem	123	123 (100.0)	0 (0.0)
Doripenem	31	31 (100.0)	0 (0.0)
Imipenem	36	36 (100.0)	0 (0.0)
Ertapenem	36	36 (100.0)	0 (0.0)
Piperacillin/tazobactam	123	122 (99.2)	1 (0.8)

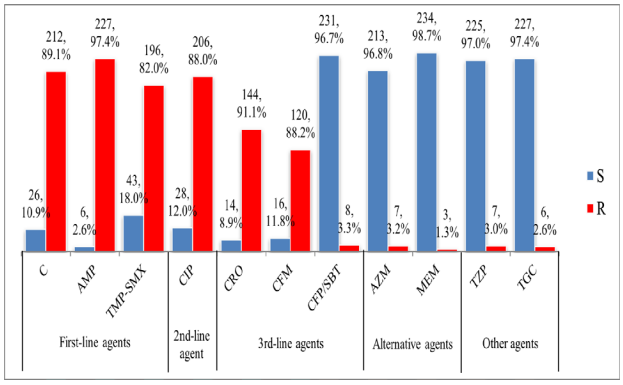

Fig 1: Antibiotic susceptibility of Salmonella Typhi isolates (Data from Khyber Teaching Hospital Peshawar, Pakistan from September 01, 2022, to March 31, 2023, N = 239)

(S = Sensitive, R = Resistant, C = Chloramphenicol, AMP = amoxicillin, TMP-SMX = Trimethoprim-Sulfamethoxazole, CIP = Ciprofloxacin, CRO = Ceftriaxone, CFM = Cefixime, CFP/SBT = Cefoperazone/sulbactam, AZM = Azithromycin, MEM = Meropenem, TZP = Piperacillin/tazobactam, TGC = Tigecycline).

same percentage had acquired resistance to third-generation cephalosporins (ceftriaxone and cefixime). Among the limited number of choices left for the treatment, carbapenems, and Tigecycline were found as superior choices for the XDR strains. Azithromycin, cefoperazone/sulbactam, and piperacillin/tazobactam combinations were also found to have excellent activity with minimum resistance.

In our results, we saw positive isolates of S. Typhi to be predominant among male and younger patients. The majority of the articles encountered during the literature search have obtained consistent findings like ours for both gender and age except for a single study which showed female predominance. [23-26, 27] A possibility for increased incidence among males would be that males spend more time outside the home and observe more unhygienic practices. [28] Likewise, a weak immune system in childhood could be the leading factor that predisposes them to infections more compared to other age groups. [29]

A high resistance against the three first-line agents which makes its usage obsolete has already been reported by many authors during the past years. Khan M et al. (2020-2021) showed absolute resistance to chloramphenicol and ampicillin. [24] Another study by Hussain A. et al. found 80% resistance to all three agents. [25] These and other studies [13, 30] further reinforce the contraindication of the first-line agents anymore for typhoid treatment. In addition, our findings about fluoroquinolone resistance are also closely aligned with the past study in Karachi, which showed 89% resistance to ciprofloxacin [30] while another reported 100% resistance to it [24], slightly higher than our findings thus making this class of drug a poor choice for the prevalent strains.

Many past national studies have shown the prevalence of XDR strains to be 5% (Islamabad) [31], 9% (Karachi) [23], 40% (Lahore) [13], and 48% (Karachi) [25, 30] while only a bunch of cases have been reported in other countries. [14, 16] In comparison to these studies, our data shows 78% prevalence of XDRs proving that alarmingly it has now taken over as the dominant strain in Peshawar region as well. Third-generation cephalosporins which have long been the drug of choice and still are in non-endemic countries are also demonstrated in our study to have high resistance against it i.e., 91.1% to ceftriaxone and 88.2% to cefixime. Past studies indicate a progressive rising trend in their resistance with time after XDR's emergence in 2016 as an epidemic in Sindh. Fatima G et al. in 2017-18 found a resistance of 55% and 50%, respectively [30] while later in 2019, a resistance of 63% and 71%, respectively were observed for these two agents. [23] Our findings in this regard are coherent with this rising trajectory and suggest that as no longer an option for treatment. Further, the majority of our data come from winter months i.e., September to March, depicting that XDR cases are no longer appearing based on seasonality, i.e., post-rainy months but also in winter as endorsed by other studies. [24, 30]

In our study, resistance against azithromycin was detected in 7 (3.2%) isolates overall while only 1 was XDR as well. Meropenem had on the other hand 3 resistant strains, imipenem and ertapenem 1 each while all carbapenems were the most stable with 100% sensitivity against XDRs. Similar results have been obtained by other studies that show 100% sensitivity of these two classes of drugs towards XDR. [23, 24, 30] Then there are others where resistance has been detected to azithromycin in Karachi [25], Lahore [13], and Nepal [32]. Similarly, there are now case reports of meropenem resistance as well. [15, 33] Resistance to azithromycin and carbapenems is a new emerging problem where we have already started heading. While azithromycin resistance has increased across Southeast Asian countries during these years after its emergence in Bangladesh in 2019, [34] a contributing factor to it was its injudicious use during the COVID-19 pandemic. A multi-national study across 71 countries found that most sold broad-spectrum antibiotics during the pandemic were macrolides. [35] The rate of bacterial co-infection is only 8% with COVID-19 [36], but an Egyptian study estimated 75% of them receive antibiotics with azithromycin being on the top of the list. [37] It is noteworthy that although carbapenem resistance is lowest at this point, its resistance has been witnessed in non-typhoidal salmonella, and soon, it is feared that it will spread to S. Typhi thus marking the emergence of pan-drug resistant strains [38] as 14 of its cases are previously reported from Pakistan. [39] Our study is based on in vitro activity and not on clinical experience. The clinical response to these last-resort drugs might not be as anticipated.

Many studies have proposed the importance of multi-drug regimens for XDR typhoid and its control. The regimen of azithromycin (bactericidal) plus meropenem (bacteriostatic) has been suggested by a study at Aga Khan University Hospital (AKUH), Karachi, to have superior activity with better clinical outcomes than azithromycin

alone. [40] Even before the emergence of XDR, a comparative study by Zmora N et al. in Nepal showed the superiority of azithromycin plus ceftriaxone over either alone because of their synergistic mechanism of action. It reduced the time to defervescence, hospital stay, decreased chronic carrier state, and hence the chance of resistance. [41] But the reason why carbapenems could not be prescribed to the large scale population is that the per day cost of azithromycin is just US$5.87 whereas for meropenem it is US$88.46 in Pakistan. [40] At present, due to its low cost, oral azithromycin is still the preferred optimal treatment and not intra-venous meropenem in countries with poor socioeconomic status like ours.

XDR has spread to other countries from Pakistan through travelers. Data from the Centre for Disease Control (CDC's) Surveillance system from 2018 to 2021 regarding XDR typhoid cases reported that 88% of the patients had travel history to or from Pakistan. While all were susceptible to azithromycin and meropenem. [27] A review article about the history of XDR S. Typhi has concluded that 2018 and 2019 as the years when intercontinental transmission of XDR typhoid cases occurred to the USA, UK, and Canada from Pakistan. [38] Keeping that in mind, the right empiric treatment choice for cases of typhoid among travelers to XDR endemic regions would be azithromycin, a carbapenem, or both.

One of our significant findings was the astonishing activity of cefoperazone combination with a beta-lactamase inhibitor, salbactam. We could not find an article looking into the details of this third-generation cephalosporin therapeutic function against XDR S. typhi strains but previously rather poor sensitivity has been reported. [42] Due to our compelling results, its curative potential should be explored by other such studies to ascertain its clinical capability. Among the other drugs tested, we found good in vitro activity of the piperacillin/tazobactam combination (with only one XDR as resistant) and Tigecycline. The piperacillin/tazobactam effectiveness has been proven in the past by Jabeen K et al. with 98% sensitivity [13] and others with 100% sensitivity against XDR. [27, 30] Similarly, the excellent activity of Tigecycline has also been repeatedly reported. [43, 44] But like carbapenems, the impediment to their clinical application is cost and affordability.

Apart from active treatment for XDR containment, vaccine campaigns have been carried out in endemic regions of the country which have yielded success to an extent. [45] Other prevention strategies, including mass hygiene awareness, sanitation, and provision of clean drinking water have been conducted that had a positive impact in this regard. [46] But given the worsening situation, for such activities to have a meaningful impact, they should be encouraged on a national scale in a pro-active approach, instead of being limited to only endemic parts.

This study has several limitations. Firstly, the data retrieved from the laboratory had only two categories (sensitive/resistant) without including the intermediate category for the resistance status. Secondly, all isolates were not tested against the full panel of 21 antibiotics. Hence care should be taken while interpreting resistance to individual antibiotics with attention to their percentage and isolates tested. Thirdly, since our study used the disc diffusion method, which could only provide qualitative data about the resistance, we could not obtain minimum inhibitory concentration (MIC) values which are more important to see the true quantitative extent of resistance. Another limitation is that it is not a multi-centric study and could not reflect the situation in the rest of the country.

CONCLUSION

XDR salmonella typhi has taken over as the dominant strain in the Peshawar regions as well. Oral azithromycin is still the drug of choice for empiric treatment of typhoid in endemic regions. A more proactive approach is necessary to ensure the safety of this drug. Moreover, enhanced surveillance of the pathogen resistance patterns and prescribing antibiotics only after culture and sensitivity (C/S) reports are recommended for all the suspected cases. The catastrophe of azithromycin resistance is already upon us. Other viable but expensive treatment options left are carbapenems, tigecycline, and piperacillin/tazobactam which should be provided by the government looking at its cost. We should start looking into alternative cheap treatment options and observe their clinical outcomes for the XDR strains.

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CONFLICT OF INTEREST: Authors declare no conflict of interest

GRANT SUPPORT AND FINANCIAL DISCLOSURE: NIL

Authors Contribution:

Following authors have made substantial contributions to the manuscript as under

Authors	Conceived & designed the analysis	Collected the data	Contributed data or analysis tools	Performed the analysis	Wrote the paper	Other contribution
Khan M	✓	×	✓	×	✓	×
Idrees M	✓	✓	×	✓	✓	×
Rehman MU	×	✓	×	×	✓	×
Osama M	✓	✓	✓	×	✓	✓
Khan P	✓	✓	×	✓	✓	×
Khan HA	×	✓	×	×	✓	×

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.

Ethical Approval:

This Manuscript was approved by the Ethical Review Board of Khyber Medical College, Peshawar. Vide No. 408/DME/KMC. Dated: 13 07 2023