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An Assessment of the Current Trends of Antibiotic Resistance of Salmonella Typhi Against 1st Line Antimicrobial Agents in Metropolitan City of Karachi

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Author`s Contribution

[1,2]Substantial contributions to the conception or design of the work, acquisition, analysis, or interpretation of data for the work, Final approval of the version to be published, [3]Drafting the work or revising it critically for important intellectual content, [4,5] Active participations in active methodology

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ABSTRACT

Objectives: To assess current trends of antibiotic resistance against S. typhi in Pakistani population.

Methodology: This was a cross-sectional, observational descriptive research conducted in various diagnostic facilities in Karachi-Pakistan from January 2019 to November 2020. A data of 1223 patients, who were reported positive for typhoid fever, was collected from various diagnostic facilities with in the area. Clinical isolates were examined for culture sensitivity by using BACTEC™ and Versa TREK™. Isolates of the Salmonella typhi and para typhi were recovered from blood of the patients. The collected data was analyzed using SPSS version 22.0.

Results: Among all isolates, 92.7% (n=1134) isolates were identified as S. typhi and the remaining 7.3% (n=89) as S. paratyphi. Study population suffering from typhoid fever were 58.5% (n=716) male trailed by 41.5% (n=507) female. Similarly, among 1223 clinical isolates, 14.2% (n=174) were non-resistant, 5.4% (n=66) were multi-drug resistant (MDR), 56.4% (n=690) were extensive drug resistant (XDR), 12.7% (n=155) were moving towards multi-drug resistant species trend and 5.7% (n=70) were heading towards XDR. A further 5.6% (n=68) were moving towards ESBL positive typhoid fever. Moreover, 49.1% of the S. typhi isolates were found to be resistant to all the first line antibiotics agents (ampicillin, chloramphenicol and co-trimoxazole) with only 15.2% found to be sensitive to all first line antibiotics drugs.

Conclusion: Strains of Salmonella typhi in Karachi are resistant to most of the drugs recommended by local infectious disease society for treatment of typhoid. This is alarming for healthcare policy makers because of lack of newer drugs discovered for treatment of typhoid fever and pathogens isolated for this highly infectious disease being resistant to most of the recommended anti-microbial agents.

Keywords: Typhoid fever, Drug resistance, XDR, Salmonella typhi.

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Introduction

Salmonella typhi is a specific *Salmonella serovar* that results in typhoid fever, which is a major public health concern in technologically developing countries.[1] Typhoid fever is a systemic disease, which may last for three to four weeks, if remained untreated. Ultimately, upsurges the death rate from 12% to 30%. Even though the global load of typhoid fever has compacted, appearance of multidrug resistant *S.typhi* (MDRST) is still a danger to public health. *S.typhi* is a motile, facultative human anaerobe that is vulnerable to numerous antibiotics. At present, 107 strains of this bacterium have been isolated; several comprising variable metabolic physiognomies, levels of virulence, and

multi-drug resistance genes that obscure treatment in areas that resistance are widespread.[2] It can identified diagnostically by its growth on MacConkey and Eosine Methylene Blue agars.

Regardless of the development of novel antibacterial drugs, typhoid fever has continued to be a foremost health concern.[3] *S.typhi* is resistant to numerous antibiotics including ceftriaxone, ampicillin, and co-trimoxazole, beyond emerging resistance to some effective drugs like ciprofloxacin as well. The advent of multidrug resistance to the frequently used antibiotics has further convoluted the treatment and management of typhoid fever and this has documented as one of the utmost challenges in managing the disease.[4]

Various factors contribute towards the cases and deaths due to typhoid fever among people. These factors include no access to drinking water, absence of proper sanitation and unhygienic conditions. An estimated 21.5 million infections and 200,000 deaths occurs from typhoid fever globally. In the tropical areas of Pakistan, typhoid fever often become more common particularly in hot and dry as well as during rains. In hot and dry season, the concentration of bacteria upsurges in streams and rivers while during heavy rains, flooding water more often dispenses sewage to the imbibing water. In certain regions, the prevalence of typhoid fever might be as huge as 1000/100,000 population in a year. In these regions, enteric fever is predominantly a disease of the children. The cases are commonly milder and self-limiting. In clement countries, insistent carriers are a further significant pool of infection.[5] For nomads in Pakistan, the highest attack rates are 10/105 visits.

Typhoid fever have a tendency to cluster in families[6], apparently reflecting a communal cause of the infection and is connected with poverty and poor housing. Typhoid fever can be diagnosed clinically by culturing the organism from patient's urine, blood or bone marrow. Positive stool cultures are more or less 60% of children and 25% of adults. Secretion of *S.typhi* in the stool is more probable with greater blood bacterial counts, and children have a tendency to have higher bacteremia than adults.[7] Lysis plating and lysis centrifugation methods hasten the identification of *S.typhi* from blood.[8] *S.typhi* is usually present in the duodenum and string test is usually used to recover it. It is occasionally excreted in the urine. Serological diagnosis is extensively trusted upon. Widal test, which measures the antibody titers to the somatic O and flagella H antigens, is trusted upon extensively, although there are different opinions on its usefulness. There have been several well-documented spates in which the Widal was usually negative, but other epidemiological settings where it has proved useful.

The rationale of the study was to assess the current trends of antibiotic resistance against *Salmonella typhi* in Karachi-Pakistan.

Methodology

This was an cross-sectional, observational descriptive study piloted on culture sensitivity data that was recorded from various diagnostic facilities of Karachi-Pakistan. The twenty-three months data from January 2019 to November 2020 was collected from the laboratories. Sample size was, calculated using WHO calculator, was 1223 out of which, 7 samples were discarded because of unavailability of certain results. Clinical isolates were examined for culture sensitivity by using BACTEC™ and Versa TREK™. Isolates of the *Salmonella typhi* and *para typhi* were recovered from blood of the patients and were subjected to antibiotic susceptibility testing. The study was approved by the IRB through letter no. JUW/IERB/035/2021.

Data from patients of all ages and gender diagnosed with typhoid and all typhoid patients reported during January 2019 to November 2020 was the inclusion criteria for the study. Culture reports from non-residents of Karachi, culture reports tested positive with other gram-negative bacteria, and typhoid patients before or after study period was defined as exclusion criteria. The principal author collected all the desired data herself by revisiting reports of each patient that was reported in 2019–2020 with positive *S. typhi* on blood culture and sensitivity reports in Karachi-Pakistan. Information was collected by visiting "Essa laboratory" and "Murshid hospital" in Karachi.

Antibiotics incorporated for assessment were: Chloramphenicol (C), Ampicillin (AML), Co-trimoxazole (SXT) Cefixime (CFM), Aztreonam (ATM), Cefotaxime (CTX), Ceftriaxone (CRO), Ofloxacin (OFL), Ciprofloxacin (CIP), Moxifloxacin (MXF), Piperacillin Tazobactam (TZP), Meropenem (IPM), Azithromycin (AZM), Fosfomycin (FOS). This study was performed at various diagnostic facilities in Karachi-Pakistan.

Data was analyzed using SPSS version 22.0. Categorical variables were presented in frequencies and percentages whereas; numerical variables were presented in mean & standard deviation. Cross tabulation was done to see the association of age and gender spread with all approved drugs for the treatment of typhoid by local guidelines. Cross-tabulation was also done among first line, second

line and third line drugs as per local treatment guidelines. Significant p-value for this study was < 0.05. Appropriate test of significance was applied at 95% confidence interval.

Results

Male typhoid cases led at 58.5%, with 41.5% female. Among 1223 cases, 14.2% were non-resistant, 5.4% MDR, 56.4% XDR, 12.7% moving towards MDR, 5.7% towards XDR, and 5.6% heading to ESBL positive. See Figure 1 for details.

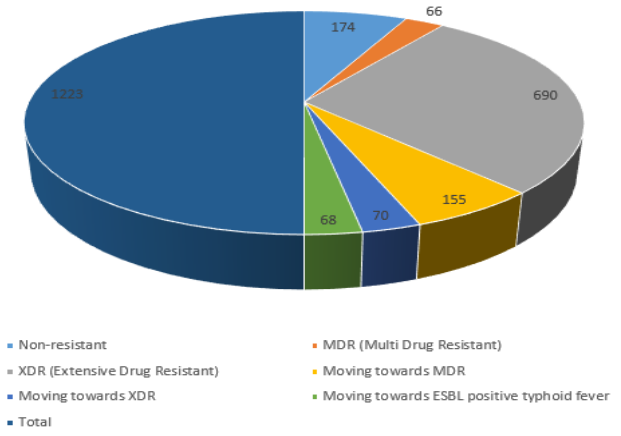

Figure 1. Susceptibility pattern and frequency of S. typhi.

All the isolates tested for first-line agents including ampicillin, chloramphenicol and co-trimoxazole. 10.1% were resistant to ampicillin, 1.6% were resistant to chloramphenicol and 1.9% were resistant to co-trimoxazole. Additionally, 11.2% were resistant to ampicillin and chloramphenicol, 7.2% were resistant to ampicillin and co-trimoxazole and 3.6% were resistant to chloramphenicol and co-trimoxazole respectively. Moreover, 49.1% of the *S. typhi* isolates were found to be resistant to all the first line antibiotics agents while 15.2% were found to be sensitive to all first line antibiotics agents. The results are displayed in table I.

The study found the highest number of isolates (43.5%) in the 1–5 years age group, followed by 27.1% in 6–10 years, and decreasing percentages in older age groups. Ambiguous data accounted for 4.0% of clinical isolates. The results are mentioned in table II.

Out of 1223 clinical isolates of *Salmonella serovars,* 49.1% *(n*=601) were resistant to all first-line agents (ampicillin, chloramphenicol and co-trimoxazole). In these resistant isolates, 99.2% (n=596) were *S. typhi* and 5(0.8%) were *S. paratyphi A.* the results are shown in table III,

Table I: Resistance pattern against first line antibiotics.

First Line Antibiotics (AML, C, SXT)	(N)	(%)
Resistant or Intermediate to Ampicillin(AML)	124	10.1
Resistant or Intermediate to Chloramphenicol(C)	20	1.6
Resistant or Intermediate to Co-trimoxazole(SXT)	23	1.9
Resistant or Intermediate to Ampicillin and Chloramphenicol	137	11.2
Resistant or Intermediate to Ampicillin and Co-trimoxazole	88	7.2
Resistant or Intermediate to Chloramphenicol and Co-trimoxazole	44	3.6
Resistant to All 3	601	49.1
Sensitive to All	186	15.2
Total	**1223**	**100**

Table II: Age-wise Distribution of Isolates in Study.

Age (in years)	N	%
0.1-5	532	43.5
6-10	331	27.1
11-15	144	11.8
16-20	54	4.4
21-25	43	3.5
26-30	22	1.8
31-35	12	1.0
36-40	9	0.7
41-45	5	0.4
46-50	5	0.4
51-55	4	0.3
56-60	1	0.1
61-65	9	0.7
66-70	1	0.1
71-75	1	0.1
76-80	1	0.1
Not known	49	4.0
Total	**1223**	**100**

Discussion

Salmonella typhi, a human host- restricted pathogen is responsible for Typhoid fever. About 21 million people are affected by this enteric fever annually, among other Asian countries, its prevalence in Pakistan is at high risk.[9] Typhoid fever is caused by polluted water, unhygienic food and poor sanitation. Nowadays, the emergence in the resistance pattern of this pathogen is challenging as it shows resistance with not only first line antibiotics but also with fluoroquinolones and third generation cephalosporin.[10] For a long-term control on typhoid fever, strategies have to be made for improving the hygiene, food safety, sanitation and water supply sources etc. In low and middle-income countries, the accessibility and affordability of typhoid conjugate vaccines should be made by World Health Organization to reduce the burden. For treating the uncomplicated typhoid fever, the only effective antibiotic left is Azithromycin. Multi-drug resistant (MDR)

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Table III: Resistant pattern against first-line antibiotics.

		Salmonella Enterica Serovars		Total	*P*-Value
		Salmonella typhi	*Salmonella Paratyphi A*		
Resistant pattern for First-line antibiotics	Resistant or Intermediate to AML	99	25	124	**0.000**
		79.8%	20.2%	100.0%	
	Resistant or Intermediate to C	15	5	20	
		75.0%	25.0%	100.0%	
	Resistant or Intermediate to SXT	19	4	23	
		82.6%	17.4%	100.0%	
	Resistant or Intermediate to AML and C	137	0	137	
		100.0%	0.0%	100.0%	
	Resistant or Intermediate to AML and SXT	73	15	88	
		83.0%	17.0%	100.0%	
	Resistant or Intermediate to C and SXT	44	0	44	
		100.0%	0.0%	100.0%	
	Resistant to All 3	596	5	601	
		99.2%	0.8%	100.0%	
	Sensitive to All	151	35	186	
		81.2%	18.8%	100.0%	
Total		1134	89	1223	
		92.7%	**7.3%**	**100.0%**	

determinants of H58 Salmonella typhi can persist and transmit within the population. Only because of ignoring this threat, the emergence in the extensively drug-resistant (XDR) strains occurs.[11]

As previously, it has been found that that there is a high prevalence of *Salmonella typhi* (76.5%) as compared to *Salmonella Paratyphi A* (23.5%) in a study conducted on 251 *Salmonella* isolates.[10] The results of the present study displays the same pattern of incidence for these serovars i.e. among 1223 clinical isolates of *Salmonella Enterica Serovars*, 92.7% were found to be *Salmonella typhi* while 7.3% clinical isolates were found to be *Salmonella Paratyphi A*.

Globally MDR and XDR *Salmonella Typhi* can cause increase in risk to human health. Although there is an increased in the efforts universally for improving the protection by typhoid vaccine but still in many areas there is a continuous spread and outbreak in typhoid fever. This spread is because of the lack of effective strategies to control the Typhoid fever in asymptomatic carriers who are shedding it for years. For complete eradication of *S. typhi* we should understand its pathogenic mechanism which should be helpful in designing the improved vaccines and new therapies against it (Cohen, 2018). WASH strategies were initiated in 1990 as Sustainable Development Goals.[12]

According to WHO report (2018), 5274 (64.4%) XDR cases (extensively drug-resistant) were reported positive out of 8188 cases in Pakistan. The present study is in accordance with the WHO report giving a similar pattern of XDR prevalence (56.4%).

Typhoid fever is a serious but common disease in developing countries that affects mainly children and adolescents. *S. typhi* is responsible for enteric fever but *Salmonella Enterica Serovars Paratyphi A* has also been described as developing infectious agent of concern. A recent systematic review shows that Enteric fever is a disorder of school-age children and of adults. It is a significant and a common cause of morbidity between 1 -5 years of age.[13] A common view of pediatric typhoid fever showed that enteric fever is common among children less than 5 years of age in many localities. The present study shows the similar pattern of typhoid prevalence which is found to be highest in the clinical isolates of children.

An outbreak of 5,274 cases of XDR *S. typhi* have been reported in Pakistan, mainly in Sindh, including Hyderabad and Karachi, since 2016, is an alarming condition. MDR-*S. typhi* which are resistant to first-line drugs (including chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole) are managed by Cephalosporin's and Azithromycin. Recently an emergence of XDR strains of *S. typhi*, which are resistant to both first and second line agents, are difficult to manage in developing countries due to lack of resources and cost of medication[14] and abundance risk of complication and death (190). To deal with immuno-compromised patients (i-e children, elderly and pregnant women) the situation become complicated. In present study, susceptibility pattern of Typhoid pathogens against antibiotics shows the highest prevalence of extensively drug resistant (XDR) *Salmonella typhi* (resistant to all first and second-line agents) followed by Multi drug resistant (MDR) *Salmonella typhi* (resistant

to all first line agents) and non-resistant strains. Present study further reported that though the considerable number of cases are not completely multiple or extensive drug resistant but are moving towards MDR (showing resistance to either of the three first-line agents) and moving towards XDR (showing resistance to either generation of cephalosporin or fluoroquinolones and first line agents). However small proportion of clinical isolates also shows progression towards ESBL (extended-spectrum beta-lactamase) positive typhoid fever.

An outbreak of typhoid fever was reported in Pakistan in 2018 shows the resistance to fluoroquinolones, chloramphenicol, ampicillin, third-generation cephalosporin and trimethoprim-sulfamethoxazole. 339 cases of XDR (extensively drug resistant) typhoid fever were reported in Hyderabad and Karachi in Pakistan.[9]

In present study, clinical isolates of *Salmonella typhi* tested for all first line agents (ampicillin, chloramphenicol and co-trimoxazole) shows considerable high percentages of resistant species. Furthermore, the highest proportion of these clinical isolates though not resistant to all first line agents but still showed resistance to at least two first line agents. These findings are extension of previous findings that shows the increasing pattern of resistance to all first line agents.

The present study also revealed that the children aged 0-5 years were found to have a more chance of resistance to all first line agents as compared to other age groups whereas other age groups showed less resistance. The reason for this age association with resistance pattern might be linked with the high prevalence of disease cases in children as compare to older. In the studied clinical isolates resistant to all first line antibiotics, *Salmonella typhi* showed more resistance as compared to negligible number of *Salmonella Paratyphi A.*

Conclusion

Strains of *Salmonella typhi* bacteria in Karachi are found to be resistant to most of the first line drugs, which are recommended by the local infectious disease society for treatment of typhoid. XDR cases having resistance to even 3rd-line drugs have also been reported. This is an alarming situation for healthcare policy makers because of lack of newer drugs discovered for the treatment of typhoid fever and pathogens isolated for this highly infectious disease, which is being resistant to most of the recommended anti-microbial agents. It is of profound importance as the population vastly effected by the resistant strains are young children of developing age group. This makes it a public health emergency concern for prevention and timely cure of disease as well as for breaking the infectious circle and halting the disease spread.

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