

RESEARCH ARTICLE

Frequency and Antimicrobial Resistance Pattern of Extensive-Drug Resistance *Salmonella typhi* Isolates

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ABSTRACT

Objectives: Extensive drug resistance (XDR) typhoid fever, caused by resistance strain of *Salmonella typhi*, is an emerging public health concern in Pakistan. XDR typhoid fever severely limits the therapeutic options in outbreak cities of Pakistan. Antimicrobial resistance (AMR) surveillance has been carried out at study hospital in Karachi and here reporting the 15 months surveillance data.

Methods: A cross-sectional study was conducted on 969 *Salmonella typhi* isolates obtained from the blood culture samples of suspected typhoid fever cases at Kharadar General Hospital (KGH) from Jan 2018 to Mar 2019. Isolates were tested against 8 antimicrobial agents by quality controlled disk diffusion technique. *Salmonella typhi* Isolates resistance to first-line drugs; ampicillin, chloramphenicol, and co-trimoxazole referred as Multi-drug resistant (MDR), Whereas first-line, second-line drug (Ciprofloxacin) and third generation Cephalosporin (Ceftriaxone) resistance were considered as XDR.

Results: Of total 969 isolates, 777 (80%) were MDR showed resistance to all three first-line antibiotics and 517 (53%) were XDR resistant to five antibiotics (Ampicillin, Chloramphenicol, Co-trimoxazole, Ciprofloxacin and Ceftriaxone). Azithromycin and Imipenem were seen sensitive to all *Salmonella typhi* isolates (100%) among tested antibiotics. More than half of study *S. typhi* isolates 513 (53%) came from children under five years of age, among them 284 (55%) were XDR.

Conclusions: A large proportion of *S. typhi* isolates showed XDR-ST strain. Only Azithromycin and Imipenem were sensitive against XDR-ST strain. Current limited antibiotic option poses a serious threat of complete antibiotic failure in future against rapidly changing strain of *S. typhi*. *J Microbiol Infect Dis* 2020; 10(2):68-74.

Keywords: Typhoid fever, Multi-drug resistance, Extensive-drug resistance, *Salmonella typhi*, Surveillance

INTRODUCTION

Typhoid and paratyphoid fever are caused by *Salmonella enterica* serovars Typhi (*S. typhi*) and Paratyphi (*S. Paratyphi*). Typhoid fever (TF) is considered as an important global public health problem and a major cause of death and disability particularly in developing countries. Globally, 14.3 million cases of typhoid fever and paratyphoid fever occurred in 2017 including 136,000 deaths [1]. South Asia reported the largest number of typhoid cases, 10.3 million, in 2017 which accounts for 72 % of global burden in 2017 with the highest incidence rate of 549 cases per 100 000 Population [1]. Typhoid fever is endemic in Pakistan with the incidence rate of

451.7 typhoid fever cases per 100,000 persons/year [2]. TF is also most common bacterial infection found in children in Pakistan, with incidence rates as high as 1000 cases per 100,000 child per years reported from Karachi [1].

An increasing trend of antimicrobial resistant pattern in *S. typhi* poses a serious threat of complete antibiotics failure in future, to the public health concern. Multi drug resistance (MDR) with reduce susceptibility to fluoroquinolone in *S. typhi* and *S. Paratyphi* has been reported since 2000 in several countries including Pakistan [3-11]. Sporadic occurrence of extensively drug resistance (XDR) stains of *S.*

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typhi, was initially reported from Bangladesh and India [12-14]. A largest XDR outbreak was reported from Pakistan [15]. This XDR outbreak was started in November 2016 from district Hyderabad then spread to other districts of the Sindh province. So far till Mar 2019, total 9,815 typhoid fever cases have been reported to the provincial health authorities from different district of Sindh province [16]. Out of these reported typhoid fever cases, 6,480 (66%) were XDR-TF. Mostly XDR-TF 4514 (69.7%) cases were reported from Karachi, the provincial capital of Sindh, with the overall XDR typhoid attack rate of 22/100,000 population [16].

Increasing number of typhoid fever cases and isolation of XDR strain of *Salmonella typhi* in Karachi and other area of Sindh province have been continuously reporting with a serious threat of complete antibiotics failure against the rapid genetic mutation stain of *S. typhi*. This is an emerging public health emergency and demands proper surveillance of antimicrobial resistance pattern of XDR strain of *Salmonella typhi* particularly in the outbreak city of Karachi. The aim of current study was to report the antimicrobial resistance surveillance data of *S. typhi* from a private tertiary care hospital.

METHODS

A retrospective observational study was conducted on 969 archived *Salmonella typhi* isolates obtained from the blood culture samples of suspected typhoid fever cases reported at Kharadar General Hospital (KGH) during the period from Jan 2018 to Mar 2019. Kharadar General Hospital Karachi, is located in the district south of Karachi which is comprises of Layari, Saddar and Kemari towns (Sub-district). Layari and Saddar towns are the most densely populated towns of Karachi. Study hospital mostly received patients from these two towns. *Salmonella typhi* isolates were tested on automated blood culture system (Bactec Dickinson, USA) against 8 antimicrobial agents by quality controlled disk diffusion technique based on Clinical and Laboratory Standards Institute (CLSI) guidelines [17]. The antibiotics (Oxoid Ltd., Basingstoke, UK) tested against *S. typhi* isolates were included Ampicillin (10 µg), Chloramphenicol (30 µg), Co-trimoxazole (25 µg), Ceftriaxone (30µg), Cefixime (5 µg), Ciprofloxacin (5 µg), Azithromycin (15 µg) and Imipenem (10 µg). The Antimicrobial Susceptibility Testing (AST) cut-off values, provided by the CLSI guidelines, were used to interpret the AST results as sensitive, intermediate and resistant.

Multidrug-resistant typhoid fever (MDR-TF) is defined as typhoid fever caused by *Salmonella enterica* serovar Typhi strains (*S. typhi*), which are resistant to the first-line recommended drugs for treatment such as ampicillin, chloramphenicol, and co-trimoxazole. [11,16,18]. Whereas Extensive Drug Resistance typhoid fever (XDR-TF) is caused by *S. typhi* strains resistance to first-line drugs, along with second-line drug (fluoroquinolone) and third generation Cephalosporin (Ceftriaxone) [16]. The study was approved by Ethics Review Committee (ERC) of KGH.

Data Analysis

Study data was entered on Microsoft Excel 2013. SPSS version 16 was used to analyse the data. Frequency and proportion were calculated for categorical variables (like gender/ MDR-TF/ XDR-TF). Whereas mean and standard deviation were taken for continuous variables (example age), Chi-square test was used to see the correlation between dependent (MDR-TF/ XDR-TF) and independent variables and P value <0.05 was considered statistically significant. Data was presented in tables and graph.

RESULTS

A total of 969 typhoid fever cases were identified by isolation of *Salmonella typhi* from blood culture of 7217 suspected typhoid fever cases reported at KGH during the study period of 15 months. Of total 969 typhoid cases, more than half of the cases 538 (55.5%) were male. Mean age was 6.6 ± 8.06 years (Table 1).

Majority of typhoid fever cases 885/969 (91.3%) were children less than 14 years of age. Among them, MDR-TF was 711 (80.3%) and XDR-TF was 473 (53.4%). Of total 885 pediatric typhoid fever cases, 513 (57.9%) were children under five years of age and 87 (9.8%) were less than two years of age. Of less than five years, MDR-TF was reported 415 (46.9%) and XDR-TF was 284 (32.0%) respectively. Whereas under two years, MDR-TF were reported 70 (7.9%), and XDR-TF cases were 52 (5.8%). Of total 969 typhoid cases, majority of MDR-TF 345 (35.6%) and XDR-TF 232 (23.9%) were fallen in the age group of 2-5 years (Table 1).

Of total 969 typhoid fever cases, 777 (80.2%) were MDR-TF cases, showed resistance to all three first-line antibiotics (Ampicillin, Chloramphenicol and Co-trimoxazole), and 517 (53.3%) were XDR-TF cases, resistant to first

line drugs, second line drugs (Ciprofloxacin) and the third generation Cephalosporin (Ceftriaxone). Among total 969 cases, only 38 (4.1%) typhoid fever cases have shown sensitivity to all tested antibiotics. There was no statistically significant correlation found between MDR-TF/ XDR-TF with different age groups and with gender (Table 2).

Highest *Salmonella typhi* resistance were seen against Ampicillin 863 (89.1%), followed by Cotrimoxazole 803 (82.9%), Chloramphenicol 800 (82.6%), Cefixime 709 (73.2%), Ceftriaxone 699 (72.1%), and Ciprofloxacin 686 (70.8%). Only two antibiotics (Azithromycin and Imipenem) were seen sensitive to all *Salmonella typhi* cases (Table 3).

During the 15 months surveillance period, on average 65 typhoid fever cases were reported in a month with minimum 14 (1.4%) cases in Jan 2018 and maximum 136 (14.0) cases in Jun 2018. Of total 969 typhoid fever cases in 15 months, nearly half of the of typhoid fever cases 462 (47.6%) were seen during only four months period from May to Aug-2018. Among these 462 typhoid cases, MDR were 366 (79.2%) and XDR were 209 (45.2%) respectively (Figure 1).

DISCUSSION

Current study reported the contribution of Kharadar General Hospital in notifying ongoing outbreak of XDR typhoid fever cases caused by the resistance strains of *S. typhi* in Karachi, Pakistan. Study reported the 15 months surveillance data of antimicrobial resistance pattern of *S. typhi* against tested antibiotics at study hospital. In Karachi during the study period (Jan-2018-Mar 2019), total 4339 XDR-TF cases were reported from different hospitals to the Provincial Disease Surveillance and Outbreak Response Unit (PDSRU) in Sindh, Pakistan [16]. Among these notified XDR-TF cases, study hospital contributed 12% (517 XDR-TF Cases) in the city XDR-TF notification. Mostly XDR-TF cases reported from the lower socio-economic neighborhood of study hospital from Lyari and Saddar towns (Sub-district) of district South, Karachi. This ongoing largest outbreak of XDR-TF was started from Hyderabad city of Sindh in November 2016 and then spread in different district of Sindh including Karachi. [15]. So far (Nov 2016-Mar 2019) 6480

XDR-TF cases have been reported from all district of Sindh among them 4514 (70%) XDR-TF cases were from Karachi [16]. Before this outbreak, a study from Aga Khan University Hospital (AKUH) reported the three years (2009-2011) clinical laboratory review data which revealed 2 cases of ceftriaxone-resistant *S. typhi* [11]. Another study from AKU reported only 4 cases of ceftriaxone-resistant *S. typhi* from Pakistan during the last 3 years (2012–2015) [19].

Typhoid fever infection occurs through oro-fecal route and spread through drinking contaminated water and/or food, poor hygiene (hand wash), and poor sanitation. The risk of typhoid fever is considered high in densely populated district south of Karachi because of unavailability of safe drinking water, poor disposal of waste water and mixing of sewerage water in drinking water, poor personal hygiene (poor practice of hand wash before eating and after defecation) practices in children, very low typhoid vaccination coverage and limited surveillance of typhoid fever in the area. AKUH has tested 13 drinking water samples from the household of typhoid fever cases located in Lyari town, Karachi, and reported the presence of high quantity of Coliforms and/or thermos-tolerant *E. coli* in tested water samples. AKUH also reported the molecular analysis of 55 community water samples using Polymerase Chain Reaction (PCR), and detected *S. typhi* DNA in 12 (21.8%) water samples [16].

Children are the most vulnerable population group among typhoid fever cases. Study reported more than 91% of salmonella isolates came from children ≤ 14 years of age and 53% under five years of age. Among pediatric typhoid fever cases, nearly one-third (32%) cases were XDR TF. Similar findings were reported by previous studies conducted in typhoid endemic areas, have shown around one-quarter to one-third of pediatric typhoid fever cases were under five years of age [20]. During 15 month's surveillance period, peak typhoid fever cases so did MDR-TF and XDR-TF cases, were seen during the summer period from May to Aug 2018 (Figure 1). These months are considered moon soon rainy season in Pakistan when drinking water contamination and diarrheal disease cases are increases.

Table 1. Demographic characteristics of *Salmonella typhi* isolates, MDR-TF & XDR-TF Cases at study hospital, Karachi (N=969).

Characteristics	<i>Salmonella typhi</i> Isolates N (%)	MDR-TF [*] , N (%)	XDR-TF ^{**} , N (%)
Sex			
Male	538 (55.5)	433 (44.7)	285 (29.4)
Female	431 (44.5)	344 (35.5)	232 (23.9)
Total	969 (100.0)	777 (80.2)	517 (53.3)
Age			
Mean Age with SD	6.51 ± 7.68		
<2 years	87 (9.0)	70 (7.2)	52 (5.4)
2-5 years	426 (44.0)	345 (35.6)	232 (23.9)
6-14 years	372 (38.4)	296 (30.5)	189 (19.5)
>14 years	84 (8.7)	66 (6.8)	44 (4.5)
Total	969 (100.0)	777 (80.2)	517 (53.3)
Quarter Period			
Q1-2018	70 (7.2)	49 (5.1)	12 (1.2)
Q2-2018	279 (28.8)	216 (22.3)	76 (7.8)
Q3-2018	304 (31.4)	249 (25.7)	201 (20.7)
Q4-2018	170 (17.5)	140 (14.4)	118 (12.2)
Q1-2019	146 (5.1)	123 (12.7)	110 (11.4)
Total	969 (100.0)	777 (80.2)	517 (53.3)

*MDR-TF= Multi-drug resistance-Typhoid Fever

** XDR-TF= Extensive-drug resistance-Typhoid Fever

Table 2. Age, gender & quarter base distribution and correlation with MDR-TF & XDR-TF cases (N=969).

Characteristics	MDR-TF N (%)	P-Value	XDR-TF N (%)	P-value
Age groups				
<2 years	70 (9.0)		52 (10.1)	
2-5 years	345 (44.4)		232 (44.9)	
6-14 years	296 (38.1)	P=0.940	189 (36.6)	P=0.449
>14 years	66 (8.5)		44 (8.5)	
Total	777 (100.0)		517 (100.0)	
Gender				
Male	433 (55.7)		285 (55.1)	
Female	344 (44.3)	P=0.795	232 (44.9)	P=0.791
Total	777 (100.0)		517 (100.0)	
Quarter Period				
Q1-2018	49 (6.3)		12 (2.3)	
Q2-2018	216 (27.8)		76 (14.7)	
Q3-2018	249 (32.0)	P=0.075	201 (38.9)	P < 0.05
Q4-2018	140 (18.0)		118 (22.8)	
Q1-2019	123 (15.9)		110 (21.3)	
Total	777 (100%)		517 (100.0)	

*XDR-TF= Extensive-drug resistance-Typhoid Fever

Table 3. Antibiotics sensitivity and resistance pattern among *Salmonella typhi* Isolates (N=969).

Antibiotics	Sensitivity N (%)	Intermediate N (%)	Resistance N (%)
Ampicillin	106 (10.9)	0 (0.0)	863 (89.1)
Chloramphenicol	169 (17.4)	0 (0.0)	800 (82.6)
Co-trimoxazole	166 (17.1)	0 (0.0)	803 (82.9)
Ciprofloxacin	249 (25.7)	34 (3.5)	686 (70.8)
Cefixime	259 (26.7)	1 (0.1)	709 (73.2)
Ceftriaxone	268 (27.7)	2 (0.2)	699 (72.1)
Azithromycin	969 (100.0)	0 (0.0)	0 (0.0)
Imipenem	969 (100.0)	0 (0.0)	0 (0.0)



Figure 1. Showing monthly trend of Typhoid Fever, MDR-TF & XDR-TF Cases over 15 months surveillance period at KGH, Karachi (N=969)

Current study also revealed that XDR-TF proportion was initially low (17%) in quarter 1 (Jan-Mar-2018) and 27% in quarter 2 (Apr-Jun-2018) of surveillance period then exponentially rose to 66% in Quarter 3 (Jul-Sep-2018) and finally steady at 72% in Quarter 4 (Oct-Dec-2018) and quarter 1 (Jan-Mar-2019) (Figure 1). This wide difference of rate of XDR-TF cases during surveillance period of Q1-2018 and Q1-2019 was due to rapid spread of genetic mutation strain of XDR-ST in the study catchment area of Lyari and Saddar towns of Karachi. Therefore, WHO recommend the antimicrobial resistance (AMR) pattern monitoring of *Salmonella typhi* in the outbreak area.

In a recent study, [21] the scientist has identified the genetic basis of a resistance strain of XDR typhoid that emerged in Pakistan. They conducted genome sequencing of XDR *Salmonella typhi* strains isolated from the outbreak region (Hyderabad and Karachi) of Sindh province of Pakistan. Genome sequencing revealed that all of the isolated belong to the H58 clade containing genes that impart resistance to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole. Further analysis showed that they harbored a plasmid (IncY) that carries the blaCTX-M15 gene, which mediates resistance to ceftriaxone and qnrS fluoroquinolone resistance gene.

In Karachi only 10 hospitals have been reporting the typhoid fever cases to PDSRU without any

formal reporting format. General practitioners (GPs) and private medical centers have received majority of typhoid fever cases from the community and there is no mechanism exist to report these cases to the concern health authorities. Also these GPs are treating patients presented with fever with broad spectrum antibiotics without any investigation. Furthermore antibiotics are easily available over the counter of medical stores without any physician prescription. Overuse or misuse of antibiotics without any rational can be a risk factor for emergence of XDR strain of *S. typhi*. Majority of physicians are still diagnosing the typhoid fever cases on clinical basis or through Widal test. They don't prescribe blood culture test either due to non-affordability of blood culture test cost by the patients or due to limited access of laboratories having antimicrobial susceptibility testing capacity, which ultimately limit the surveillance of XDR *S. typhi*.

Emergence of XDR stains of *S. typhi*, would severely limit the therapeutic option in Pakistan. Only available antibiotic options are Azithromycin and Carbapenem against XDR *S. typhi*. Cost effectiveness and only oral drug has made Azithromycin the current drug of choice for the treatment of XDR Typhoid fever in Pakistan. Carbapenem is expensive and give intravenously so use in admitted patients. Moreover expensive antibiotics option for XDR-TF treatment would further raised the economic health burden of the country.

As the therapeutic options become more limited, further preventive measures should be urgently needed to take by the concern authority for the control of this prevail XDR-TF outbreak in Sindh. These measures may include availability of safe drinking water, improve sanitation, mass vaccination campaign and health education to the affected communities regarding personal hygiene (hand washing before meal and after defecation), boiling drinking water, washing eatable fruits and vegetables, limit eating out particularly from street vendors. Also need to provide current updates on XDR-TF management to GPs and other typhoid treating physicians. At Kharadar general hospital during Sep 2018 to April 2019, nearly 10,000 children age 6 months to 10 years from the neighbourhood of Saddar and Lyari towns, have received the single dose of WHO prequalified

Typhoid Conjugate Vaccine (TCV) [1], having long-lasting immunity, with the support of AKU Project "Surveillance for Enteric Fever in Asia Project" (SEAP II).

Limitation: Re-confirmation of *S. typhi* resistance cases through minimum inhibitory concentration (MIC) of the antibiotics was not determined.

Conclusion

A large proportion of *S. typhi* isolates were XDR-ST with only sensitivity to Azithromycin and Imipenem antibiotics. Current limited treatment option would pose a serious threat in future for no choice of antimicrobial treatment option for the treatment of rapidly changing resistance strain of *S. typhi*. There should be a need of continuous surveillance of antimicrobial resistance pattern of *S. typhi* and enhance XDR-ST reporting network by including more hospitals and private practitioners in the outbreak areas.

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Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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