

# Emergence of Azithromycin Resistance in progressing trends of extensively drug resistant typhoid in Lahore, Pakistan

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## ABSTRACT

**Aim:** To study the trends of progression of Extensively Drug Resistant (XDR) Salmonella typhoid and identify the alarming emergence of Azithromycin Resistance in Lahore, Pakistan.

**Methodology:** In this multicentral study 400 Salmonella typhoid positive blood cultures from January 1<sup>st</sup>, 2017 to June 1<sup>st</sup>, 2019 from individuals of all ages were collected, from three major academic microbiology centres from Lahore, Pakistan. The entire culture reports from these centres were analysed including the drug sensitivity patterns and relevant demographic data. The case was further classified using the NIH guidelines into non-resistant, multidrug resistant (MDR), and extra drug resistant (XDR) typhoid and azithromycin resistance depending on antibiotic susceptibility patterns.

**Results:** In the study 300 (75%) of samples were fulfilled of the selection criteria. Among selected samples 22 (7.3%) cases were azithromycin resistant XDR, 100 (33.33%) were MDR, 56 (18.67%) were non-resistant species of Salmonella. Alarmed to notice the emergence of azithromycin resistant XDR Salmonella in samples from 2019. Furthermore, there is a proportional progressive increase in the ratio of blood culture positive XDR salmonella in our region.

**Conclusion:** A progressive increase in the blood cultures positive for XDR salmonella is alarming as is the emergence of azithromycin resistant XDR Salmonella in samples from 2019. We would like to stress the need for substantive sustained & timely local and international interventions to curb this resistant organism and established menace.

**Keywords:** Azithromycin resistance, typhoid, XDR, Pakistan.

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## INTRODUCTION

Typhoid is a persistent health concern in South Asia and is one of most common bacterial infectious endemics in Pakistan<sup>1, 2</sup>. With the emergence of Extensively-Drug Resistance (XDR) Typhoid, the threat is transcontinental. Despite preventive and therapeutic advancements, enteric fever remains a challenge both locally and globally. Rapid emergence of resistant strains has created a unique academic and clinical state of alarm.

Antimicrobial resistance is a global phenomenon and Pakistan is significantly affected. There is a 65% increase in antibiotic consumption from 2000 to 2015 mainly due to excessive empirical use by professionals and easy availability of prescription grade antibiotics over the counter<sup>3, 4</sup>.

The first XDR case in the country was reported in Hyderabad in 2016.<sup>5</sup> Multiple studies have since identified a remarkable increase in antimicrobial resistance in Karachi, Hyderabad and other districts across Sindh<sup>5</sup>. Current literature shows cases reported in North America, Europe and China<sup>6</sup>. This information highlights the need for urgent global priority of this issue rather than just a mere concern. The gold standard for typhoid fever diagnosis is the isolation of Salmonella typhi in blood culture samples<sup>7</sup>,

followed by antimicrobial susceptibility testing. Using this method, the trend of the antibiogram of the strains circulating in the city was evaluated.

Azithromycin is a macrolide antibiotic and is currently the only known effective oral option to treat XDR Salmonella.<sup>8</sup> Resistance to Azithromycin further calls upon healthcare for attention to the status of XDR trends and the critical nature of its management.

This study aims at reporting and confirming this emerging trend of XDR typhoid in the city, along with identification of the emergence and possible rapid progression of azithromycin resistant XDR globally. This has the potential of being labelled as a sub classification of enteric fever.

## METHODOLOGY

This retrospective study was conducted in Lahore, the most populous city of the province of Punjab in Pakistan. The current population of the city is estimated to be 11.3 million (2017 Census)<sup>9</sup>.

The study was conducted over a period of 30 months, between 1<sup>st</sup> Jan 2017 to 1<sup>st</sup> June 2019. Data was extracted from three major leading private hospital-based microbiology laboratories which largely serve the people residing in the greater city of Lahore. These tertiary-care centres are Chughtai Laboratory, Hameed Latif Hospital and Doctors Hospital. Each centre contributed their patient

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data to an independent research body and was granted expedited ethical approval from their relevant IRB / ethical committees. A total of 22,000 blood cultures were conducted at these labs, in this study period.

These laboratories follow the standardised microbiological procedure protocol. Blood culture was done using BACT-ALERT BD BACTEC system (Becton Dickinson, MD, USA). On positive signalling, blood culture bottles were pulled on and sub-cultured accordingly. Aerobic bottle signalling growth was sub-cultured on blood agar, MacConkey agar and Chocolate agar. The first two plates were incubated aerobically and the last plate in 5% CO<sub>2</sub> atmosphere at 37°C for 24 h.<sup>10</sup> Anaerobic bottle signalling growth was sub-cultured on blood agar, MacConkey agar and Chocolate agar and an additional blood agar plate was incubated anaerobically at 37°C for 48 h.<sup>11</sup>

Identification of *Salmonella* from lactose non-fermenting colonies on MacConkey agar was done according to standard microbiological procedures using the API 20E (BioMerieux, France) and VITEK-2. Antibiotic susceptibility testing was done according to the Kirby-Bauer disc diffusion method on Muller Hinton agar (Oxoid, UK).<sup>12</sup>

All isolates identified as *Salmonella typhi* and *Salmonella Paratyphi A, B and C* were included in this study. According to National Institute of Health (NIH) Islamabad's surveillance FELTP-Pakistan Epidemiological reporting, typhoid fever is caused by *Salmonella Typhi* or *Salmonella Paratyphi A, B or C* strains. It is further classified into three types; 1: Non-Resistant, 2: Multi-Drug Resistant (MDR) and, 3: Extensively Drug Resistant (XDR) typhoid fever.<sup>13</sup> Non-Resistant type is sensitive to first-line agents (ampicillin, chloramphenicol & trimethoprim-sulfamethoxazole) and second-line agents (cefixime & ceftriaxone) irrespective of its sensitivity to the fluoroquinolone group. The MDR type is resistant to first line recommended agents but are sensitive to second line agents irrespective of their sensitivity to fluoroquinolones. The XDR type is resistant to first line, second line drugs and fluoroquinolones. XDR typhoid is sensitive currently to all the carbapenem agents and only azithromycin out of the macrolide class. Data was entered and descriptive statistics were determined using SPSS version 25.0.

## RESULTS

Out of 22,000 blood cultures, a total of 400 (1.81%) cases were positive for typhoid during a 30-month period study period between 1<sup>st</sup> Jan 2017 to 1<sup>st</sup> June 2019. There were

217(54.25%) males and 183(45.75%) females. The predominant etiological agent was *S. Typhi* with 391 cases (97.8%) and 9 cases (2.3%) due to *S. Paratyphi A*. No cases were reported as *S. Paratyphi B* or *C*.

Age range: 1 to 85 years; mean 13.27 years. (Table 1)  
Year-wise: 24 cases in 2017, with a sharp rise to 233 in 2018 and 142 in the first 5 months of 2019. This study noted patterns of antibiotic resistance. (Figure 1)  
Drug resistance pattern: Of first line drugs, a high resistance of 73.7% was noted.

Resistance pattern of second line drugs revealed 42.2%. The fluoroquinolones showed sensitivity in only 6.1%. The remainder were either resistant 59.8% or intermediate 34.1%. The carbapenems revealed 100% sensitivity. Azithromycin resistance was noted in only 2019 with a calculated 5.75% overall resistance in the study period. (Table 2).

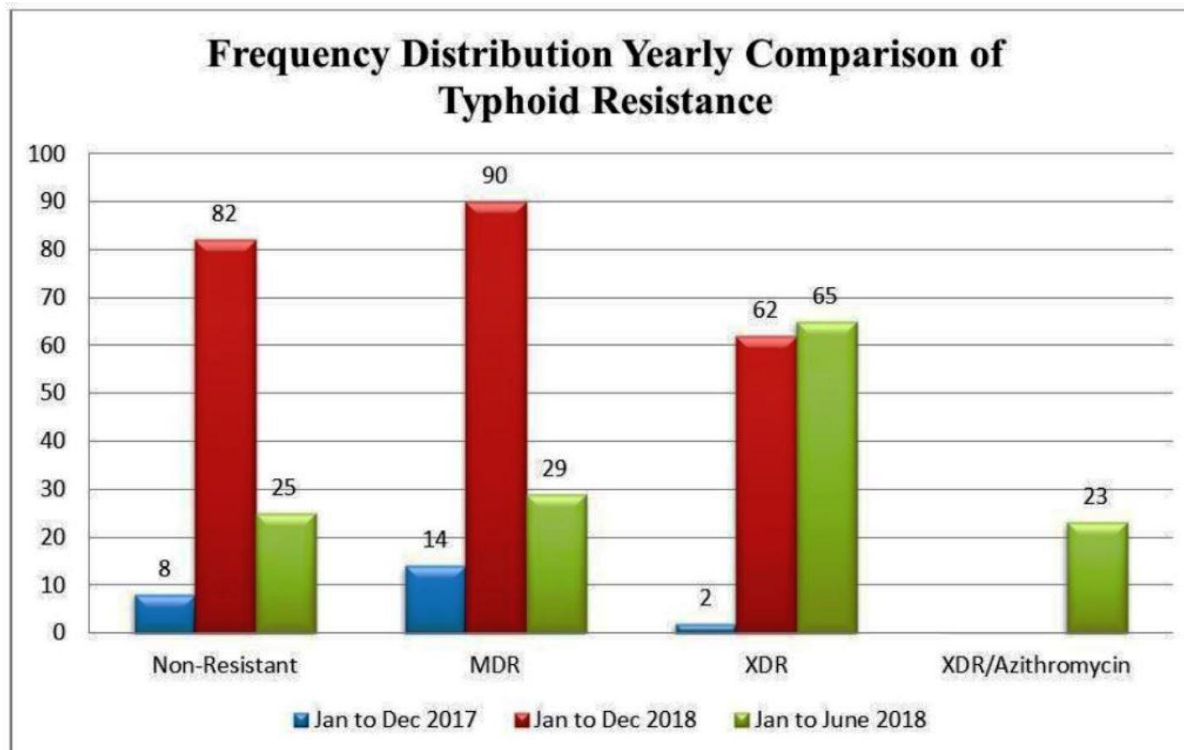
Table 1: Social demographics of the study

Characteristics	Categories	Frequency
Gender	Male	217 (54.3%)
	Female	183 (48.5)
Age Groups	≤5	119(29.8%)
	6 – 10	98(24.5%)
	11 – 15	59(14.8%)
	16 – 20	41(10.3%)
	21 and above	83(20.8%)
Hospitals	Hameed Latif Hospital	105(26.3%)
	Chughtai Lab	263(65.8%)
	Doctors Hospital	32(8.0%)
City	Lahore Main City	245(61.3%)
	Lahore District	155(38.8%)
Species	<i>Salmonella Paratyphoid A</i>	9(2.3%)
	<i>Salmonella Typhoid</i>	391(97.8%)

Table 2: Frequency distribution regarding antibiotic and drug resistance

	Drugs Resistance		
	I	R	S
Ampicilin	0 (0.0%)	294 (10.0%)	105 (3.7%)
Azithromycin	1 (0.2%)	23 (0.8%)	103 (3.6%)
Cefixime	0 (0.0%)	145 (4.9%)	218 (7.6%)
Ceftriaxone	2 (0.4%)	165 (5.6%)	224 (7.8%)
Chloramphenicol	1 (0.2%)	69 (2.3%)	18 (0.6%)
Ciprofloxacin	134(30.5%)	235 (8.0%)	24 (0.8%)
Imipenem	0 (0.0%)	0 (0.0%)	136 (4.8%)
Meropenem	0 (0.0%)	0 (0.0%)	137 (4.8%)
Trimethoprim-sulfamethoxazole	0 (0.0%)	259 (8.9%)	117 (4.1%)
Miscellaneous	300(68.4%)	1729 (59.2%)	1770 (62%)

I, R=resistant, S=Sensitive



## DISCUSSION

The retrospective analysis showed that there is a significant socio-economic impact of enteric fever. Higher incidence was seen in males as compared to females. This could be because males may be more susceptible due to various risk factors including life style, exposure or employment<sup>14</sup>. The largest number of affected population were children between the age of 1-15 years; 29.5% of these were in 1–5 years of age. This is in-line with published literature, revealing that males of 5 to 15 years and school-going children were the most affected<sup>15</sup>. Studies reported from India and Bangladesh also reveal that children younger than five years are the most affected<sup>16</sup>.

The lack of availability of the conjugate typhoid vaccine around the world, especially in South Asia including Pakistan can be considered as failure of employing effective preventive measures. This vaccine is not available in public, private or government sectors in Pakistan. The conjugate vaccine gives protection to all age groups, especially below 2 years<sup>17</sup>. Polysaccharide vaccine commonly available in Pakistan has poor immunogenicity especially for children less than 2 years of age. This vaccine comes at a personal cost and is not yet part of the standard cost-free (EPI) immunization program.

Cases above 21 years of age show 20.8% incidence in our study. Young adults and older age groups have a greater exposure because they like to explore food options with likely unhygienic eating practices<sup>18</sup>.

In 2016, the first case of XDR was reported from Hyderabad and in the following two years, 339 isolates were reported from Sindh, the Southern province of

Pakistan<sup>5</sup>. Since then cases are popping up globally as predicted.

Our study showed 24 cases in 2017, with a spike increase to 233 cases in 2018 and 142 cases in the first 5 months of 2019. Amongst these, in 2017 there were 14 cases of MDR and 2 cases of XDR. In 2018, there were 90 cases of MDR and 62 cases of XDR, showing an increase from the previous year. The remaining cases during 2017 and 2018 were non-resistant. In both 2017 and 2018, MDR cases exceeded the non-resistant cases. Till June in 2019, there are 65 cases of XDR, exceeding both the 29 cases of MDR and 25 non-resistant cases. A pattern of Azithromycin resistant XDR was also reported in 2019, 23 cases out of the 65 cases of our total XDR typhoid in 2019 were Azithromycin resistant. Earlier this year, Azithromycin resistance was also reported in China and Bangladesh, showing that this emergence can turn into a global pattern<sup>19</sup>.

Furthermore, an interesting scientific aspect to typhoid progression is the genetics of *Salmonella typhoid*. The predicted anticipation of a progressively resistant typhoid global epidemic risk is a realistic threat once we study the genomic trends that allows *S. typhi* to acquire resistance. *Salmonella typhoid* has acquired resistant genes likely transferred from other gram-negative organisms via plasmid transference. The likely culprit is *E. coli*, although PCR analysis of various genes is noted in literature. Azithromycin resistance with PCR analysis was studied in Bangladesh and China<sup>6,19</sup>.

Whether transfer of such genes is the reason behind azithromycin resistance here locally, still needs medical evidence-based analysis. PCR analysis of all cultures that show azithromycin resistance in typhoid fever, should be

further investigated to look for known genes rendering resistance<sup>6,19</sup>.

Salmonella Para-typhi has not yet shown any XDR type of resistance in the study conducted, which is in consistency with literature reported.

Attempts have been made to evaluate the factors contributing towards the XDR strains. Studies show various risk factors including male gender, contamination from an infected person, food from outside home, and significant patient history of frequent/overuse of antibiotics.<sup>20</sup>

In Pakistan, XDR Typhoid is believed to have started from Sindh as the first case was reported from there and propagated locally due to various hygiene related issues including the widespread abuse of antibiotics. Antibiotic use is a prevalent practice in the country due to unrestricted public antibiotic access, pharmacists or other personnel dictating antibiotic prescriptions, self-medication, and parental administration of antibiotics without seeking relevant professional medical advice.<sup>21</sup> To top it, compliance to antibiotic regimen in patients on typhoid treatment is poor resulting in incomplete courses of antibiotics.<sup>22</sup> In addition, professional capacities in Infectious Disease, Microbiology and Medicine in general are locally substandard. Training programs for these specialities are lacking. This stands itself as a major concern for the healthcare community at large.

Most developed countries have regulations on prescription authorities for medical professionals and dispensing rules for pharmacies. Such regulations are either in their infancy, not enforced or completely lacking in Pakistan. Amendments and enforcements in these are the dire need of this time, to monitor the sources of prescriptions that are being submitted. There is a practice of prescribing second- or third-line agents, before prescribing first line agents to a patient. Regulatory authorities like the PMDC should look into monitoring prescriptions of such physicians. Proper implementation of tapping the PMDC number, medical practicing license or higher certifications should be regularly done to keep check. Improper Continued Medical Education (CME) of health professionals is another untold tale, which results in lack of their awareness regarding new and upcoming guidelines to manage suspected or diagnosed typhoid cases.

Our media has a lacking component of public health issues. Community awareness programs are essentially non-existent. Campaigns should be organized by the government and health authorities. Lack of education is a self-created health hazard towards exacerbating this issue. All forms of media should be used to send out public messages. Such a modality will have a significant impact to curb this menace.

Mass immunization is one other method that should be implemented. Typhoid conjugate vaccine was anticipated to be incorporated into the current EPI schedule back in 2017. This is still being waited upon. The availability of the conjugate vaccine for private use isn't there, let alone its incorporation into the EPI schedule to date.<sup>10</sup> Furthermore, in order of impact, improvements have to be enforced in water quality hygiene, better sanitation, overcrowding management and surveillance measures like were done in Sindh.

Limitations of our study primarily stem from the retrospective nature of the data which does not take into account the clinical presentation and the duration of symptoms or antibiotic use.

We were unable to evaluate if the blood cultures were done before the administration of antibiotics or after the ineffectiveness of antibiotics. Practice in the periphery is to start antibiotics and then monitor for symptoms. Also, suspected cases are often given empirical antibiotics as the blood culture results arrive late depending upon the laboratory, usually after 2 to 7 days since the time of the sample is taken and sent for testing. The financial status of the patient and hospital resources is another factor due to which blood cultures are done only infrequently. Therefore, typhoid diagnosis is often clinical and less specific tests like Widal test are frequently used as basis for reaching a diagnosis for treatment. These numbers may be an under-estimation of the true magnitude of the problem as blood culture and sensitivity is rarely performed and usually done after empiric treatment failure.

## CONCLUSION

There is a proportional progressive increase in the ratio of blood culture positive XDR Salmonella Typhoid in Lahore, Pakistan. Furthermore, it is alarming to notice the emergence of Azithromycin Resistant XDR Salmonella Typhoid in blood culture samples from 2019, which is consistent with the reports coming from China and Bangladesh for this year. If no measures are taken, this leaves no current oral antibiotic option to treat S. Typhi. Intravenous options of one class only, will be the sole treatment left for typhoid management.

Hence, the results of this study highlights the need for substantive sustained & timely local and international interventions to be taken in order to curb this resistant organism and established menace.

**Disclaimer:** None to declare.

**Conflict of interests:** None to declare

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