Extensively Drug Resistant Typhoid Fever Seen at Tertiary Care Hospital in Lahore

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Abstract

Background
Typhoid fever is endemic in Pakistan. Multi-drug resistance is commonly observed in Salmonella Typhi (*S.Typhi*) isolates, due to which first line of drugs, ampicillin, trimethoprim-sulphamethoxazole and chloramphenicol become ineffective for treatment. Towards the end of 2016 these MDR strains also developed resistance to ceftriaxone and quinolones and were called extensively drug resistant (XDR). Since first reported outbreak of XDR *S.Typhi*, it continues to increase in number in Sindh.

Objectives
Present study was planned to see the sensitivity pattern of *S. Typhi* isolated in a tertiary care hospital, Lahore.

Study Design
Retrospective analysis

Setting and Duration
The study was done at Microbiology Department of Services Institute of Medical sciences from January 2018 to April 2019.

Materials & Methods
All 16288 blood culture samples received from indoor and outpatient department were analyzed for *S. Typhi* by standard microbiological techniques. Sensitivity of *S. Typhi* to ampicillin, trimethoprim-sulphamethoxazole, chloramphenicol, ciprofloxacillin, ceftriaxone, azithromycin and imipenem was assessed Kirby-Bauer method according to CLSI guidelines 2018.

Results
During Jan-Dec 2018, number of *S. Typhi* isolated were 29 out of these 13 were MDR and 14 XDR. In the first four months of 2019, 69 *S. Typhi* were isolated; of these 6 were MDR and 58-XDR. Theses findings are significant *p*-value <0.00017. Another alarming finding was the presence of one azithromycin resistant XDR. Most of *S. Typhi* were isolated from children less than 15 years old.

Conclusions
With increasing XDR *S. Typhi* being isolated in Lahore, coordinated efforts are needed to control it. This will include vaccination and awareness campaign for better hygiene, safe water and improved sanitary conditions. Antibiotic stewardship should be taken seriously at Government level and over the counter sale of antibiotics must be stopped.

Key Words
Typhoid, Salmonella typhi, extensively drug resistant, XDR

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Background
The Indo-Pak subcontinent has seen a decline in prevalence of typhoid fever from the turn of the century to 2015. In a multi -center study conducted in Pakistan on blood culture isolates; the percentage of *Salmonella Typhi* (*S. Typhi*) was 1.32% in 2015, a decrease from 6.42% in 1992.[1] In India this decline has been accompanied with a decreased incidence of multi-drug resistant strains (MDR) of *S. Typhi*.[2] However, in Pakistan research has shown an increase in MDR *S. Typhi* over the years.[1,3,4] The estimated incidence of *S. Typhi* infections is more than 500/100000 both in Pakistan and India.[5]

Salmonella enterica serotype Typhi is a Gram negative bacterium that causes typhoid fever. It causes fever, malaise, headache and rash and can in severe cases progress to life threatening complications including encephalitis and intestinal hemorrhage.[6,7,8] *S. Typhi* spreads via food and water contaminated with human feces. Diagnosis of typhoid fever is by blood & bone marrow culture in first week followed by stool/urine culture in second week of illness. Sensitivity of blood cultures is 40-80% but this drops to 30% if tested after the first week of infection.[9,10,11,12] Diagnosis of typhoid in low to middle income countries (LMIC) is mostly done by serological tests, Widal and Typhi dot, which are of low sensitivity and specificity and are not recommended for diagnosis of typhoid fever.[19]

In Pakistan we are increasingly seeing MDR strains of *S. Typhi* which are resistant to ampicillin, trimethoprim sulphamethoxazole and chloramphenicol. Some cases of extensively drug resistant (XDR) *S. Typhi* have also been reported. XDR strains are defined as MDR strains exhibiting additional resistance to quinolone and ceftriaxone.[13,14] All risk factors associated with transmission of *S. Typhi* are present in Pakistan; there is often sewage mixing with water pipes, wide

spread poor hygiene, and a lack of awareness of basic preventative measures.[1,15,16,17,18]

Since reports of sporadic cases of one to two XDR *S. Typhi* over the years[3,20,21,22] an outbreak started on 30 Nov 2016 to March 2017. In this 101 cases of XDR *S. Typhi* were reported from Latifabad and Qasimabad, Sindh.[15] XDR salmonella has now spread with travel to USA, UK and Canada.[13,23,24]

This paper intends to add to the growing field of research in this area by measuring the prevalence of *S. Typhi* and especially drug resistant strains in a 1450 bed tertiary care hospital in Lahore.

Materials & Methods

This retrospective cross-sectional study of blood culture isolates was done at Microbiology Department of Services Institute of Medical Sciences, Lahore.

All blood cultures received and processed from January 2018 to April 2019 were analyzed for *S. Typhi*. A total of 16288 blood cultures were received in aerobic, tryptic soya broth from admitted patients and outpatient department.

Blind subcultures were made on blood agar and MacConkey agar plates after incubation of blood culture bottles at 35^0C for 24 hours. Inoculated plates were examined after overnight incubation at 35^0C. Identification of isolated growth was made on colony morphology and Gram stain reaction. All non -lactose fermenter on MacConkey agar were tested for oxidase production. Further biochemical tests done on oxidase negative colonies were urease, citrate utilization, motility ant triple sugar iron (TSI) tests.[25] If results were ambiguous API20E was set up. In 2019 second subculture was done on day 7 before discarding blood culture bottle as sterile. Procedure for second subculture was the same as the first subculture.

Sensitivity of *S. Typhi* was carried out on Mueller Hinton agar by Kirby–Bauer method according to CLSI guidelines 2018 (26). Antibiotic discs used were ampicillin (AMP 10ug, oxoid), trimethoprim sulphamethoxazole, chloramphenicol (C 30ug), iprofloxacillin (CIP5ug), ceftriaxone (CRO 30ug), azithromycin (AZM 15ug) and imipenem (IPM 10ug).

Statistical Analysis was done by percentage and chi square. Numbers and percentages are reported in this study along with year wise trends.

Results

Most of the typhoid cases were seen in children under 15 years old as seen in Fig.1. More males, 58%, were seen than females as shown in Fig 2. The *p*-value of this result is 0.10604 hence it is not significant at $p<0.05$

In Jan-Dec 2018, 29 laboratories confirmed cases of *S. Typhi* were seen in 14020 blood cultures received. In comparison the

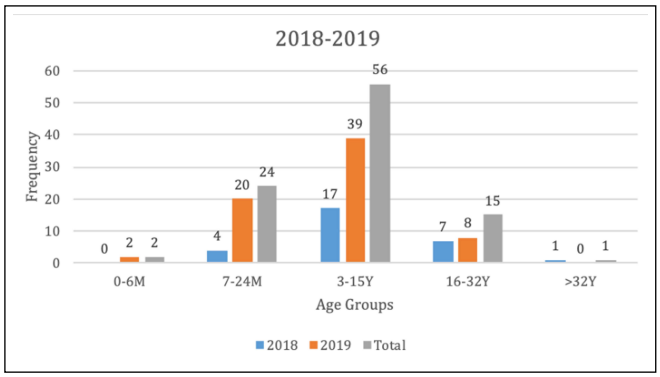

Fig 1. Distribution of *Salmonella* Typhi according to Age N=98

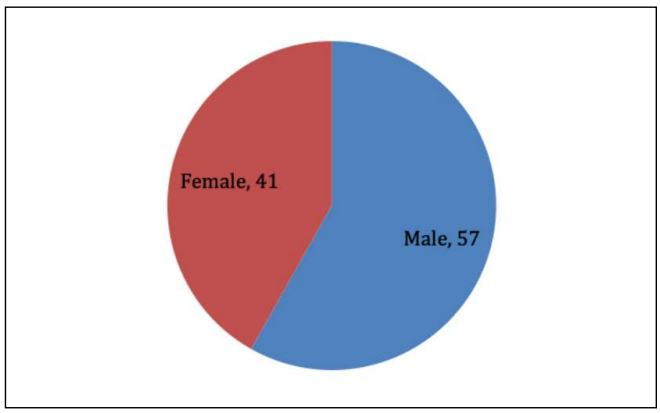

Fig 2. Distribution of *Salmonella* Typhi according to Gender. N=98

period between Jan-April 2019 saw 69 *S. Typhi* cases isolated from 2268 blood culture specimens. Sensitivity of these isolates to antibiotics is shown in Fig.3. Blue bars indicate the sensitive strains isolated in 2018 whilst red bars represent sensitive strains isolated in 2019.

The ratio of MDR to XDR cases is shown in Table1. There was a significant increase in XDR in 2019; 58 cases isolated in comparison to 14 the year before. The number of MDR isolated conversely decreased with only 6 isolated in 2019, down from 13 in 2018. The difference came back as significant with a *p*-value of 0.00017.

Month wise trend of XDR *S.Typhi* isolated is shown in Fig 4. It shows not only an increase incidence of XDR *S.Typhi* in 2019 but also shows the increasing number of cases seen each month.

Discussion

Typhoid is a systemic illness associated with serious complications.[6] Morbidity and mortality increase significantly in the presence of MDR and XDR strains. This is associated with an increased financial burden disproportionately affecting low to middle income countries.[8,27,28]

Table1. Ratio of Non-Resistant, MDR TO XDR *Salmonella* Typhi

	Non –Resistant	MDR	XDR
2018 (N29)	2	13	14
2019 (N69)	5	6	58
2018-19 (N 98)	07	19	72

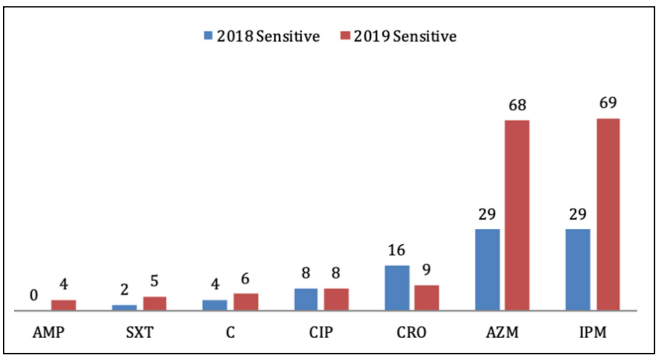

Fig 3. Sensitivity Pattern of *Salmonella* Typhi

AMP Ampicillin, SXT trimethoprim sulphamethoxazole, C chloramphenicol, CIP ciprofloxacillin, CRO ceftriaxone, AZM azithromycin, IPM imipenem.

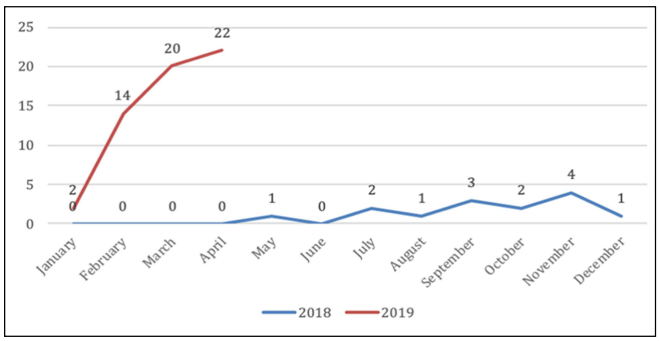

Fig 4. Month wise trend of XDR *Salmonella* Typhi isolated (JAN2018-APRIL 2019)

One common theme in the majority of the studies carried out has been the high rate of infection in children less than 15 years old[3,6,15, 29, 30] as seen in our study. What is even more concerning are the numerous reports of cases seen in 2-year old and even in under 6-month-old babies.[15,31] In a systematic population based study conducted in in Karachi the incidence of infections seen in infants under 12 months age was 506.4/100000. Low rates of exclusive breast feeding were pointed out as a possible explanation for the high rates seen.[30] In the present study 2 cases were seen in under 6-month-old infants, with one of them being a 12-day old neonate. The young age of patients seen at presentation also raises questions about vaccination and prevention strategies. Currently vaccinations in Pakistan can only be given to children over two years old yet, as highlighted above, a significant number of cases got infected before they reached that age. Furthermore, vaccination against typhoid is not part of the routine program of immunization in Pakistan in contrast to countries like China, India and Vietnam.[17]

Typhoid fever was seen more in male, 58%, as compared to female children. (Fig 2). This gender distribution of typhoid cases is similar to 56.4% reported by Yousafzai *et al* [15] and has been reported to be as high as 65.7% in another study.[22] This could be due to outdoor activities of male children making them more susceptible to getting the infection and/or male children being more likely to be brought for treatment.

The ratio of non-resistant to MDR and XDR *S. Typhi* is shown in Table 1. It reveals a significant increase in XDR *S. Typhi* and decrease in MDR *S. Typhi* in 2019 as compared to 2018. In Pakistan the most concerning finding has been the increase in cases of XDR *S. Typhi* seen in recent years and, in particular, 2019 since the outbreak of XDR typhoid fever in Sindh.[29] The first case of XDR *S. Typhi* in our microbiology laboratory was detected in Feb. 2018 and till 31 December 2018 a total of 11 XDR strains were isolated from 29 laboratory confirmed typhoid cases. However, there has been a significant increase in XDR *S. Typhi* isolated in 2019. Since 1st Jan to 30th April 2019 there have been 58 XDR *S. Typhi* isolated out of 69 laboratory confirmed typhoid fever cases as shown in Fig 3 & Fig 4. So far there is no published data from Punjab on XDR S Typhi after the outbreak reported in Sindh. In Sindh the burden of XDR *S. Typhi* is continuing to increase despite preventive measures having been adopted. According to latest report, there are 7646 laboratories confirmed *S. Typhi* cases of which 4763 are of XDR *S. Typhi*.[32]

There is an urgent need to conduct further studies in other hospitals to determine if this increase is an isolated finding at a particular hospital or part of a regional trend across Punjab. An alarming finding is the detection of one azithromycin resistant XDR strain of *S. Typhi* seen in 2019, which indicates the evolving drug resistance in this microorganism. Although azithromycin resistance has been reported previously[33], in presence of XDR resistance it gains added significance.

Conclusion

Considering the increase in incidence of MDR and XDR *S. Typhi* there is a need to form and implement basic guidelines to prevent this from becoming an epidemic.[34] Typhoid can be prevented by good sanitation, safe water and proper hygiene. People need to be educated on the modes of spread of typhoid helping increase public awareness.[9,18] Vaccination drive is the immediate solution to reduce the impending typhoid outbreak in monsoon season and attention should be given in increasing

uptake of the typhoid vaccine.[29] With judicious use of antibiotics in hospitals and community, resistance can be controlled and reverted as has been shown in studies in Pakistan and India.[2,6,35]

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