JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

*Original Article*

# Extended-spectrum beta-lactamases producing extensively drug-resistant *Salmonella* Typhi in Punjab, Pakistan

Muhammad Saeed[1], Muhammad Hidayat Rasool[1], Farhan Rasheed[2], Muhammad Saqalein[1], Muhammad Atif Nisar[1], Ambereen Anwar Imran[2], Saba Tariq[3], Afreenish Amir[4], Aamer Ikram[4], Mohsin Khurshid[1]

[1] *Department of Microbiology, Government College University Faisalabad, Pakistan*
[2] *Department of Pathology, Allama Iqbal Medical College, Lahore, Pakistan*
[3] *Centre for Applied Molecular Biology, University of the Punjab, Lahore, Pakistan*
[4] *National Institute of Health, Islamabad, Pakistan*

## Abstract

Introduction: The multidrug-resistant (MDR) *Salmonella enterica* serovar Typhi isolates have been increasingly reported from the Asian and African countries. The emergence of isolates with decreased susceptibility to fluoroquinolones and cephalosporins has worsened the situation. Recently, an outbreak from Sindh, Pakistan was reported caused by extensively drug-resistant (XDR) *S.* Typhi strains.

Methodology: In the present study, a total of 82 cases of typhoid have been investigated during 2018 from the febrile children referred to a tertiary care hospital in the population-wise largest province (Punjab) of Pakistan. *S.* Typhi was identified by standard microbiological techniques and isolates were characterized for antimicrobial resistance profiling and minimum inhibitory concentrations were determined. The presence of various ESBL genes in *S.* Typhi was confirmed by the PCR.

Results: Out of the 82 isolates tested, 35 (43%) were found to be XDR; resistant to the first-line drugs. The resistance to third-generation cephalosporins was mainly mediated by extended-spectrum beta-lactamases i.e. *bla*TEM and *bla*CTX-M genes.

Conclusions: The higher prevalence of ESBL producing *Salmonella* typhi clinical strains raises the concern about transmission prevention and infection management in the community as well as clinical settings. Moreover, the study highlights the problem concerning the declining antibiotic arsenal for the therapeutic management of typhoid fever and the emergence and spread of XDR strains in Pakistan.

**Key words:** Salmonella; cephalosporins; ESBL; typhoid.

*J Infect Dev Ctries* 2020; 14(2):169-176. doi:10.3855/jidc.12049

(Received 24 September 2019 – Accepted 31 January 2020)



## Introduction

The occurrence of Typhoid fever is alarmingly high in developing countries with approximately 200,000 deaths each year [1]. *S.* Typhi is transmitted by a fecal-oral route usually by the consumption of contaminated water. the emergence of multidrug-resistant (MDR) *S.* Typhi strains has been increasingly reported particularly from the low-income as well as middle-income countries. The first-line treatments including the beta-lactams such as ampicillin along with chloramphenicol and co-trimoxazole (trimethoprim-sulfamethoxazole) remained effective till the 1970s, however, the multidrug-resistant (MDR) isolates (showing resistance to first-line drugs) were increasingly being observed [2]. The fluoroquinolones have been used as second-line therapeutic agents in the regions, but fluoroquinolone resistance has also been increasingly reported [3]. The third-generation cephalosporins are now being used for the management of typhoid fever especially in the absence of other options; however, sporadic cases and outbreaks of cephalosporin-resistant *S.* Typhi have also been reported recently [4].

The extended-spectrum β-lactamase (ESBL) producing *S.* Typhi strains were quite uncommon from the introduction of cephalosporins till the first decade of the 21st century and were reported only from few studies from Asian countries or the patients with a history of travel to that region [5-8]. The ESBLs are known to confer cephalosporin resistance; although were previously uncommon among *Salmonella enterica* serovar Typhi strains and this transfer is facilitated especially if these resistant determinates are associated with transposon or plasmids, for example, *bla*CTXM-15 [9].

The surveillance data from Karachi, Pakistan demonstrated an increase in the percentage of MDR *S.* Typhi, sporadic resistance to ceftriaxone was found in

2 isolates obtained from children during 2009-2011 [10]. Since 2016, the cephalosporin-resistant isolates were reported from a large proportion from Sindh Province of Pakistan, mainly from the larger cities i.e. Karachi and Hyderabad. Additionally, the case was also reported in the United Kingdom from a person with a recent travel record to Pakistan [4]. In addition to the cephalosporin resistance, these *S.* Typhi strains were nonsusceptible to ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, and fluoroquinolones and were termed as Extensively drug-resistant typhoid (XDR). The present study was aimed to determine the susceptibility of *S.* Typhi isolates towards normally used antimicrobial agents from the febrile children attending the tertiary care hospital at Lahore, Pakistan. We report the emergence of extensively drug-resistant (XDR) isolates obtained from the blood cultures obtained from these febrile children and have delineated the genetic basis of cephalosporin resistance.

## Methodology

*Bacterial Isolates*

Between July-December 2018, a total of 82 *Salmonella enterica* serovar Typhi were isolated from the febrile children suspected from typhoid fever either admitted directly or referred to a tertiary care hospital (Allama Iqbal Medical College/Jinnah Hospital, Lahore) at Lahore (Punjab), Pakistan. The blood samples were inoculated in the tryptic soy broth bottles immediately after the collection. The blood culture bottle was detected as positive by transferring the broth on a glass slide for Gram staining and sub-culture onto Blood agar (Oxoid, UK), Chocolate agar (Oxoid, Hampshire, UK) and MacConkey agar (Oxoid, Hampshire, UK) incubated at 35-37 °C. The strain identification was initially performed by Gram-staining, and routine biochemical tests and by using API 20E (Biomérieux, Lyon, France) and VITEK® consistent with the manufacturer instructions. For the serovar confirmation, agglutination assays were performed using antisera (Denka Seiken Co Ltd., Tokyo, Japan) as per user guidelines. This study was conducted using the bacterial strains which have been isolated for treatment purpose. Additionally, the study was completely anonymous, and the data or identifiable information was not obtained therefore informed consent, or the ethics approval are not required for such type of study according to the local legislation.

*Molecular Characterization*

Molecular characterization of the isolates was done at Microbiology Department, Government College University Faisalabad. The identity of *S.* Typhi was further confirmed by PCR using specific primers as described in Table 1 for the *fliC-d* gene of the *Salmonella* serovar Typhi reference strain. The bacterial DNA was isolated from the bacterial colonies

**Table 1.** List of primer used in the study.

| Target gene | Primer name | Primer sequence (5'-3') | Amplicon (bp) | Annealing Temperature (°C) |
|---|---|---|---|---|
| *fliC* | H-For | ACTCAGGCTTCCCGTAACGC | 763 | 50 |
| | Hd-Rev | GGCTAGTATTGTCCTTATCGG | | |
| *bla*TEM | TEM-F | TCAACATTTCCGTGTCG | 860 | 56 |
| | TEM-R | CTGACAGTTACCAATGCTTA | | |
| *bla*SHV | SHV-F | ATGCGTTATATTCGCCTGTG | 896 | 56 |
| | SHV-R | AGATAAATCACCACAATGCGC | | |
| *bla*CTXM | CTXMU-F | ATGTGCAGYACCAGTAARGT | 593 | 52 |
| | CTXMU-R | TGGGTRAARTARGTSACCAGA | | |
| *bla*CTXM1 | CTXM1-F | CCGTTTCCGCTATTACAAACCGTTG | 944 | 56 |
| | CTXM1-R | GGCCCATGGTTAAAAAATCACTGC | | |
| *bla*CTXM2 | CTXM2-F | ATGATGACTCACAGCATTCG | 833 | 56 |
| | CTXM2-R | TCCCGACGGCTTTCCGCGTT | | |
| *bla*CTXM8 | CTXM8-F | TTTGCCCGTGCGATTGG | 368 | 50 |
| | CTXM8-R | CGACTTTCTGCCTTCTGCTCT | | |
| *bla*CTXM9 | CTXM9-F | ATGGTGACAAAGAGAGTGCA | 870 | 50 |
| | CTXM9-R | CCCTTCGGCGATGATTCTC | | |
| *bla*CTXM10 | CTXM10-F | GCAGCACCAGTAAAGTGATGG | 524 | 56 |
| | CTXM10-R | GCGATATCGTTGGTGGTACC | | |
| *bla*CTXM14 | CTXM14-F | GAGAGTGCAACGGATGATG | 941 | 56 |
| | CTXM14-R | TGCGGCTGGGTAAAATAG | | |
| *bla*CTXM15 | CTXM15-F | CACACGTGGAATTTAGGGACT | 996 | 55 |
| | CTXM15-R | GCCGTCTAAGGCGATAAACA | | |

using FavorPrep™ Genomic DNA Extraction Kit (Favorgen Biotech Corporation, Ping-Tung, Taiwan) according to the instructions provided. The eluted DNA was stored at −20 °C till further experiments and was run on 1% agarose gel followed by staining using ethidium bromide and visualized by UV transillumination. The PCR was performed in a total reaction mixture of 30 µL having 15 µL of 2X master mix (New England Biolabs, Hertfordshire, UK), 200 nM of each forward and reverse primers and 1 µL of DNA was added in T100™ Thermal Cycler (Bio-Rad Laboratories Inc., Hercules, California, United States). following amplification, steps were used: 1 cycle of 94 °C for 5 minutes; 35 cycles of 94 °C for 30 seconds, 50 °C for 40 seconds, and 72 °C for 45 seconds; and a final cycle of 72°C for 5 minutes. The obtained amplicons were separated using 1.2% agarose gel electrophoresis and were stained with the dye (ethidium bromide) to visualize under the gel documentation system.

*Antimicrobial Susceptibility*

The antimicrobial susceptibility testing for the *S.* Typhi clinical strains was performed using the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) standards [11] for the following antibiotics; ampicillin, amoxicillin, piperacillin-tazobactam, ceftriaxone, cefotaxime, cefixime, cefepime, imipenem, meropenem, ciprofloxacin, chloramphenicol and trimethoprim-sulfamethoxazole (Oxoid, Hampshire, UK). (In case the interruptive criteria were unavailable for *S.* Typhi, the criteria described for Enterobacteriaceae was followed for the interpretation of results). The isolates which were resistant to the first-line antimicrobial agents including ampicillin, co-trimoxazole, and chloramphenicol were classified as multidrug-resistant (MDR) as described previously. The *S.* Typhi isolates resistant to two more groups of antibiotics (fluoroquinolones and 3rd generation cephalosporins) were considered as "extensively drug-resistant" (XDR) as proposed by previous studies [4].

*Determination of minimum inhibitory concentrations*

The MICs of the following beta-lactam antimicrobial agents were determined: ampicillin, amoxicillin, piperacillin-tazobactam, ceftriaxone, cefotaxime, cefixime, cefepime, imipenem and meropenem by broth microdilution method using the freshly prepared antibiotic stocks and Mueller-Hinton broth as per CLSI 2018 guideline. *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) were used as quality control strains and the susceptibility results were interpreted consistently with the CLSI 2018 guidelines.

*PCR Amplification of ESBLs*

The isolates including the cephalosporin-resistant as well as sensitive isolates were screened by PCR to determine the presence of *bla*TEM, *bla*SHV and *bla*CTX-M genes using specific primers as shown in table 1. The PCR amplification reactions were carried out in a 30 µL volume having 15 µL 2X master mix, 200 nM of each ESBL primer and 1 µL of template DNA. All the amplification reactions were completed in T100™ Thermal Cycler (Bio-Rad Laboratories, Inc. California, United States). The *bla*CTX-M positive isolates were further characterized for the occurrence of *bla*CTX-M types including *bla*CTX-M-1, *bla*CTX-M-2, *bla*CTX-M-8, *bla*CTX-M-9, *bla*CTX-M-10, *bla*CTX-M-14, and *bla*CTX-M-15 using species primers as listed in Table 1.

**Table 2.** Antimicrobial susceptibility profiling of *Salmonella* Typhi isolates from febrile children.

| Antimicrobial agents | Susceptible n (%) | Intermediate n (%) | Resistant n (%) |
|---|---|---|---|
| Ampicillin | 21 (25.61) | 0 (0) | 61 (74.39) |
| Amoxicillin | 21 (25.61) | 0 (0) | 61 (74.39) |
| Piperacillin-tazobactam | 47 (57.32) | 4 (4.88) | 31 (37.80) |
| Ceftriaxone | 47 (57.32) | 0 (0) | 35 (42.68) |
| Cefotaxime | 47 (57.32) | 0 (0) | 35 (42.68) |
| Cefixime | 47 (57.32) | 0 (0) | 35 (42.68) |
| Cefepime | 47 (57.32) | 0 (0) | 35 (42.68) |
| Imipenem | 82 (100) | 0 (0) | 0 (0) |
| Meropenem | 82 (100) | 0 (0) | 0 (0) |
| Ciprofloxacin | 3 (3.66) | 5 (6.10) | 74 (90.24) |
| Azithromycin | 82 (100) | 0 (0) | 0 (0) |
| Chloramphenicol | 3 (3.66) | 0 (0) | 79 (96.34) |
| Trimethoprim-sulfamethoxazole | 3 (3.66) | 0 (0) | 79 (96.34) |

**Table 3.** MIC distribution of various beta-lactam agents for *Salmonella* Typhi isolates.

| Antimicrobial agents | Breakpoints (µg/mL) | No. of isolates from which the MIC (µg/mL) were | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ≤ 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | ≥ 128 |
| Ampicillin | ≥ 32 | 0 | 0 | 0 | 0 | 0 | 8 | 13 | 0 | 0 | 13 | 11 | 37 |
| Piperacillin-tazobactam[a] | ≥ 128/4 | 0 | 0 | 0 | 0 | 4 | 21 | 13 | 3 | 6 | 0 | 4 | 31 |
| Ceftriaxone | ≥ 4 | 0 | 0 | 21 | 23 | 3 | 0 | 0 | 0 | 0 | 0 | 18 | 17 |
| Cefotaxime | ≥ 4 | 0 | 0 | 12 | 34 | 1 | 0 | 0 | 0 | 0 | 0 | 19 | 16 |
| Cefixime | ≥ 4 | 0 | 0 | 0 | 14 | 33 | 0 | 0 | 0 | 0 | 0 | 23 | 12 |
| Cefepime | ≥ 16 | 0 | 0 | 0 | 15 | 32 | 0 | 0 | 0 | 0 | 6 | 18 | 11 |
| Imipenem | ≥ 4 | 0 | 28 | 38 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Meropenem | ≥ 4 | 0 | 9 | 45 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a]The MIC value of piperacillin-tazobactam are expressed as MIC values of piperacillin with equal (4 µg/mL) concentration of tazobactam.

**Table 4.** Clinical characteristics and distribution of MIC and extended-spectrum β-lactamases (ESBL) genes among extreme drug resistant (XDR) *Salmonella enterica* serovar Typhi clinical isolates.

| No. | Gender | Age (Years) | Departments | MIC (µg/mL) of Beta-lactams | | | | | | | | ESBL genes | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | AMP | TZP | CRO | CTX | CFM | FEP | IMP | MEM | TEM | CTXMU | CTXM1 | CTXM15 |
| 1 | Female | 5 | ICU | 128 | 128 | 64 | 64 | 64 | 64 | 0.25 | 0.25 | + | + | + | - |
| 2 | Male | 3 | OPD | 256 | 256 | 64 | 128 | 64 | 64 | 0.25 | 0.5 | + | + | + | - |
| 3 | Female | 9 | OPD | 256 | 128 | 128 | 128 | 128 | 128 | 0.125 | 0.25 | + | + | + | + |
| 4 | Male | 4 | OPD | 128 | 128 | 64 | 64 | 64 | 32 | 0.5 | 0.5 | + | + | + | - |
| 5 | Female | 0.1 | Emergency | 256 | 256 | 128 | 64 | 64 | 128 | 0.25 | 0.25 | + | + | + | + |
| 6 | Male | 1 | Post-Operative | 256 | 256 | 128 | 128 | 128 | 64 | 0.5 | 0.5 | + | + | + | + |
| 7 | Female | 1 | ICU | 128 | 64 | 64 | 64 | 64 | 32 | 0.125 | 0.25 | + | + | + | - |
| 8 | Male | 1 | ICU | 256 | 256 | 128 | 128 | 128 | 128 | 0.5 | 0.5 | + | + | + | + |
| 9 | Female | 10 | Pediatric | 256 | 256 | 64 | 64 | 64 | 64 | 0.25 | 0.25 | + | + | + | + |
| 10 | Male | 2 | ICU | 256 | 128 | 128 | 128 | 64 | 128 | 0.25 | 0.25 | + | + | + | + |
| 11 | Male | 4 | ICU | 128 | 128 | 64 | 64 | 64 | 64 | 0.25 | 0.5 | + | + | + | - |
| 12 | Female | 2 | Pediatric | 256 | 64 | 128 | 128 | 64 | 64 | 0.5 | 0.5 | + | + | + | + |
| 13 | Male | 6 | OPD | 256 | 128 | 128 | 128 | 128 | 128 | 0.125 | 0.25 | + | + | + | + |
| 14 | Female | 2 | Emergency | 128 | 128 | 64 | 64 | 64 | 64 | 0.25 | 0.5 | + | + | + | - |
| 15 | Male | 13 | ICU | 128 | 64 | 64 | 64 | 64 | 64 | 0.25 | 0.5 | + | + | + | - |
| 16 | Male | 4 | Emergency | 256 | 128 | 128 | 128 | 128 | 128 | 0.25 | 0.25 | + | + | + | + |
| 17 | Male | 0.1 | Emergency | 256 | 128 | 128 | 64 | 64 | 128 | 0.25 | 0.25 | + | + | + | + |
| 18 | Female | 2 | Pediatric | 128 | 64 | 64 | 64 | 64 | 32 | 0.125 | 0.25 | + | + | + | - |
| 19 | Female | 7 | Emergency | 128 | 128 | 64 | 64 | 64 | 64 | 0.25 | 0.25 | + | + | + | - |
| 20 | Female | 2 | ICU | 128 | 128 | 64 | 64 | 64 | 32 | 0.125 | 0.25 | + | + | + | - |
| 21 | Female | 2 | Emergency | 128 | 128 | 64 | 64 | 64 | 32 | 0.125 | 0.25 | + | + | + | - |
| 22 | Female | 4 | ICU | 256 | 128 | 64 | 64 | 64 | 64 | 0.25 | 0.25 | + | + | + | + |
| 23 | Female | 3 | Emergency | 128 | 128 | 64 | 64 | 64 | 32 | 0.125 | 0.25 | + | + | + | - |
| 24 | Female | 10 | Emergency | 256 | 128 | 64 | 64 | 64 | 64 | 0.25 | 0.25 | + | + | + | + |
| 25 | Female | 6 | Emergency | 256 | 256 | 128 | 128 | 128 | 128 | 0.125 | 0.25 | + | + | + | + |
| 26 | Male | 3 | ICU | 256 | 128 | 64 | 128 | 64 | 64 | 0.25 | 0.5 | + | + | + | - |
| 27 | Male | 5 | ICU | 256 | 128 | 128 | 128 | 128 | 128 | 0.25 | 0.25 | + | + | + | + |
| 28 | Male | 4 | Pediatric | 256 | 128 | 128 | 128 | 128 | 64 | 0.5 | 0.5 | + | + | + | + |
| 29 | Male | 2 | Emergency | 256 | 256 | 128 | 128 | 128 | 64 | 0.5 | 0.5 | + | + | + | + |
| 30 | Male | 5 | Post-Operative | 256 | 128 | 128 | 128 | 128 | 128 | 0.25 | 0.25 | + | + | + | + |
| 31 | Male | 3 | Emergency | 256 | 128 | 128 | 128 | 128 | 64 | 0.5 | 0.5 | + | + | + | + |
| 32 | Male | 9 | Emergency | 128 | 128 | 64 | 64 | 64 | 64 | 0.25 | 0.5 | + | + | + | - |
| 33 | Female | 8 | Emergency | 256 | 128 | 64 | 64 | 64 | 64 | 0.25 | 0.25 | + | + | + | + |
| 34 | Male | 1 | ICU | 256 | 128 | 128 | 128 | 128 | 64 | 0.5 | 0.5 | + | + | + | + |
| 35 | Male | 0.3 | ICU | 256 | 128 | 128 | 64 | 64 | 128 | 0.25 | 0.25 | + | + | + | + |

Following the PCR amplification, 5 µL of each amplicon was separated by using 1.2% agarose gel electrophoresis in at 120 V in 1X TAE buffer for 35 minutes and visualized using UV-transilluminator. The PCR products were purified and sequenced from Macrogen Inc. (Seoul, South Korea) and were confirmed by using the NCBI BLAST tool.

## Results

In this study, a total of 82 *S.* Typhi isolates were included which were isolated from the children, belonging to multi cities of Punjab, Pakistan over a period of 6 months. Among the 82 isolates of *S.* Typhi, thirty-five isolates were classified as extensively drug-resistant (XDR) and confirmed for the presence of ESBL genes by genotypic methods.

The antimicrobial susceptibility testing has shown that all the *Salmonella* Typhi isolates were susceptible to carbapenems (imipenem & meropenem) and azithromycin. Among 82 isolates, only three isolates were susceptible to fluoroquinolone and sulfamethoxazole-trimethoprim (Table 2). Thirty-five isolates were resistant to the third-generation cephalosporins and were classified as XDR.

The MIC of ampicillin ranged between 2 µg/mL to ≥ 128 µg/mL. Overall 61 (74.39%) isolates were resistant to ampicillin (MIC ≥ 32 µg/mL), whereas all the *bla*TEM positive isolates had a MIC ranging between 64 µg/mL to ≥ 128 µg/mL. The isolates positive for both *bla*TEM and *bla*CTX-M had a MIC of ≥ 128 µg/mL for ampicillin (breakpoints; ≥ 32 µg/mL), ≥ 64 for ceftriaxone (breakpoints; ≥ 4 µg/mL), and ≥ 32 for cefepime (breakpoints; ≥ 16 µg/mL). The distribution of MIC for the beta-lactam agents is presented in Table 3.

To determine the diversity of ESBL enzymes among the *Salmonella* Typhi isolates, the *bla*TEM, *bla*SHV, and *bla*CTX-M genes were screened followed by screening for the *bla*CTX-M types using PCR. Overall a total of 42 (51%) isolates were *bla*TEM positive, whereas 35 isolates (42.6%) were positive for *bla*CTX-M genes. None of the 82 isolates harbored the *bla*SHV gene. Among the 35 XDR strains of *S.* Typhi, a *bla*SHV was completely absent whereas the *bla*TEM and *bla*CTX-M were present in all the 35 strains. The further evaluation of *bla*CTX-M types showed that *bla*CTX-M-1 was present in all these 35 strains while 21 strains were positive for *bla*CTX-M-15. The *bla*CTX-M-2, *bla*CTX-M-8, *bla*CTX-M-9, *bla*CTX-M-10, and *bla*CTX-M-14 was not found in any of the isolates. The distribution of the ESBL genes among the XDR *Salmonella* Typhi strains and the distribution of MIC to various beta-lactam drugs is shown in Table 4.

## Discussion

The typhoid fever is a critical ongoing health issue as the World Health Organization (WHO) has suggested that there were 27 million cases of typhoid fever in 2010 [12]. The issue of endemicity of typhoid caused by an extensively drug-resistant (XDR) strains foretells the possibility that soon the treatment of typhoid will be practically and economically difficult especially in developing countries that might result in a situation like the pre-1948 era when typhoid fever was an untreatable disease with higher mortality [13].

In Pakistan, the flare-up of XDR *S.* Typhi was reported initially in Hyderabad, Sindh in 2016. This up-surge further spread to other parts of the province and Karachi; the largest city of the country was vastly affected. It is an unfortunate and astonishing condition that few cases of XDR *S.* Typhi were identified in the United Kingdom and it seems that all the diagnosed cases have a travel history to Pakistan and/or Asian countries. The origin or development of these XDR strains in Pakistan is uncertain and might be attributed to the hampered health care system in Pakistan which contributed to the development of these XDR phenotypes [4].

In this study, we reported the appearance of XDR *S.* Typhi isolates from Punjab (population-wise largest province), Pakistan which is non-susceptible to the ampicillin, third-generation cephalosporins, fluoroquinolones and trimethoprim-sulfamethoxazole that might be linked to the previously reported isolates from the region of Pakistan as well as the United Kingdom [4,10]. The treatment of typhoid fever was dependent on the first-line antimicrobials agents including ampicillin, co-trimoxazole, and chloramphenicol till the early 1990s. However, the extensive therapeutic implications of these drugs resulted in the development of resistant strain for all three first-line treatment options and were termed as MDR *S.* Typhi [14].

The fluoroquinolones were endorsed as an option for typhoid during the late 1990s consequent to the emergence of MDR strains which could be orally administered and were found highly effective with minimal side effects; although the use was initially restricted in children due to the possible adverse effects on the growth of long bones [15]. Fluoroquinolone resistance among *S.* Typhi isolates is now widespread in Asian countries and in Africa approximately 10% isolates are reported to develop resistance to the

fluoroquinolones as a result of mutations in the *gyrA* gene [16,17]. The accumulation of mutations in the quinolone-resistant determining region (*gyrA* and *parC*) is resulting in a gradual increase of the MIC of ciprofloxacin for *S.* Typhi strains. The ciprofloxacin susceptible strains having a MIC ≤ 0.06 μg/mL are widely known to acquire a single mutation in *gyrA* gene (S83F) with an increase in MIC values whereas the further mutations in *gyrA* and *parC* can cause an increase in the MIC values (≥ 4 μg/mL) [3].

The azithromycin as well as third-generation cephalosporins have been preferred choices for the therapeutic management of typhoid due to the emergence of MDR phenotypes and fluoroquinolone resistance and mainly because of their broader spectrum as well the choice of oral or intravenous use. However, the cephalosporin-resistant *S.* Typhi isolates have been reported in past few years especially in South Asian countries with the sporadic case from Pakistan and India and the outbreak of typhoid being reported from Karachi (Sindh province), Pakistan [4, 18-20]. The third-generation cephalosporins, for instance, cefixime and ceftriaxone are currently the drug of choice for the treatment of enteric fever in South Asian countries and are generally usually for empirical therapy which is likely to drive the cephalosporin resistance among *S.* Typhi and many other Gram-negative bacteria [14]. The literature has proposed the term "extensively drug-resistant" (XDR) for the S. Typhi isolates which are resistant to five antimicrobial agents consistent with the nomenclature recommended for other pathogenic bacterial species [4,14]. The sporadic cases of cephalosporin-resistant have been reported in few studies and of which few individual cases have found XDR *S.* Typhi isolates which were resistant to cephalosporins and fluoroquinolone in addition to the MDR phenotypes [5,7,20-22].

So far, the resistance to third-generation cephalosporins was mainly mediated by the extended-spectrum β-lactamases (ESBLs) among *S.* Typhi isolates from Pakistan and India [18,20]. The Indian isolates were reported to carry IncA and IncX3 plasmids and harboring *bla*SHV-12, *bla*CMY-2, *bla*DHA-1 and *bla*TEM-1B determinants [18,19]. An *S.* Typhi isolates encoding *blaC*TX-M15 gene on an IncY plasmid was recovered from the Democratic Republic of Congo [9]. Few other studies have reported the *bla*CTX-M producing *S.* Typhi isolates from Nigeria, Japan, and Southern India as well as from the travelers having a travel history to Iraq and Guatemala [5,23-26]. More recently, *S.* Typhi strains harboring the *bla*CTX-M15 gene were isolated from Pakistan that was resistant to third-generation cephalosporin and fluoroquinolones as well as MDR phenotypes therefore and has been categorized as extensively drug-resistant (XDR). All the XDR isolates had a composite transposon that was located on the plasmid (IncHI1) or the chromosome and an additional IncY plasmid containing *bla*CTX-M15 and *qnrS* genes [4]. The isolates from Iraq and Bangladesh were found to harbor *bla*CTX-M genes, and the Iraqi isolate was found to have an IncN plasmid whereas the isolates from Pakistan were having the *bla*CTX-M gene and the IncY plasmid [4]. A similar plasmid was identified from the draft genome of a single isolate from Rawalpindi, Pakistan as identified in the isolate from Sindh, Pakistan [4,22].

The high rates of bacteremic typhoid fever are suggested in children especially among the school-age children. The present study has highlighted the occurrence of XDR *Salmonella* Typhi from the toddlers and young infants which suggests the exigency of prevention strategies including immunization plans, especially in the impoverished school-age children. The data related to antimicrobial resistance and surveillance of resistant strains is imperative for the assistance of clinicians for the treatment of infected patients. Although, the XDR isolates in the present study were fully susceptible to azithromycin the strains non-susceptible to azithromycin have been reported in some Asian countries [27,28]. It seems that the increasingly common use of azithromycin for the treatment of these XDR strains will accelerate the emergence of azithromycin-resistant strains as well in the near future.

The incidence rates in some countries of Asia and Africa indicate the critical gaps in knowledge and surveillance system in these regions [29,30]. The evidence-based approaches are essential to bridge the gaps and to screen the broader regions in these continents. The notional replacement of orally administered antibiotics by the parenterally administered expensive antimicrobial agents such as carbapenems and tigecycline would be prohibitive for the treatment of typhoid fever cases in the developing countries owing to the higher cost. The XDR cases from Punjab, Pakistan highlight the emergence of widespread cephalosporins resistance among *S.* Typhi strains, which necessitates the implication of an urgent action plan before such strains become the usual phenotypes which will ultimately lead to treatment failure for typhoid fever with the available antimicrobial agents.

## Conclusion

Despite the higher prevalence and emergence of XDR cases, the surveillance of typhoid is weak in the Asian countries, and the vaccination plan is inadequate. Moreover, the progress toward the prevention and control of typhoid fever especially in the children will involve the commitment at a national level as well as the international support to enhance the surveillance, better use of vaccines, implementation of routine immunization programs, and provision of safe water, sanitation facilities, and hygiene measures.

### Ethical approval

The study was ethically approved by the "Ethical Review Board" under reference number 47/ERB from Allama Iqbal Medical College, Jinnah Hospital Lahore, Pakistan.

### References


1. Mweu E, English M (2008) Typhoid fever in children in Africa. Trop Med Int Health 13: 532-540.
2. Yang YA, Chong A, Song J (2018) Why is eradicating typhoid fever so challenging: Implications for vaccine and therapeutic design. Vaccines 6: 45.
3. Cuypers WL, Jacobs J, Wong V, Klemm EJ, Deborggraeve S, Van Puyvelde S (2018) Fluoroquinolone resistance in Salmonella: insights by whole-genome sequencing. Microb Genom 4: e000195.
4. Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, Wong VK, Dallman TJ, Nair S, Baker S, Shaheen G, Qureshi S, Yousafzai MT, Saleem MK, Hasan Z, Dougan G, Hasan R (2018) Emergence of an extensively drugresistant Salmonella enterica Serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. mBio 9: e00105-18.
5. Pfeifer Y, Matten J, Rabsch W (2009) Salmonella enterica serovar Typhi with CTX-M beta-lactamase, Germany. Emerg Infect Dis 15: 1533-1535.
6. Rotimi VO, Jamal W, Pal T, Sovenned A, Albert MJ (2008) Emergence of CTX-M-15 type extended-spectrum beta-lactamase-producing Salmonella spp. in Kuwait and the United Arab Emirates. J Med Microbiol 57: 881-886.
7. Ahmed D, Hoque A, Mazumder R, Nahar K, Islam N, Gazi SA, Hossain MA (2012) Salmonella enterica serovar Typhi strain producing extended-spectrum beta-lactamases in Dhaka, Bangladesh. J Med Microbiol 61: 1032-1033.
8. Al Naiemi N, Zwart B, Rijnsburger MC, Roosendaal R, Debets-Ossenkopp YJ, Mulder JA, Fijen CA, Maten W, Vandenbroucke-Grauls CM, Savelkoul PH (2008) Extended-spectrum-beta-lactamase production in a Salmonella enterica serotype Typhi strain from the Philippines. J Clin Microbiol 46: 2794-2795.
9. Phoba MF, Barbe B, Lunguya O, Masendu L, Lulengwa D, Dougan G, Wong VK, Bertrand S, Ceyssens PJ, Jacobs J, Van Puyvelde S, Deborggraeve S (2017) Salmonella enterica serovar Typhi producing CTX-M-15 extended spectrum beta-lactamase in the Democratic Republic of the Congo. Clin Infect Dis 65: 1229-1231.
10. Qamar FN, Azmatullah A, Kazi AM, Khan E, Zaidi AK (2014) A three-year review of antimicrobial resistance of Salmonella enterica serovars Typhi and Paratyphi A in Pakistan. J Infect Dev Ctries 8: 981-986. doi: 10.3855/jidc.3817.
11. Clinical and Laboratory Standard Institute (CLSI) (2018) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Eighth Informational Supplement. CLSI document M100 (ISBN 1-56238-839-8).
12. Date KA, Bentsi-Enchill AD, Fox KK, Abeysinghe N, Mintz ED, Khan MI, Sahastrabuddhe S, Hyde TB (2014) Typhoid fever surveillance and vaccine use - South-East Asia and Western Pacific regions, 2009-2013. MMWR Morb Mortal Wkly Rep 63: 855-860.
13. Levine MM, Simon R (2018) The Gathering Storm: Is Untreatable Typhoid Fever on the Way? mBio 9: e00482-18.
14. Britto CD, Wong VK, Dougan G, Pollard AJ (2018) A systematic review of antimicrobial resistance in Salmonella enterica serovar Typhi, the etiological agent of typhoid. PLoS Negl Trop Dis 12: e0006779.
15. Choi SH, Kim EY, Kim YJ (2013) Systemic use of fluoroquinolone in children. Korean J Pediatr 56: 196-201.
16. Al-Emran HM, Eibach D, Krumkamp R, Ali M, Baker S, Biggs HM, Bjerregaard-Andersen M, Breiman RF, Clemens JD, Crump JA, Cruz Espinoza LM, Deerin J, Dekker DM, Gassama Sow A, Hertz JT, Im J, Ibrango S, von Kalckreuth V, Kabore LP, Konings F, Lofberg SV, Meyer CG, Mintz ED, Montgomery JM, Olack B, Pak GD, Panzner U, Park SE, Razafindrabe JL, Rabezanahary H, Rakotondrainiarivelo JP, Rakotozandrindrainy R, Raminosoa TM, Schutt-Gerowitt H, Sampo E, Soura AB, Tall A, Warren M, Wierzba TF, May J, Marks F (2016) A multicountry molecular analysis of Salmonella enterica Serovar Typhi with reduced susceptibility to ciprofloxacin in Sub-Saharan Africa. Clin Infect Dis 62 Suppl 1: 42-46.
17. Tadesse G, Tessema TS, Beyene G, Aseffa A (2018) Molecular epidemiology of fluoroquinolone resistant Salmonella in Africa: A systematic review and meta-analysis. PLoS One 13: e0192575.
18. Rodrigues C, Kapil A, Sharma A, Devanga Ragupathi NK, Inbanathan FY, Veeraraghavan B, Kang G (2017) Whole-genome shotgun sequencing of cephalosporin-resistant Salmonella enterica Serovar Typhi. Genome Announc 5: e01639-16.
19. Devanga Ragupathi NK, Muthuirulandi Sethuvel DP, Shankar BA, Munusamy E, Anandan S, Veeraraghavan B (2016) Draft genome sequence of blaTEM-1-mediated cephalosporin-resistant Salmonella enterica serovar Typhi from bloodstream infection. J Glob Antimicrob Resist 7: 11-12.
20. Munir T, Lodhi M, Ansari JK, Andleeb S, Ahmed M (2016) Extended spectrum betalactamase producing cephalosporin resistant Salmonella Typhi, reported from Rawalpindi, Pakistan. J Pak Med Assoc 66: 1035-1036.
21. Kleine CE, Schlabe S, Hischebeth GTR, Molitor E, Pfeifer Y, Wasmuth JC, Spengler U (2017) Successful therapy of a multidrug-resistant extended-spectrum beta-lactamase-producing and fluoroquinolone-resistant Salmonella enterica subspecies enterica Serovar Typhi infection using combination therapy of meropenem and fosfomycin. Clin Infect Dis 65: 1754-1756.
22. Gul D, Potter RF, Riaz H, Ashraf ST, Wallace MA, Munir T, Ali A, Burnham CA, Dantas G, Andleeb S (2017) Draft genome sequence of a Salmonella enterica Serovar Typhi

strain resistant to fourth-generation cephalosporin and fluoroquinolone antibiotics. Genome Announc 5: e00850-17.
23. Ramachandran A, Shanthi M, Sekar U (2017) Detection of blaCTX-M extended spectrum beta-lactamase producing Salmonella enterica Serotype Typhi in a tertiary care centre. J Clin Diagn Res 11: 21-24.
24. Akinyemi KO, Iwalokun BA, Alafe OO, Mudashiru SA, Fakorede C (2015) bla CTX-M-I group extended spectrum beta lactamase-producing Salmonella typhi from hospitalized patients in Lagos, Nigeria. Infect Drug Resist 8: 99-106.
25. Morita M, Takai N, Terajima J, Watanabe H, Kurokawa M, Sagara H, Ohnishi K, Izumiya H (2010) Plasmid-mediated resistance to cephalosporins in Salmonella enterica serovar Typhi. Antimicrob Agents Chemother 54: 3991-3992.
26. Gonzalez-Lopez JJ, Piedra-Carrasco N, Salvador F, Rodriguez V, Sanchez-Montalva A, Planes AM, Molina I, Larrosa MN (2014) ESBL-producing Salmonella enterica serovar Typhi in traveler returning from Guatemala to Spain. Emerg Infect Dis 20: 1918-1920.
27. Parry CM, Thieu NT, Dolecek C, Karkey A, Gupta R, Turner P, Dance D, Maude RR, Ha V, Tran CN, Thi PL, Be BP, Phi LT, Ngoc RN, Ghose A, Dongol S, Campbell JI, Thanh DP, Thanh TH, Moore CE, Sona S, Gaind R, Deb M, Anh HV, Van SN, Tinh HT, Day NP, Dondorp A, Thwaites G, Faiz MA, Phetsouvanh R, Newton P, Basnyat B, Farrar JJ, Baker S (2015) Clinically and microbiologically derived azithromycin susceptibility breakpoints for Salmonella enterica serovars Typhi and Paratyphi A. Antimicrob Agents Chemother 59: 2756-2764.
28. Patel SR, Bharti S, Pratap CB, Nath G (2017) Drug resistance pattern in the recent isolates of Salmonella Typhi with special reference to cephalosporins and azithromycin in the Gangetic Plain. J Clin Diagn Res 11: 1-3.
29. Breiman RF, Cosmas L, Njuguna H, Audi A, Olack B, Ochieng JB, Wamola N, Bigogo GM, Awiti G, Tabu CW, Burke H, Williamson J, Oundo JO, Mintz ED, Feikin DR (2012) Population-based incidence of typhoid fever in an urban informal settlement and a rural area in Kenya: implications for typhoid vaccine use in Africa. PLoS One 7: e29119.
30. Lee JS, Mogasale VV, Mogasale V, Lee K (2016) Geographical distribution of typhoid risk factors in low and middle income countries. BMC Infect Dis 16: 732.

## Corresponding author

Mohsin Khurshid, PhD
Assistant Professor
Department of Microbiology, Liaquat Block, Government College University Faisalabad, New Campus, Jhang Road, Faisalabad-38000, Pakistan
Phone: +923334301513
Fax: +92-41-9203023
Email: mohsin.mic@gmail.com, mohsinkhurshid@gcuf.edu.pk

**Conflict of interests:** No conflict of interests is declared.