Extended-spectrum beta-lactamases producing extensively drug-resistant \textit{Salmonella} Typhi in Punjab, Pakistan

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Abstract

Introduction: The multidrug-resistant (MDR) \textit{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) \textit{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. \textit{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 \textit{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. \textit{bla}TEM and \textit{bla}CTX-M genes.

Conclusions: The higher prevalence of ESBL producing \textit{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: \textit{Salmonella}; cephalosporins; ESBL; typhoid.

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Introduction

The occurrence of Typhoid fever is alarmingly high in developing countries with approximately 200,000 deaths each year [1]. \textit{S.} Typhi is transmitted by a fecal-oral route usually by the consumption of contaminated water. the emergence of multidrug-resistant (MDR) \textit{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 \textit{S.} Typhi have also been reported recently [4].

The extended-spectrum \beta-lactamase (ESBL) producing \textit{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 \textit{Salmonella enterica} serovar Typhi strains and this transfer is facilitated especially if these resistant determinates are associated with transposon or plasmids, for example, \textit{bla}CTXM-15 [9].

The surveillance data from Karachi, Pakistan demonstrated an increase in the percentage of MDR \textit{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

| Target gene | Primer name | Primer sequence (5’-3’ | Amplicon (bp) | Annealing Temperature (°C) |
|-------------|-------------|------------------------|---------------|---------------------------|
| *fliC*      | H-For       | ACTCAGGCTTCCCGTAGACGC  | 763           | 50                        |
|             | Hd-Rev      | GGCTAGTATTGCTCTTATCGG  |               |                           |
|             | TEM-F       | TCAACATTCTCCGTGTCG     | 860           | 56                        |
|             | TEM-R       | CTGACAGTACAAATGCTTA    |               |                           |
|             | SHV-F       | ATGCGTTGATTTCCGCTGTL   | 896           | 56                        |
|             | SHV-R       | AGATAAACACACAAATGCAC   |               |                           |
| *blaTEM*    | CTXMU-F     | ATGTCGAGYACGACTAAARGT  | 593           | 56                        |
|             | CTXMU-R     | TGGTGRAARTARGTSACCAGA  |               |                           |
|             | CTXM1-F     | CGGTTCGCTATTACAAACGTTG | 944           | 56                        |
|             | CTXM1-R     | GGCCCATGGTTAAGAATCCTG  |               |                           |
|             | CTMX2-F     | ATGTGACTACACAGCATTCC   | 833           | 56                        |
|             | CTMX2-R     | TCCGAGGGCTTTCCGCGT    |               |                           |
|             | CTXM8-F     | TTTCCGCAGTGGATTGG      | 368           | 50                        |
|             | CTMX8-R     | CGATTTCTGGCCTTGCCTC    |               |                           |
|             | CTMX9-F     | ATGTTGAACAAAGAGATGCA   | 870           | 50                        |
|             | CTMX9-R     | CCCCACGGCGTATGTT       |               |                           |
| *blaCTXM*   | CTMX10-F    | GACGACCACTAAAGATGATG   | 524           | 56                        |
|             | CTMX10-R    | GCGATATCGTGTTGATG      |               |                           |
| *blaCTXM9*  | CTMX14-F    | GAGATGCAACCGATTGATG    | 941           | 56                        |
|             | CTMX14-R    | TCCAGGCTGGTAAATAG      |               |                           |
|             | CTMX15-F    | CACACGTTGAAATATTGATGAC | 996           | 55                        |
|             | CTMX15-R    | GCCGTCTAGCGGATAAAC     |               |                           |
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 *blaTEM, blaSHV* and *blaCTX-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 *blaCTX-M* positive isolates were further characterized for the occurrence of *blaCTX-M* types including *blaCTX-M-1, blaCTX-M-2, blaCTX-M-8, blaCTX-M-9, blaCTX-M-10, blaCTX-M-14*, and *blaCTX-M-15* using species primers as listed in Table 1.

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**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) | Breakpoints (µg/mL) | No. of isolates from which the MIC (µg/mL) were | 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                  |                     |                                               |                                               |
| Piperacillin-tazobactam | 128/4              |                     |                                               |                                               |
| Ceftriaxone              | ≥ 4                 |                     |                                               |                                               |
| Cefotaxime               | ≥ 4                 |                     |                                               |                                               |
| Cefixime                 | ≥ 4                 |                     |                                               |                                               |
| Ceftazidime              | ≥ 16                |                     |                                               |                                               |
| Imipenem                 | ≥ 4                 |                     |                                               |                                               |
| Meropenem                | ≥ 4                 |                     |                                               |                                               |

*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 |
|-----|--------|-------------|-------------|-----------------------------|------------|
| 1   | Female | 5           | ICU         | 128 128 64 64 64 64 0.25 0.25 | + + + -   |
| 2   | Male   | 3           | OPD         | 256 256 64 128 64 64 64 0.25 0.25 | + + + + - |
| 3   | Female | 9           | OPD         | 256 128 128 128 64 128 0.125 0.25 + + + + |
| 4   | Male   | 4           | OPD         | 256 128 64 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 64 0.25 0.25 | + + + + - |
| 10  | Male   | 2           | ICU         | 256 128 128 128 64 128 0.25 0.25 + + + + - |
| 11  | Male   | 4           | ICU         | 256 128 64 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 64 0.25 0.5 | + + + -   |
| 15  | Male   | 13          | ICU         | 128 64 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 64 32 0.125 0.25 | + + + -   |
| 19  | Female | 7           | Emergency   | 128 128 64 64 64 64 64 0.25 0.25 | + + + -   |
| 20  | Female | 2           | ICU         | 128 128 64 64 64 64 32 0.125 0.25 | + + + -   |
| 21  | Female | 2           | Emergency   | 128 128 64 64 64 64 32 0.125 0.25 | + + + -   |
| 22  | Female | 4           | ICU         | 256 128 64 64 64 64 64 0.25 0.25 | + + + + |
| 23  | Female | 3           | Emergency   | 128 128 64 64 64 64 32 0.125 0.25 | + + + -   |
| 24  | Female | 10          | Emergency   | 256 128 64 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         | 256 128 128 128 64 128 0.25 0.25 | + + + -   |
| 27  | Male   | 5           | ICU         | 256 128 128 128 128 128 0.25 0.25 | + + + + |
| 28  | Male   | 4           | Pediatric   | 256 128 128 128 64 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 64 0.25 0.25 | + + + + |
| 33  | Female | 8           | Emergency   | 256 128 128 128 64 64 0.25 0.25 | + + + + |
| 34  | Male   | 1           | ICU         | 256 128 128 128 64 64 0.5 0.5 | + + + + |
| 35  | Male   | 0.3         | ICU         | 256 128 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 blaTEM positive isolates had a MIC ranging between 64 µg/mL to ≥ 128 µg/mL. The isolates positive for both blaTEM and blaCTX-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 blaTEM, blaSHV, and blaCTX-M genes were screened followed by screening for the blaCTX-M types using PCR. Overall a total of 42 (51%) isolates were blaTEM positive, whereas 35 isolates (42.6%) were positive for blaCTX-M genes. None of the 82 isolates harbored the blaSHV gene. Among the 35 XDR strains of S. Typhi, a blaSHV was completely absent whereas the blaTEM and blaCTX-M were present in all the 35 strains. The further evaluation of blaCTX-M types showed that blaCTX-M-1 was present in all these 35 strains while 21 strains were positive for blaCTX-M-15. The blaCTX-M-2, blaCTX-M-8, blaCTX-M-9, blaCTX-M-10, and blaCTX-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 upsurge 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
flouroquinolones 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 blaSHV-12, blaCMY-2, bladHA-1 and bblaTEM-1B determinants [18,19]. An *S. Typhi* isolates encoding blaCTX-M15 gene on an IncY plasmid was recovered from the Democratic Republic of Congo [9]. Few other studies have reported the blaCTX-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 blaCTX-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 blaCTX-M15 and qnrS genes [4]. The isolates from Iraq and Bangladesh were found to harbor blaCTX-M genes, and the Iraqi isolate was found to have an IncN plasmid whereas the isolates from Pakistan were having the blaCTX-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 bactereic 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.

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