ORIGINAL ARTICLE

Prevalence of Multi-Drug Resistant and Extensively Drug-Resistant *Salmonella enterica* Serovar Typhi Recovered from Pediatrics' Septicemia Patients in Lahore

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ABSTRACT

Background: Increasing antimicrobial resistance to enteric pathogens like *Salmonella*Typhi is an important public health issue.
Aim: To report the prevalence of MDR and XDR *S.*Typhi in the pediatrics population.
Methods: This retrospective study on blood cultures provided an insight into the frequency and drug susceptibility of *S.*Typhi at Fatima Memorial Hospital, Lahore. Blood samples (1-4ml) were drawn from suspected septicemia children's patients (n=2575) aseptically. Blood was inoculated in BacT/Alert peads bottles and processed in an automated BacT/Alert system. Positive samples were further sub-cultured on blood and MacConkey agar. Isolates confirmation and antibiogram were performed by VITEK 2 compact system.
Results: Out of 2575, 292 (11.3%) blood cultures yielded bacterial growth. Among these, *S.* Typhi(170; 58.2%) and *Salmonella paratyphi*A(12; 4.1%) were isolated. *S.*Typhi(43%) was mostly isolated in the age group of 1-5 years. The majority of the *S.* Typhi and *S.paratyphi* A were isolated from peads OPD (55; 30.1%) and peads emergency wards (49; 26.7%). Overall, 23.5% and 54% were MDR and XDR *S.* Typhi respectively, and were sensitive to azithromycin and carbapenems. *S.* Typhi displayed resistance to chloramphenicol (72%), ampicillin (78%), co-trimoxazole (78%), ciprofloxacin (59%), and ceftriaxone (56%).
Conclusion: A strict surveillance plan is mandatory for the containment of the MDR and XDR spread. Public awareness, improved sanitation, safe water supply, and vaccination can play a major role in the prevention of outbreaks.
Keywords: Children, Septicemia, MIC, XDR, *Salmonella*Typhi

INTRODUCTION

Antimicrobial resistance (AMR) is becoming a serious concern around the globe. By 2050, one person will die after every three seconds due to AMR pathogens[1-3]. *Salmonella* is listed as antibiotic resistance 'priority pathogen' by the World Health Organization (WHO). Typhoid fever caused by the host-restricted bacterial pathogen;*Salmonella enterica* subsp. enterica serovar Typhi (*S.* Typhi) represents a potential threat to public health in low- and middle-income countries such as Pakistan[4]. Typhoid infections are estimated to cause more than 200,000 deaths annually around the world[5].

According to the WHO, typhoid is a major public health issue and has the greatest impact on children and young adults[6]. Pakistan has the third-highest typhoid rate among the global population and the fourth most common cause of death. The incidence of typhoid fever among children between 2-5 years was 573.2 per 100,000 people per year in Pakistan[7]. The infection caused by *S.* Typhi is critical for treatment because of the acquisition of resistant genes primarily mediated by transmissible plasmids. Multi-resistant *S.* Typhi (MDR-*S.*Typhi*)*was identified between 1970-1980, which showed resistance to first-line drugs (ampicillin, chloramphenicol, co-trimoxazole) and were susceptible to quinolones[8]. However, the Aga Khan University in Pakistan has documented that during the period 2001-2006, the incidence of MDR-*S.* Typhiincreased from 34.2% to 48.5% and resistance to ciprofloxacin also raised from 1.6% to 64.1%[9]. As a result, a third-generation cephalosporin (ceftriaxone) has beenwidely practiced to treat infections caused by such pathogens. But in 2016, the first case of extensively drug-resistant *S*. Typhi (XDR-*S.* Typhi) was reported in Sindh Province, Pakistan.

The *S*. Typhi (H58 strains) were resistant to all first-line antibiotics, ciprofloxacin, and ceftriaxone, but were sensitive to azithromycin, imipenem, and meropenem[10]. A survey in Pakistan found 69% of XDR-*S.* Typhifrom Karachi and 27% from Hyderabad[11]. Consequently, there is a significant risk of a sporadic rise in XDR-*S.* Typhi in other cities of Pakistan.So, this study was aimed to determine the prevalence of MDR and XDR-*S.* Typhi in Lahore, Pakistan.

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MATERIALS AND METHODS

Ethical approval was obtained from the Institutional Review Board, Fatima Memorial Hospital, Lahore. Blood samples (n=2575) were collected aseptically from suspected pediatric sepsis patients in BacT/Alert PF blood culture bottles between September 2018 and February 2019. Blood culture bottles were placed in the Bact/Alert automatic blood culture system (BioMerieux, France) for up to five days. Each patient's demographic data such as age, gender was also considered in this study.

All positive blood cultures were then subcultured on Blood and MacConkey agars and incubated at 37°C for 18-24 hours. Preliminary identification of isolates was made by colony morphology, cultural characteristic, and Gram staining. Also, the biochemical confirmation of bacteria was made by GN cards in the compact VITEK 2 system (BioMerieux, France).

The serotypes of Salmonella were determined by an agglutinating antiserum (Bio-Rad, Hercules, California, USA) based on the Kauffmann and White scheme. Briefly, a small drop of antiserum (20µL) was added to a glass slide and mixed with the Salmonella colony. After 5 - 10 seconds, a positive reaction was considered as a visible agglutination, whereas a negative reaction was considered homogenous milky turbidity (Kauffmann-White scheme).

MIC (µg/mL) was determined against different antibiotics (Ampicillin, Ceftriaxone, Chloramphenicol, Ciprofloxacin, Amoxicillin/Clavulanic Acid, Imipenem, Meropenem, Trimethoprim/Sulfamethoxazole) using AST cards by VITEK 2 compact system (BioMerieux, France). Manual antimicrobial susceptibility testing was performed for azithromycin (15 µg)(Oxoid, UK) using the Kirby–Bauer disc diffusion method because they were unavailable in the sensitivity panel for the Gram-negative in VITEK 2 compact system. The zones' sizes were interpreted using CLSI 2018 guidelines.

RESULTS

Of the 2,575 blood cultures, 292(11.3%) resulted in clinically important pathogens. Of that number, 182/292 (62.3%) were *Salmonella spp*., Among these, 170(93%) isolates were identified as *S.* Typhi and 12(7%) were *S.paratyphi* A. Serotypes were also confirmed for *S. Typhi* O9, 12, and *S*. paratyphi O1, 2, 12. The highest number of Salmonella (158; 87%) was found in pediatrics' age groups between 1 - 15 years of age compared to adults where incidence was observed (24;13%). Among children, the highest number of *Salmonella* enterica was isolated from age groups between1 - 5 years (78; 43%) and 6 to 10 years (58; 32%) (Table-I).

Table-I: Age-wise distribution of *S. Typhi*and *S.paratyphi A* in blood samples

Age Ranges	**n; %**
< 1 Year	4 ; 2%
1 - 5 years	78 ; 43%
6 -10 years	58 ; 32%
11 -15 years	18 ; 10%
16 -20 years	6 ; 3%
21-25 years	6 ; 3%
> 25 years	12 ; 7%

Most cases of *S.* Typhi and *paratyphi* A were isolated from peads OPD wards (55; 30.1%), followed by peads emergency (49; 26.7%), peads surgical wards (32; 17.6%), peads medical wards (31; 17%), surgical intensive care unit (ICU) (15; 8%) and peads ICU (11; 6%).

Antimicrobial susceptibility testing revealed that 72%, 78%, and 78% of *S.* Typhi were resistant to chloramphenicol, ampicillin, and co-trimoxazole, respectively. Resistance to ciprofloxacin was 59% and ceftriaxone 56%. All isolates tested for azithromycin were susceptible. *S*. *paratyphi* A was 100% sensitive to all first- and second-line drugs analyzed (Table-II).

Table-II: Percent resistant pattern of *S.* Typhi and *S. paratyphi* A

Isolates	**AMP**	**SXT**	**C**	**CIP**	**CRO**	**AMC**	**IMP**	**MEM**	**AZT**
Salmonella Typhi (n=170)	78%	78%	72%	59%	56%	53%	2%	1%	0%
Salmonella paratyphi A (n=12)	0%	0%	0%	0%	0%	0%	0%	0%	0%

AMP; Ampicillin, SXT; Co-trimoxazole, C; Chloramphenicol, CIP; Ciprofloxacin, CRO; Ceftriaxone, AMC; Amoxicillin/clavulanic acid, IMP; Imipenem, MEM; Meropenem, AZT; Azithromycin

DISCUSSION

Enteric fever is a major public health concern globally. It is a significant burden on morbidity and mortality, particularly in developing countries. The increase in MDR and emerging strains of XDR in Pakistan has created an alarming situation concerning antibiotic resistance[12]. There are few studies available on the XDR *S*. Typhi in Lahore. Therefore, the purpose of this study was to determine the regional antibiotic susceptibility profile of *S.* Typhi in Lahore. In this study,182 (62.3%) *S.*Typhiisolates were identified from 292 positive blood cultures over a short six-month period, indicating a higher incidence of typhoid fever than in recent years. Various studies documented*S.*Typhi as the most common serotype isolated over the past decade and similar results were observed in our study (*S.* Typhi =93% and *S. paratyphi* A=7%)[13-15].

As the literature indicatedthe highest incidence of typhoid in children under the age of 15 years, 87% of this study population also came from the pediatric age group. In our study, the incidence of MDR *S.* Typhiwas 23.5%, while another study carried out by Ali *et al*. also showed similar results at a rate of 37.5%[16]. The cause of MDR may be attributed to the misuse of over-the-counteravailable antimicrobials and their uncontrolled use without appropriate medical supervision. In terms of XDR *S.* Typhi,the frequency was 54% in our study, while another study conducted by Hussain *et al*. showed a similar result with 48%[17]. Even though antibiotics are life-saving medications, their excessive use may limit the effectiveness of this antimicrobial agents[18]. A resurgence of MDR *S*. Typhi has been observed on this subcontinent. Almost all first-line antibiotics showed complete resistance in many instances. In this study, resistance to ampicillin, chloramphenicol, and co-trimoxazole was 78%, 72%, and 78%, respectively. The study by Lin-Hui Su *et al.* showed upward trends in resistance to ampicillin (91%), chloramphenicol (91%), and co-trimoxazole (88%). Ceftriaxone and ciprofloxacin were considered to be the main pillars of typhoid therapy, but we also found high resistance to these antibiotics. Resistance to ciprofloxacin was observed in 59% of isolates, but increased resistance to ciprofloxacin from 1% to 69% was observed in a tertiary care hospital in Taiwan[19].

Another study from Pakistan reported 69% XDR *S.* Typhi in Karachi and 27% in Hyderabad, Sindh[11]. Our study has depicted worrisome data of these MDR and XDR strains in Punjab province. 71% of the isolates in the study were MDR strains and 54% were XDR strains of *S.* Typhi. This means that, like Sindh, Punjab is also facing a frightening increase in resistant strains of *Salmonella*. A recent study conducted in India by Makkar A *et al.* found 2.73% and 1.91% multi-resistant *S.* Typhiand paratyphi respectively which is significantly less than our study[20]. Another study by Florian et al. predicted a higher incidence of MDR *S*. Typhi in Ghana, Kenya, and Tanzania[21]. Azithromycin is one of the few remaining treatment options to deal with these superbugs. A study by Misra R *et al.* revealed high susceptibility of 92% and 92.6% in two different Indian cities[22]. Another study by Divyashree *et al.* also reported 93.6% susceptibility to azithromycin[23]. However, all isolates analyzed in this study were sensitive to azithromycin. As azithromycin is an effective oral option available for typhoid fever, its broad use in a variety of other clinical conditions should be minimized to prevent the potential emergence of resistant strains.

CONCLUSION

Our study indicated that children under the age of 5 years were primarily impacted by XDR *S.*Typhi. Meropenem and azithromycin, alone or in combination, were the most efficient antibiotics for the treatment of XDR *S.* Typhi. Generalized antibiotic stewardship programs need to be conducted and a strict surveillance plan is required to control this spread and formulate an antibiotic stewardship policy. Public awareness, improved sanitation, drinking water supply, and mass vaccination can play an important role in the prevention of epidemics.

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