Sensitivity Pattern of *Salmonella Typhi* from Blood Culture in Paediatric Population

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Abstract

Background: The bacterium *Salmonella enterica serovar typhi* causes typhoid fever which is a life-threatening systemic infection that mainly occurs in developing countries of the world and remains a major public health issue. Paratyphoid fever is caused by *Salmonella enterica serovars Paratyphi A and B* and (infrequently *C*). Appropriate and immediate antimicrobial therapy is required for the prevention of complications and mortality due to enteric fever. Therefore, this study is designed to investigate the current sensitivity pattern of *Salmonella typhi* so that appropriate antibiotics can be initiated on time.

Objective: To determine the sensitivity pattern of *Salmonella typhi* in enteric fever among the pediatric population visiting a tertiary care hospital.

Methods: This cross-sectional study was carried at the Department of Pediatrics in National Institute of Child Health Karachi from December, 2019 to June, 2020 after acquiring ethical approval from the hospital committee. There were 149 children aged 3-12 years of either gender diagnosed with enteric fever selected for this study. Patients' information was collected on pre-designed proforma. Blood of five milliliters quantity was drawn and sent to the pathology department within 12 hours of the admission. *Salmonella typhi* was identified by biochemical testing of the suspicious non-lactose fermenting colonies. Mueller Hinton Agar medium was used for testing antibiotic sensitivity. The sensitivity of the drug was interpreted as Sensitive, Intermediate and Resistant based on inhibition zone size.

Results: The average age of the children was 5.56±2.39 years. Sensitivity for meropenem, azithromycin was 100% and 93.3% respectively while the sensitivity of ciprofloxacin was 53.7%. Ampicillin, Co-trimoxazole, Chloramphenicol and Ceftriaxone were more than 80% resistant.

Conclusion: Our study confirms the sensitivity for meropenem, azithromycin, ciprofloxacin. Ampicillin, Co-trimoxazole, Chloramphenicol and Ceftriaxone showed higher resistance. This study emphasizes the need for continuous evaluation and judicious use of antimicrobials, considering the ever-changing antibiogram.

Keywords: *Salmonella typhi, enteric fever, Mereopenem, Azithromycin, drug resistance.*

INTRODUCTION

The bacterium *Salmonella enteric serovar typhi* causes typhoid fever which is a life-threatening systemic infection that mainly occurs in developing countries of the world and remains a major public health issue [1, 2]. Paratyphoid fever is caused by *Salmonella enterica serovars Paratyphi A and B* and (infrequently *C*) [3]. Paratyphoid and typhoid fever are generally considered enteric fever [1]. Worldwide, 17 million cases are estimated to occur and it causes 178,000,000 mortalities yearly [4]. It is estimated that more than 21.6 million cases of the disease occurred in the year 2000 around the world that resulting in 216,000 mortalities and above 90% of the cases occurred in Asia [5]. The incidence of the disease in some parts of South Asia is reported high which is 1600 per one lac people [1]. It is unfortunate that Pakistan is among those countries in which this disease is constant and the disease burden is high. The incidence rate of the disease for 2 to 15 years of children in Pakistan is reported as 451.7 per thousand person-years [6]. New data related to the age-specific incidence of the disease from developing countries reveal that enteric fever is likely to be frequent among 0 to 4 years of children, mainly the affected age group is 6 months to 2 years [4].

The disease is frequent in developing countries and often occurs due to unsafe supply of water, food contaminated with feco-oral sources and substandard facilities for sanitation [7, 8]. Thus, it may be concluded that the incidence of enteric fever indicates substandard care of environmental and personal hygiene. Presenting signs and symptoms of typhoid fever are similar to other febrile diseases and commonly confounded with other frequent causes of acute fever like dengue, leptospirosis, malaria, brucellosis, chikungunya, Zika and rickettsial infection [9, 10]. The similarity of presenting signs with other diseases creates trouble in making a clinical diagnosis [10]. The classical signs of enteric fever are gradual onset of high-grade fever, relative bradycardia, rose spots [11]. Signs and symptoms at presentation in children are significantly different as compared to adults. The frequent presenting complaints in enteric fever in children are gradual onset of high-grade fever over several days, vomiting, diarrhea, anemia, thrombocytopenia and hepatosplenomegaly [2].

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The resistance to at least two classes of antibiotics is multidrug resistance (MDR) and recently this resistance has risen [12]. The point of concern is the emergence of multidrug resistance to first-line antibiotics such as ampicillin, cotrimoxazole and chloramphenicol that further brings complications in the management and treatment of typhoid fever [1, 2]. Resistance to first-line antibiotics has been observed in many parts of South Asia and was linked with various epidemics in late 1980 and early 1990 [10]. The resistance to first-line antibiotics caused to adopt of fluoroquinolones as a choice for drugs. However, fluoroquinolone-resistant *Salmonella typhi* is now prevalent in Asia [4]. Complete resistance to fluoroquinolone that includes resistance to advanced generation fluoroquinolone gatifloxacin, showed after 2010 and has been related to prolonged fever and treatment failures [10]. In 2017 the WHO identified fluoroquinolone-resistant *Salmonella enterica* among the priority pathogens for research and development of new antimicrobial agents [4]. According to data published in Surveillance for enteric fever in Asia project (SEAP), more than half of the *Salmonella typhi* were multidrug-resistant in Pakistan and resistance to fluoroquinolone was observed in approximately 90% isolates of *Salmonella typhi* and *Salmonella Paratyphi* [10]. WHO recommends cefixime to treat typhoid fever which is an oral third-generation cephalosporin but infrequent resistance to these antibiotics has also been reported [9, 11]. It is reported in a study conducted in India, that sensitivity for Meropenem, Ciprofloxacin and Amoxicillin-clavulanic acid was 95%, 87.5% and 82.5% respectively [2]. The study conducted by Sudharshan RC showed no resistance to ciprofloxacin [13] while another study reported 27.5% resistance to ciprofloxacin [12]. Since 2016, outbreaks of extensively drug-resistant *Salmonella typhi* strains that are resistant to ceftriaxone and cefixime have been reported in parts of Pakistan [10]. Azithromycin is being considered as an alternative drug in uncomplicated typhoid fever that decreases the risk of poor clinical outcomes and shortens the hospital length of stay as compared to fluoroquinolones and deterioration rate than ceftriaxone when adopted for the management of enteric fever. However, there is still limited experience with the use of this drug [9].

Appropriate and immediate antimicrobial therapy is required for the prevention of complications and mortality due to enteric fever. Nevertheless, multidrug resistance is a rising issue in *Salmonella enterica* due to which antimicrobial treatment compromises. It is highly essential to monitor the pattern of resistance to the drug and figure out the involved mechanism on a continuous basis due to alterations in the pattern of antibiogram which varies from region to region and setting to setting. Thus, the study aims to investigate the current sensitivity pattern of *Salmonella typhi* in enteric fever among the pediatric population visiting a tertiary care hospital. The secondary objective is to determine the association of drug sensitivity with patients' demographic variables and duration of illness.

MATERIAL AND METHODS

This cross-sectional study was carried out in the Department of Pediatrics in the National Institute of Child Health from December, 2019 to June, 2020 with the approval of the Hospital Ethics Committee. Children of both genders of age 3-12 years diagnosed with enteric fever on blood culture report of National Institute of Child Health were included in the study. Patients who had taken antibiotics at least two weeks ago before presenting to the hospital were excluded from this study. Written consent was taken from attendants of the patients to use their data for the current study after explaining to them the purpose and benefits of the study. Considering a 3.6% [2] sensitivity rate of co-trimoxazole at 95% confidence interval and 3% precision yielded a sample size of 149 patients which was calculated by the online available calculator Open-Epi version 3.01. Non-probability consecutive sampling technique was used to enroll study subjects. Patients' information including age, gender, residence, presenting complaints, duration of illness, and pattern of drug sensitivity was collected on pre-designed proforma.

The blood sample was drawn and sent to the pathology department within 12 hours of the admission. The collected blood samples were processed on BACTEC automated blood culture system. Blood culture samples were kept incubated for a period of 7 days at a temperature of 37ºC to determine the growth of the culture. MacConkey Agar media was used for isolation of the bacteria and was kept for incubation for 24 to 48 hours at 37ºC. Non-lactose fermenting colonies were picked from a culture grown on MacConkey Agar media. Suspicious colonies were identified through biochemical testing. *Salmonella typhi* was identified by biochemical testing of these suspicious non-lactose fermenting colonies.

The method of Kirby-Bauer disc diffusion was used according to the guidelines of the Clinical and Laboratory Standards Institute to test the sensitivity of antibiotics. Mueller Hinton Agar medium was used for testing antibiotic sensitivity. Mueller Hinton Agar plates were kept for incubation at 37ºC. The strength of the antibiotic disc and the size of the inhibition zone was observed following the 24 hours of incubation. The sensitivity of the drugs was interpreted as sensitive, intermediate and resistant based on inhibition zone size according to Clinical and Laboratory Standards Institute (CLSI) 2019 guidelines [14].

All the collected data were entered into statistical package SPSS version 21 and was analyzed using this statistical package. Descriptive statistics were computed in terms of mean±standard deviation or frequencies with percentages as applicable. Quantitative variables such as age, weight, height, duration of illness were expressed as mean±standard deviation. Qualitative variables such as gender, presenting complaints,

Table 1: Comparison of drug sensitivity among age groups and gender.

Antibiotics Drugs	Age Groups			Gender		
	3-6 years n=109	6.1-12 years n=40	p-value	Male n=86	Female n=63	p-value
Ampicillin	13(11.9)	5(12.5)	0.924	8(9.3)	10(15.9)	0.224
Co-trimoxazole	17(15.6)	6(15)	0.929	11(12.8)	12(19)	0.296
Chloramphenicol	18(16.5)	6(15)	0.824	11(12.8)	13(20.6)	0.198
Ciprofloxacin	57(52.3)	23(57.5)	0.802	41(47.7)	39(61.9)	0.19
Ceftriaxone	21(19.3)	7(17.5)	0.807	15(17.4)	13(20.6)	0.622
Meropenem	109(100)	40(100)	0.999	86(100)	63(100)	0.999
Azithromycin	102(93.6)	37(92.5)	0.754	81(94.2)	58(92.1)	0.715

All values are presented as n (%)

Table 2: Comparison of drug sensitivity among residence and duration illness group.

Antibiotics Drugs	Residence			Duration of Illness		
	Rural n=40	Urban n=109	p-value	1-2 week n=127	>2 Weeks n=22	p-value
Ampicillin	4(10%)	14(12.8%)	0.637	14(11%)	4(18.2%)	0.342
Co-trimoxazole	4(10%)	19(17.4%)	0.266	19(15%)	4(18.2%)	0.699
Chloramphenicol	5(12.5%)	19(17.4%)	0.468	20(15.7%)	4(18.2%)	0.774
Ciprofloxacin	20(50%)	60(55%)	0.47	65(51.2%)	15(68.2%)	0.284
Ceftriaxone	6(15%)	22(20.2%)	0.473	21(16.5%)	7(31.8%)	0.09
Meropenem	40(100%)	109(100%)	0.999	127(100%)	22(100%)	0.999
Azithromycin	36(90%)	109(94.5%)	0.624	119(93.7%)	20(90.9%)	0.182

All values are presented as n(%)

residence, drug sensitivities of ampicillin-sulbactam, co-trimoxazole, chloramphenicol, ciprofloxacin, ceftriaxone, meropenem and azithromycin were expressed in terms of frequencies with percentages.

RESULTS

There were 149 children aged 3-12 years of either gender diagnosed with enteric fever were selected for this study. The average age, height, weight and duration of illness of the children was 5.56±2.39 years, 102.28±14.54 cm, 36.85±7.46Kg and 2±0.59 days respectively. More than half of the study participants were males (n=86, 57.72%) and belonging to Urban areas (n=109, 73.15%). The most frequent presenting complaint was fever (n=149, 100%) followed by headache (n=77, 51.7%), abdominal pain (n=96, 64.4%), diarrhea (n=87, 58.4%), loss of appetite (n=94, 63.1%), cough (n=48, 32.2%), nausea (n=51, 32.9%) and vomiting (n=49, 32.9%).

Among all the antibiotics, meropenem shows higher sensitivity (n=149, 100%) followed by azithromycin (n=139, 93.3%), ciprofloxacin (n=80, 53.7%) whereas highly resistant drugs were ampicillin (n=131, 87.9%), co-trimoxazole (n=126, 84.6%), chloramphenicol (n=125, 83.9%) and ceftriaxone (n=121, 81.2%). Ciprofloxacin (n=38, 25.5%) and Azithromycin (n=3, 3.4%) also showed an intermediate pattern. The sensitivity pattern of any antibiotic was not significantly different among younger and older children and males and females (**Table 1**). The pattern of antibiotic sensitivity was also not different among patients belonging to a rural and urban area and patients with shorter and longer duration of illness (**Table 2**).

DISCUSSION

Five countries have been declared as endemic for enteric fever and Pakistan is one of among those with an incidence of 451.7/100,000 person-years for age group of 2-15 years [15]. Top most barriers in controlling infectious diseases are poor sanitary and hygiene conditions and multi-drug resistance at individual and community level [16]. In the present study an average age at the time of presentation was 5.56±2.39 years which is in line with Comeau JL study who reported an average age of 7.5 years [17]. This study demonstrated almost same burden among age group of 5-10 years and <5 years as nearly half of cases in age group 5-10 years had enteric fever frequency 52.2%. This finding of age pattern is consistent with other studies. Rangantha A *et al.* showed nearly half of the cases were aged between 5-10 years (47%) [18]. Carl Britto reported in a meta-analysis that the highest prevalence among 5-9 years of age group, followed by 10-14 years and <5years [19]. In the present study a sensitivity of 100% and 93.3% was observed for meropenem, azithromycin respectively. Whereas a sensitivity of 53.7% was seen for ciprofloxacin. Moreover, a resistance of more than 80% was observed for ampicillin, co-trimoxaole, chloramphenicol and ceftriaxone. The findings for sensitivity of ceftriaxone, cefixime and azithromycin were similar to previous available literature in which 100% sensitivity was observed for ceftriaxone [20] and cefixime [21] and 96.3–100% sensitivity to azithromycin [21, 22]. Azithromycin was also reported equivalent or superior to fluoroquinolones, extended-spectrum cephalosporins, chloramphenicol for managing uncomplicated enteric fever with rapid resolution of signs and symptoms

[23, 24]. Another similar study reported a higher susceptibility of *S. typhi* and *S. paratyphi A* isolates against chloramphenicol and cefixime was observed in contrast to ofloxacin [25].

Thus, unnecessary usage of these antibiotics should be avoided in order to not to influence the efficacy of these against *S. typhi*. A study from India demonstrated a sensitivity of 95%, 87.5% and 82.5% for meropenem, ciprofloxacin and amoxicillinclavulanic acid respectively [2]. No resistance to ciprofloxacin was observed in study of Sudharshan RC [13] whereas a resistance of 27.5% to ciprofloxacin was also reported [12]. Since 2016, resistance to ceftriaxone has been documented from different institutes of Pakistan [10]. In a study conducted by Laghari *et al.* [26], it was reported that one third of the study subjects received ceftriaxone which showed a sensitivity of 65.8%. 50.1% *S. typhi* isolates showed sensitivity for ciprofloxacin. There were 2.6% cases of MDR typhoid and 0.9% cases of XDR typhoid. A 100% sensitivity was seen against ofloxacin in *S.typhi* and *S. paratyphi* [27, 28]. However, a contrasted finding was reported by Dutta *et al.* [29] with 56% resistance of olfaxcin in *S. paratyphi A* isolates and 18.2% in *S. typhi* isolates. Therefore, evidences of higher resistance against ofloxacin raised the concerns for its usage as first line treatment choice for managing enteric fever. Health authoritis should take corrective measures for this practice and restrict the irrational usage of fluoroquinolones.

CONCLUSION

The study confirms the sensitivity for meropenem, azithromycin, ciprofloxacin. Ampicillin, Co-trimoxazole, Chloramphenicol and Ceftriaxone showed higher resistance. This study stressed over the necessity for constant monitoring and sensible antimicrobials usage, in accordance with the ever-changing antibiogram.

ETHICS APPROVAL

The study was conducted after obtaining approval from Hospital Ethics Committee. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Helsinki declaration.

CONSENT FOR PUBLICATION

Written consent was taken from attendants of the patients to use their data for the current study after explaining to them the purpose and benefits of the study.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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