Short communication

Pattern of Anti-microbial Drug Resistance in Childhood Typhoid Fever in a selected Hospital in Karachi, Pakistan

Abstract

Introduction

Typhoid fever is a major public health issue in Pakistan. Variations in clinical manifestations make diagnosis a challenging task. Over use of antibiotics make the organism resistant. Antibiotic resistance is currently the most threatening issue as regards to infection control and our study would be helpful in the understanding of this feature of the microbes. The main purpose of this study was to determine the antimicrobial drug resistance and sensitivity pattern in *Salmonella typhi* and *S. paratyphi.*

Methods

This is a descriptive study carried out in a private hospital in Karachi, Pakistan. One hundred consecutive patients, children from age one day till 12 years admitted in the hospital with the history of fever and had positive blood culture for *Salmonella typhi* and *S.paratyphi* were included, 9 antimicrobial drugs were taken into account to check their sensitivity. Statistical analysis was performed using SPSS (IBM SPSS Statistics 20.0). Data was expressed in frequencies and percentages.

Results

Most of the children belong to middle class 58% with 62% male and common age group (40%) was 1 day to 4 years. Nearly half of them drinking unboiled water and had ladder pattern of high grade fever. Most pronounced symptoms were abdominal pain, nausea and anorexia. Resistance pattern was ciprofloxacin 100%, chloramphenicol 89.1%,Ampicillin 87.1% Ceftriaxone76.2%, Cefixime 75.2%,Amoxicillin 65.3% .

Conclusion

Typhoid fever is most commonly observed with unhygienic practices, eating of unhealthy outside food and contaminated water. Pattern of anti microbial resistance gives us a little choice to select antibiotic for typhoid fever. Typhoid fever still remains the commonest bacteraemic illness in Pakistan with children being especially susceptible. Antimicrobial non-susceptibility continues to complicate management.

Keywords

Typhoid fever, Pakistan, antimicrobial resistance, children

Background

Typhoid fever is a type of enteric fever, along with paratyphoid fever. The cause is the bacterium *Salmonella typhi*, also known as *Salmonella enterica* serotype *typhi,* growing in the intestines and blood [1]. Typhoid fever is a systemic infection caused by *Salmonella typhi,* usually through ingestion of contaminated food or water. The acute illness is characterized by prolonged fever, headache, nausea, loss of appetite, and constipation or sometimes diarrhea [2-3]. Typhoid is spread by eating or drinking food or water contaminated with the feces of an infected person. About 10% of people have recurrent symptoms after feeling better for one to two weeks. Relapses are actually more common in individuals treated with antibiotics. Risk factors related to safe water, adequate sanitation, appropriate personal and food hygiene, migration [4-5].

Public health authorities in Pakistan are identifying possible typhoid fever cases, starting typhoid vaccination campaigns in the most affected districts, and spreading educational messages about proper handwashing and safe food and water practices [6-7]. There is a need for routine antimicrobial susceptibility surveillance of enteric fever isolates and close review. The high proportions of *S. typhi* strains demonstrating MDR from Iraq (83%) and Pakistan (52%) relative to those from other countries in this study (≤17%) are consistent with a recent report indicating that 80% of patients infected with MDR *S. typhi* originate from the Asian continent and the remainder occur mostly in Africa and Latin America. Self-medication and unguided antibiotic treatment are two main reasons for MDR development. The high disease burden in pre-school children in certain sites highlights the importance of vaccines and delivery systems that can reach this age group, as well as older children and adolescents. The site in Pakistan had the most significant problem with antimicrobial agent resistance, with nearly two-thirds of the isolates being multidrug resistant. Nalidixic acid resistance, which is associated with a poorer treatment outcome for fluoroquinolone therapy was observed in high proportions of isolates from the sites in India, Pakistan and Viet Nam disease prevention, early diagnosis and appropriate treatment can prevent typhoid mortality and possibly reduce the severity of the disease, even in areas with limited resources findings highlight the complex geographical variation in the disease incidence, age groups affected, and level of antimicrobial resistance, all of which need to be considered in deliberations about the deployment of typhoid vaccines as tools to assist in the control of typhoid fever.

Treatment with antibiotics is life-saving but the recent advent of resistance against traditional antimicrobial agents has led to limited treatment options. Multidrug resistance (MDR), defined as resistance to the three first-line classes of antimicrobial agents (chloramphenicol, ampicillin,

and trimethoprim/sulfamethoxazole [TMP/SMX]), has become prevalent in most of South Asia, with figures reaching 13% in India and 44% in Pakistan. There has been previous research in Pakistan demonstrating increase in resistance against Ciprofloxacin. The trend of antibiotic resistance is on the rise with emerging MDR strains of salmonella. Antibiotic resistance is currently the most threatening issue as regards to infection control and our study would be helpful in the understanding of this feature of the microbes. The current study was planned to determine the antimicrobial drug resistance and sensitivity pattern in *Salmonella typhi* and *S. paratyphi.*

Methodology

This is a descriptive study carried out in one of a private hospital in Karachi, Pakistan. One hundred one patients were selected based on available database in which the inclusion criteria was children aged between one day to 12 years admitted in the hospital with the history of fever more than one week duration and had positive blood culture for *Salmonella typhi* or *S. paratyphi* were included in the study. Study was approved from institutional review board and data was collected by taking relevant history and reviewing charts and computer based data from April 2018 till September 2018. Total number of patients presented during this period was 452 out of which 100 blood culture were positive for *Salmonella typhi.*

Those parents who did not give consent were excluded from the study. Data was collected on survey questionnaire. The questionnaires was designed based on references of published literature and consultancies with experts in the field; which incorporating important risk factors for typhoid fever. Information was collected using face-to-face interviews with parents. Questionnaire included demography (gender, age, economic status, father and mother literacy), history of taking food from outside, drinking water. Second part included clinical features: fever, diarrhea, constipation, abdominal pain, nausea, vomiting, body ache, headache, anorexia, rashes, hepatosplenomegaly. Third part of questionnaire included Full Blood Count (FBC) / blood picture, blood culture and anti-microbial sensitivity.

Blood Sample Collection

Patients with the history of fever or any associated factor with fever were observed for study. The blood cultures of patients were sent to pathology department of the hospital on the very first day when patient came to OPD or admitted through Emergency without administration of antibiotics.3cc to 4cc venous blood was drawn from patients between the age of 1day to 12 years,by using sterile technique and directly injected into Becton Dickinson (BD) BACTEC PEDS PLUS/F vial after wiping the top of vial with alcohol swab. Each vial contained 40 ml of enriched brain-heart infusion broth.

Isolation of Salmonella paratyphi and Salmonella typhi

Each bottle was processed on BD BACTEC FX 40 automated blood culture system. Broth only from positive vials was directly examined by gram stain and subcultured on Sheep Blood Agar, MacConkey's Agar, and Chocolate Agar. Positive cultures for Gram negative bacteria suggestive of *Salmonella paratyphi* and *Salmonella typhi* were confirmed serologically with specific Salmonella paratyphi Antisera A (2-0), *Salmonella paratyphi* Antisera B (4-0), *Salmonella typhi* Antisera (O 9).

Antimicrobial drugs susceptibility test

Salmonella isolates were tested for antimicrobial drugs susceptibility by Kirby- Bauer disc diffusion method on Mueller Hinton Agar plates and incubated at 37°C and then antibiotic disc incor-

poration and zone of inhibition were measured in millimeters after 24 hours of incubation. A total of 9 different antibiotic discs were used to check the susceptibility and resistance of clinical isolates obtained. Isolates were classified into sensitivity, resistant and intermediate pattern according to zone of inhibition.

All positive blood culture for *Salmonella typhi* and *Salmonella paratyphi,* had 9 antimicrobial drugs were taken into account to check their sensitivity for *Salmonella typhi.* They were ceftriaxone, ciprofloxacin, chloramphenicol, cefixime, meropenem, azithromycin, imipenem, amoxacillin. Statistical analysis was performed using SPSS (IBM SPSS Statistics 20.0). Data was expressed in frequencies and percentages.

Results

One hundred and one (n=101) participants who met the study's inclusion criteria were participated, of which 62.4% (n=63) participants were male and 40.6% (n=41) were aged between 1 day to 4 years. More than one third (38.6%) of the study participants were belongs to low socioeconomic family. Nearly one-third of participants' mother (31.7%) and father (36.6%) were graduated (Table 1).

Table1. Selected Demographics of Study Participants

	Number	Percentages
Gender		
Male	63	62.4
Female	38	37.6
Age		
1day to 4 years	41	40.6
5 years to 8 years	25	24.8
9 years to 12 years	35	34.7
Residence		
Low Socioeconomic	39	38.6
Middle Class	59	58.4
Porsche area	3	3.0
Mother Literacy		
Nil	24	23.8
Middle School	8	7.9

Matric	19	18.8
Intermediate	18	17.8
Graduation	32	31.7
Post-Graduation	0	0
Father's Literacy		
Nil	16	15.8
Middle School	7	6.9
Matric	16	15.8
Intermediate	19	18.8
Graduation	37	36.6
Post-Graduation	6	5.9
Number of days of outside food/snacks in last month		
Nil	4	4.0
1 day to 10 days	26	25.7
11 days to 20 days	19	18.8
21 days to 30 days	52	51.5
Drinking Water		
Boiled	19	18.8
Mineral	36	35.6
Unboiled	46	45.5

More than half (55.4%) of patients diagnosed with typhoid had ladder pattern fever, high grade fever; temperature more than 103°F, abdominal pain (53.5%) and nausea (67.3%) (Table 2). Other common complaints were constipation (36.6%), diarrhea (27.7%), and body ache (29.7%). More than one third (37.6%) had hepatomegaly and one quarter with splenomegaly (26.7%).

Table2. Clinical Presentation of Study Participants

	Number	Percentages
Fever		
Continuous	0	0
Intermittent	45	44.6
Ladder Pattern	56	55.4
Temperature		
99 °F- 101 °F	0	0
102 °F – 103 °F	37	36.6
More than 103 °F	64	63.4
Abdominal Pain		
Yes	54	53.5
No	47	46.5
Constipation		
Yes	37	36.6
No	64	63.4
Diarrhea		
Yes	28	27.7
No	73	72.3
Rashes		
Yes	2	2.0
No	99	98.0
Nausea/ Vomiting		
Yes	68	67.3
No	33	32.7
Anorexia		
Yes	91	90.1

No	10	9.9
Body ache		
Yes	46	45.5
No	55	54.5
Headache		
Yes	30	29.7
No	71	70.3
Hepatomegaly:		
Yes	38	37.6
No	63	62.4
Splenomegaly		
Yes	27	26.7
No	74	73.3

In this cohort of study all patients were *Salmonella typhi* positive; 99% had normal WBC, neutrophils and platelets count (Table 3). More than one third (41.6%) of study participants had hemoglobin less than 10mg/dl and 29% had lymphocytosis.

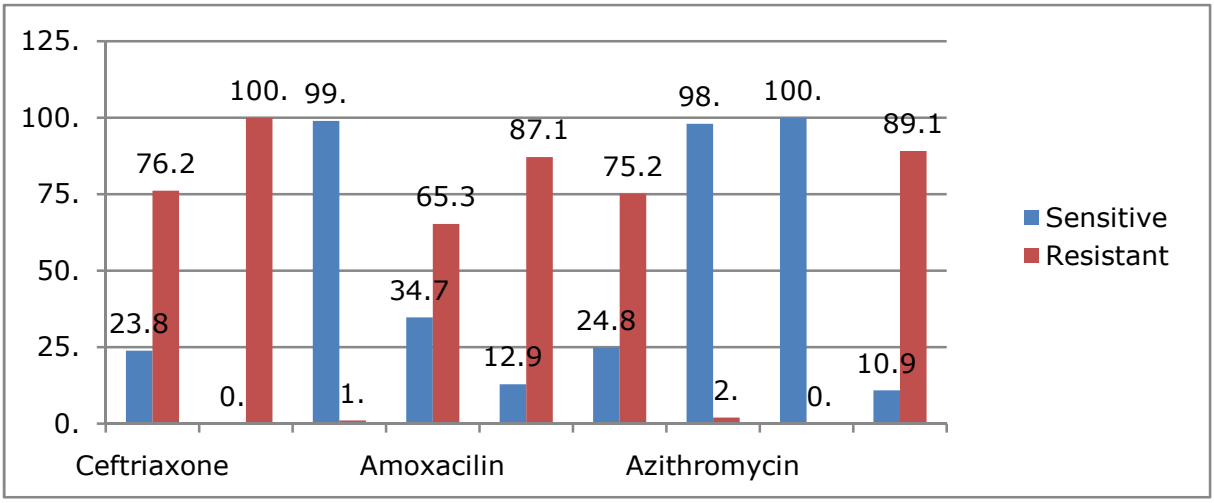
Table 3. Blood markers of study participants

	Number	Percentages
WBC		
Leukopenia (< 4.0/uL)	1	1.0
Normal (4.0 - 10.0/uL)	100	99.0
Leukocytosis (>10.0/uL)	0	0
Neutrophils		
Neutropenia (<40%)	1	1.0
Normal (40-75%)	100	99.0

Neutrophilia (>75%)	0	0
Lymphocytes		
Lymphocytosis (> 45 %)	20	19.8
Normal (20- 45%)	81	80.2
Lymphopenia (< 20%)	0	0
Platelets		
Thrombocytopenia	0	0
Normal	101	100
Thrombocytosis	0	0
Hemoglobin		
Less than10gm/dl	42	41.6
More than or equal to 10gm/dl	59	58.4
Blood C/S		
Salmonella typhi	101	100
Salmonella paratyphi	0	0

Thirty-five (19%) *S. typhi* cases were sensitive to all six antibiotics tested, and 83 (44%) were multidrug-resistant (Figure 1). Nine other cases of *S. typhi* were resistant to nalidixic acid only (Figure 2). Two *S. paratyphi* cases were resistant to chloramphenicol, and one was resistant to ampicillin. We did not find multidrug-resistant (MDR) *S. typhi* in the Hijrat colony and Sultanabad sites, and 66% of the MDR *S.typhi* strains were found in Bilal colony alone.

Figure 1. Antibiotic Sensitivity of study participants



Discussion

According to recent estimates, between 11 and 21 million cases and 128 000 to 161 000 typhoid-related deaths occur annually worldwide. A similar but often less severe disease, paratyphoid fever, is caused by *Salmonella paratyphi* A and B [12]. The strain of *Salmonella typhi* does not respond to most antibiotics used to treat typhoid fever. The outbreak has spread to the city of Karachi and to multiple districts, and several deaths have been reported. In 2018, three cases of XDR typhoid fever were reported in travelers. Antimicrobial resistance emerging in areas endemic for typhoid, leading to treatment failure [13].

In this study done in a private hospital Karachi Pakistan, majority are male and age one day to 4 years. Nearly half of them belongs to middle class and drink unboiled water. Only 18.8% drink boiled water and one third afford to drink mineral water. Typhoid is spread by eating or drinking food or water contaminated with the feces of an infected person. About 10% of people have recurrent symptoms after feeling better for one to two weeks [14-16].

In symptomatology more than half patients in our study presented with ladder pattern high grade fever with abdominal pain. The most frequent symptoms were anorexia, nausea vomiting and body ache. One third has shown hepatomegaly and one fourth of participants had splenomegaly. The acute illness is characterized by prolonged fever, headache, nausea, loss of appetite, and constipation or sometimes diarrhea. Symptoms are often non-specific and clinically non-distinguishable from other febrile illnesses [17-19]. Complete blood picture of our study participants has shown Hb of less than 10gm% in 41.6% and lymphocytosis.

Blood culture in study participants has shown sensitivity as follows; Imipenum 100% , Meropeum 99%, Azithromycin 98% has shown sensitivity. The resistance pattern is Ciprofloxacilin 100%, Chloromphenicol 89.1%, Ampicillin 87.1%, Ceftriaxone 76.2%, Cefixime 75.2% and Amoxicillin 65.3%.

Rehman et.al (2014) reported 80% of patients infected with MDR *S. typhi* originate from the Asian continent The high proportions of *S. typhi* strains demonstrating MDR from Iraq (83%) and Pakistan (52%) Our findings showed that almost all isolates were susceptible (99.7%) to ceftriaxone (a third-generation cephalosporin), which remains the drug of choice for the treatment of typhoid fever [20-22] .

Literature has shown that pattern of AMR is not uniform globally and evolved at different rates in endemic regions from South Asia to Africa, with resistance to first line antibiotics (co-trimoxazole, ampicillin and chloramphenicol). Britto and his colleague in 2018 reported that over

prescription of antibiotics has played a key role in the increasing burden of drug and MDR strains of *S. typhi* being observed in Sub-Saharan Africa and South Asia and remains a major public health concern [23-25].

Our study has shown 100% resistance to ciprofloxacillin , ceftriaxone 76.2% and amoxacillin 65.3% . Nearly same as reported by Abdullah et al reported i.e. ciprofloxacin, 96.47%, cefixime, 96.62% ; ceftriaxone, 98.79%; amoxicillin was 96.48% and about 62.64% of the isolates were MDR strains [26-27]. Evidence of high antimicrobial resistance levels and disease severity support the need for continued surveillance; and improved diagnostics for typhoid multidrug resistance (resistance to ampicillin, chloramphenicol, and cotrimoxazole) was observed in 52% *S. typhi* isolates and 2% *S. paratyphi* isolates [28-29].

In our study the resistance for chloramphenicol is 89.1% while sensitive to azithromycin and meropenem . Aziz et al in 2018 published that culture proven *Salmonella typhi*, of whom 44.52% showed sensitivity only to meropenem, 32.8% to azithromycin, sensitivity to ceftriaxone only3.64% [30-31]**.** Hospitalized children data has shown significant drug resistance of antibiotic commonly used in typhoid fever.

Conclusion

This case report study has shown significant antibiotic resistance. The emergence of drug resistance and changing patterns of sensitivity of antimicrobial agent for *Salmonella typhi* and *S.paratyphi* making typhoid fever treatment more challenging. Typhoid fever still remains the commonest bacteraemic illness in South Asian countries with children being especially susceptible. Antimicrobial non-susceptibility continues to complicate management protocols and limited therapeutic options.

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