Clinical Characteristics and Antibiotics Sensitivity of Culture Positive Typhoid Fever Patients in Baghdad Teaching Hospital - A Single Center Study

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Abstract

BACKGROUND: Enteric fever caused by *Salmonella* Typhi is an endemic disease in Iraq. Variations in presentations make it a diagnostic challenge. If untreated or treated inappropriately then it is a serious disease with potentially life-threatening complications. The recent emergence of drug resistant strains of *S.* Typhi is a rising public health problem and a clinical concern to the physician.

AIM: The objectives of the study were to assess and describe the patterns of antimicrobial resistance, clinical characteristics, epidemiological distribution, and complications of typhoid fever.

PATIENTS AND METHODS: Fifty cases of typhoid fever (culture proven) were collected during the period from February 2019 to November 2019 in the medical wards of Baghdad Teaching Hospital. Detailed history, physical examination, and laboratory investigations were conducted and statistical analysis of the results was done, prospective observational study was conducted.

RESULTS: During the study period, 50 cases of typhoid fever were documented, mean age of presentation was 30.7 ± 12.8, 60% of the cases were male gender, gastrointestinal complications were the most common (90%) followed by hematological complications (71%). Mortality of typhoid fever in our study was 2%. High percentage of resistance to third generation cephalosporins, ciprofloxacin, and azithromycin was found (96%, 56%, and 56%, respectively) while good sensitivity to trimethoprim and meropenem was found (94% and 76%, respectively). Significant association was also found between the development of typhoid fever complications and the presence of anemia, thrombocytopenia, lymphopenia, and eosinopenia. Significant association was also found between the complications and the infection with strains resistant to cephalosporins, ciprofloxacin, and azithromycin.

CONCLUSIONS: There is a concerning increase in resistance toward cephalosporins, ciprofloxacin, and azithromycin while meropenem and trimethoprim are emerging as effective drugs. There was high incidence of complications found (84%).Lymphopenia, anemia, eosinopenia, and thrombocytopenia are independent risk factors for the development of complications of typhoid fever.

Introduction

Typhoid fever is a potentially fatal disease caused by the infection with *S.* Typhi and salmonella paratyphi to a lesser extent. The disease was initially called typhoid fever because of its clinical similarity to typhus. In the early 1800s, typhoid fever was clearly defined pathologically as a unique illness on the basis of its association with enlarged Peyer's patches and mesenteric lymph nodes. In 1869, given the anatomic site of infection, the term enteric fever was proposed as an alternative designation to distinguish typhoid fever from typhus. However, to this day, the two designations are used interchangeably. No known hosts other than humans were identified. It is mostly foodborne or waterborne transmission which results from fecal contamination by ill or asymptomatic chronic carriers. Heathcare workers occasionally acquire enteric fever after exposure to infected patients or during processing of clinical specimens and cultures [1].

S. typhi is a Gram-negative rod, facultative intracellular microorganism that can invade the macrophages and survive and resist inactivation. The bacterium is serologically positive for lipopolysaccharide antigens O9 and O12, protein flagellar antigen Hd, and polysaccharide capsular antigen Vi. The Vi capsular antigen is largely restricted to *S.* typhi, Polysaccharide capsule Vi has a protective effect against the bactericidal action on the serum of infected person [2].

Between 1000 and 1 million organisms are required to create the disease typhoid in a human being, which, therefore, is said to be the infectious dose of *Salmonella enterica* serotype typhi. Obviously, *S.* typhi Vi-positive strains are more infectious and more virulent than Vi-negative strains of *S. enterica* serotype typhi. High gastric acidity is one important barrier against invasion of *S.* Typhi and a low gastric pH

is, therefore, an important defense mechanism. Aging, gastrectomy, proton-pump inhibitors, or antacids leads to achlorhydria and facilitates typhoid infection. In the small intestine, the bacteria first adhere to mucosal cells and then invade the mucosa following which they rapidly penetrate the mucosal epithelium through either microfold cells or enterocytes and arrive in the lamina propria, where they rapidly elicit an influx of macrophage that ingest the bacilli but do not generally kill them. Some bacilli remain within the macrophage of the small intestinal lymphoid tissue and some microorganisms translocate to the intestinal lymphoid follicles and the draining mesenteric lymph nodes and by which they enter the thoracic duct and the general circulation [3], [4].

Global estimates of typhoid fever burden range between 11 and 21 million cases and approximately 128,000 to 161,000 deaths annually. High incidence correlates with poor sanitation and lack of access to clean water and it is rare in developed countries with the infrastructure improvement. A consistent finding of typhoid fever disease burden studies in the past two decades has been the high incidence of typhoid fever in South and South-East Asia with marked intra-country heterogeneity in both age-specific and geographic incidence. New data from sub-Saharan Africa have improved the understanding of the burden and risk factors in that region. Furthermore, new data confirm that typhoid fever with severity sufficient for an outpatient visit or hospital admission is common [5].

The protean manifestation of this disease makes it a true diagnostic challenge as it resembles several other illnesses. It caused prolonged fever (38.5–40) which can continue for up to 4 weeks associated with abdominal pain, nausea, and vomiting, constipation or diarrhea, headache, cough, and myalgia. The incubation period is typically about 10–14 days but can be longer, and the onset may be insidious. The temperature rises in a stepladder fashion for 4–5 days with malaise, increasing headache, drowsiness, and aching in the limbs. Constipation may be caused by swelling of lymphoid tissue around the ileocecal junction, although in children diarrhea and vomiting may be prominent early in the illness. The pulse is often slower than would be expected from the height of the temperature, that is, a relative bradycardia.

Clinical manifestations can be divided according to the weeks of fever into manifestations of the 1st week that includes: Fever, headache, myalgia, relative bradycardia, constipation or diarrhea and vomiting in children, manifestations of the 2nd week that include: Rose spots on trunk, abdominal distension, splenomegaly, diarrhea, and cough. Manifestations of the 3rd week include: Delirium, complications then coma and death (if untreated) [6].

However; clinical severity varies and severe cases may lead to GI bleeding or perforation (which results from necrosis of ileocecal payer patches mostly in the 3rd or 4th week and might require surgical intervention), hepatitis, pancreatitis, and subclinical cholecystitis. Hematological complications include anemia, thrombocytopenia, leukopenia, pancytopenia disseminated intravascular coagulation (DIC) (clinical or usually subclinical), and hemolytic uremic syndrome. Respiratory complications include bronchitis, pneumonia, and pleural effusion. Neuropsychiatric manifestations including Guillain-Barre syndrome, meningitis, myelitis, encephalopathy, delirium (coma vigil), ataxia, cranial or peripheral neuritis, and psychosis. Cardiovascular complications including endocarditis, myocarditis, pericardial effusion, and asymptomatic ECG changes (prolonged QT interval). Rare complications may include acute kidney injury and nephritis, osteomyelitis, reactive or septic arthritis, splenic or hepatic abscess, parotitis, or death [2], [7].

Relapse occurs in 5–10% of patients, usually two to 3 weeks after the resolution of fever. The relapse is usually milder than the original attack, and the *S. enterica* serotype typhi isolate from a patient in relapse usually has the same antibiotic-susceptibility pattern as the isolate obtained from the patient during the original episode. Reinfection may also occur and can be distinguished from relapse by molecular typing [3]. About 10% of untreated patients continue to excrete *S.* typhi in stool for 3 months and 2–5% develop chronic asymptomatic carriage shedding bacteria for more than 1 year especially in patients with biliary abnormalities or concurrent bladder infection with *Schistosoma haematobium* associated with increased gallbladder cancer *S.* typhi adapted to survive in the urinary bladder by forming biofilms and invade the gallbladder epithelial cells [1].

Confirmation of typhoid fever requires isolation of *S.* typhi from blood, bone marrow, stool, or duodenal fluid. Cultures from skin above rose spots, buffy coats, and blood clots treated with streptokinase have been used [8]. Sensitivity of blood culture is 40–80% due to increased antibiotics use and small number of organisms in the blood (<15/ml) [1]. *S.* typhi in the blood are associated with mononuclear cells/platelets fraction so centrifugation of blood and culture of Buffy coat can reduce the time to isolation but does not increase the sensitivity [1]. The volume of blood and the ratio of blood to broth determines blood culture yield: 10–15 mL of blood is necessary to maintain an optimum ratio of 1–12 [9].

Stool culture become positive in the 3rd week in untreated patients. The sensitivity of stool culture depends on the amount of stool cultured, and the positivity rate increased with the duration of illness. Stool cultures are positive in 30% of patients with acute typhoid fever [2]. Stool isolation of *S.* typhi alone is insufficient for diagnosis and only marginally improves diagnosis by blood culture. However, it is confirmatory for carrier detection. Serological tests based on agglutination of Vi antigens have 70–80% sensitivity

and up to 95% specificity in identifying carriers of *S.* typhi [10].

Bone-marrow aspirate culture is positive in 80–95% of typhoid patients. Culturing more than 10 mL of blood is necessary to match the positivity rate with that of 1 mL of bone marrow. Bone-marrow cultures are useful for lengthy illness and antibiotic treatment. Culture of intestinal secretions which are best obtained by duodenal string test can be positive despite negative bone marrow culture [1].

The classic widal test is more than 100 years old. It detects agglutinating antibodies to the O and H antigens of *S.* typhi. The levels are measured by using doubling dilutions of sera in large test tube [4]. Although easy to perform, this test has moderate sensitivity and specificity especially in endemic areas [1]. It can be negative in up to 30% of culture proven typhoid fever, because of blunted antibody response by prior use of antibiotic. Moreover, patients with typhoid may show no detectable antibody response or have no demonstrable rise in antibody titer. Unfortunately, *S. enterica* serotype typhi shares these antigens with other salmonella serotypes and shares these cross-reacting epitopes with other Enterobacteriaceae. This can lead to false positive results. If paired serums are available a fourfold rise in the antibody titer between convalescent and acute sera is diagnostic [2], [3], [4].

New diagnostic tests for typhoid fever have evolved; recent advances in molecular immunology have led to the identification of sensitive and specific markers for typhoid fever and technology to manufacture practical and inexpensive commercial kits for rapid diagnosis, namely: Tubex test and TyphiDot test. TyphiDot is a DOT enzyme immunoassay that detects IgM and IgG antibodies against 50 kD antigen on the outer membrane of *S.* typhi. Tubex is a semi quantitative test that uses polystyrene particle agglutination to detect IgM antibodies to O9 antigen [11]. This test performed better than Widal test in both sensitivity and specificity [1].

Recently, DNA probes and polymerase-chain-reaction (PCR) have been developed to detect *S.* typhi directly in the blood. Urine antigen detection has 65–95% sensitivity. PCR has still not been used in clinical practice [2].

Before the advent of antimicrobial therapy, case fatality rates for typhoid fever exceeded 20% in many areas, since untreated disease led to complications such as intestinal perforation [1]. In 1948, the first effective antimicrobial therapy for typhoidal salmonella, chloramphenicol, started a new era in the management of enteric fever and remained the mainstay of therapy for the next two decades. In the early 1970s, outbreaks of chloramphenicol-resistant typhoid with the evidence of horizontal transfer of resistance genes were reported around the world. Ampicillin and trimethoprim–sulfamethoxazole emerged as alternatives. By the late 1980s, resistance to all three antibiotics (multidrug-resistant typhoid) was increasingly reported, with associated increases in case fatality rates [12]. Fortunately, fluoroquinolones, including ciprofloxacin, proved to be highly effective against enteric fever. Fluoroquinolones thus became the primary therapy for typhoid globally for two decades; in recent years, however, decreased susceptibly to ciprofloxacin (minimal inhibitory concentration [MIC] 0.125–0.5 μg/ml) or ciprofloxacin resistant (MIC > 1 μg/ml) have emerged in the Indian subcontinent so clinicians became reliant on azithromycin and cephalosporins [5].

Now, reports of resistance to these antibiotics are increasing, in addition to the ongoing outbreak in Pakistan since November 2016, cephalosporin-resistant *S.* Typhi has been identified in cases acquired in India, Bangladesh, the Philippines, and Guatemala [12]. For treatment of severe disease, clinicians are turning to carbapenems, which are expensive and often inaccessible in the resource-limited settings where typhoid is most common. Decreased susceptibility to azithromycin has also been reported [7]. These reports suggest that a limited window may be closing. A lack of oral antibiotic options will shift the treatment of suspected enteric fever from outpatient settings, where more than 90% of patients with typhoid are typically treated, to inpatient settings, thereby overwhelming hospitals in places where nosocomial infections with drug-resistant bacteria are common. The cost and effect on health systems in countries where typhoid is endemic could be devastating. In addition, the acquisition of carbapenem-resistance genes, which has already been seen in nontyphoidal salmonella serovars, may not be far off [12]. We now face the very real prospect that untreatable typhoid fever will reemerge if the acquisition of carbapenem resistant genes developed.

Objectives

The study was done with the aim of:

1. Documenting recent trends in antibiotics susceptibility and therapeutic response,
2. Clinical profile
3. Complications of typhoid fever.

Patients and Methods

Study design and sample

This study is a prospective study conducted at the medical units of Baghdad teaching hospital during the period from February 2019 to November 2019. It included 50 adult patients (age more than 14 years) who were admitted due to clinical suspicion of typhoid fever and they were all proved by culture.

Procedure and measurement

After obtaining informed consent, documentation of the symptoms and the signs was made and recoded in pro forma and they were sent for laboratory investigations including complete blood picture, blood film, liver and renal function tests, virology screen for those who presented with jaundice or elevated liver enzymes, general urine examination (GUE), and imaging studies that included chest radiograph (CXR), abdominal ultrasound and echocardiography whenever indicated. 10 ml of blood were drawn with aseptic technique and sent for culture and sensitivity testing. Trypticase soya broth which establishes the growth of all common pathogens was used as a culture medium. Culture on MacConkey agar was done as per the standard method. Suspected Gram-negative non lactose fermenting colonies were further processed and identified by biochemical reactions and confirmed by group and type specific salmonella anti sera.

All the isolates of *S.* typhi were tested for their antimicrobial susceptibility pattern by disk diffusion method against ceftriaxone (30 µg), ampicillin (10 µg), cefotaxime (30 µg) ciprofloxacin (5 µg), cotrimoxazole (25 µg), chloramphenicol (30 µg), ceftazidime (30 µg), nalidixic acid (30 µg),meropenem (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), levofloxacin (5 µg), azithromycin (15 µg), gentamicin (10 µg), amikacin (30 µg), and MIC was assessed for the antibiotic tested. The MIC was determined by the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute. Bacteria grown overnight were suspended in phosphate-buffered saline to an optical density at 600 nm (OD600) of 0.60, which was equivalent to 5.0×10^8 CFU/ml, and then diluted in Mueller-Hinton broth to a final concentration of 5.0×10^5 CFU/ml. The bacteria were treated with antibiotics in 96-well plates, and the plates were incubated at 37°C for 24 h. The lowest concentration with no visible bacterial growth was defined as the MIC. Multiple drug-resistant (MDR) isolates of *S.* Typhi were those resistant to all three first line antityphoid drugs (ampicillin, chloramphenicol, and trimethoprim–sulfamethoxazole). Low-level resistance to ciprofloxacin was defined as an MIC of 0.125–1 mg ml 21.

All patients received empirical treatment after admission for typhoid fever that included either cephalosporins or fluroquinolones until the results of blood culture and sensitivity became available. The antibiotics were changed according to the results of culture and clinical response. Patients' medical records were reviewed for demographic, clinical, and laboratory features. All patients were followed for clinical improvement and development of complications in the hospital and response to culprit antibiotic. Defervescence was defined as the number of days required for fever subsides after starting a certain antibiotic.

Statistical analysis

We evaluated variable factors and studies their association with the development of complications. T test was used to compare continuous variables and Chi-square test was used to compare categorical variables. Statistical analyses were performed using SPSS for Windows (SPSS for Windows Inc. Version 22. Chicago, Illinois). p value of less than 0.05 was considered significant.

Ethical statement

This was an observational study, during which patient's health, safety, and privacy were not harmed.

Results

During the study period, 50 patients with culture positive typhoid fever who were admitted to the medical unit were evaluated from admission to discharge.

Demographic characteristics

According to our study, there were gender differences in the incidence of typhoid fever with predominance of male (60%) over female (40%) as shown in Figure 1.
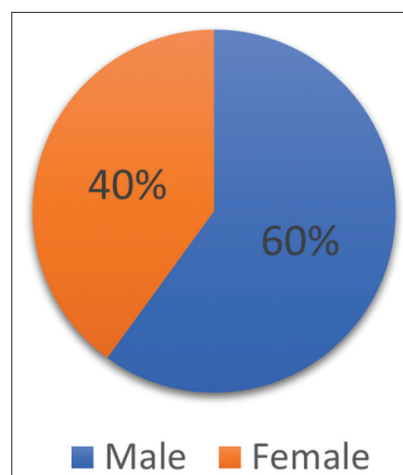

Figure 1: Gender difference among the population of our study

There were also variations in the age group of patients with typhoid fever illustrated in Figure 2. The highest isolation was seen in younger age groups. The majority of patients fell in the range of 15–20 years, mean age was 30.7 ± 12.8.

Typhoid fever tended to show seasonal variation in our study. Highest incidence was noticed in April and May followed by September, October. Monthly distribution of typhoid fever in our study is described in Figure 3.
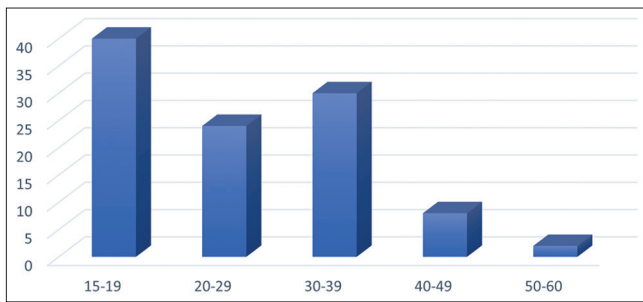
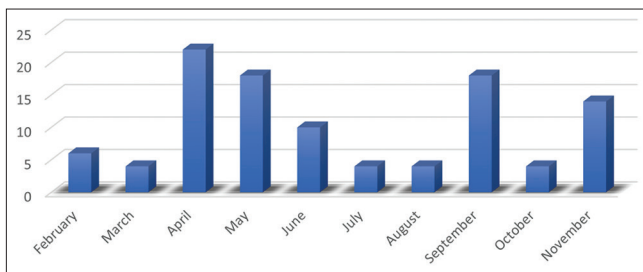Figure 2: Distribution of typhoid fever according to age group

Figure 3: Month-wise distribution of typhoid fever cases in our study

Clinical characteristics

The most common presenting symptoms beside fever were abdominal pain (96%), headache (70%), constipation (70%), vomiting (50%), and jaundice (24%) (Figure 4).
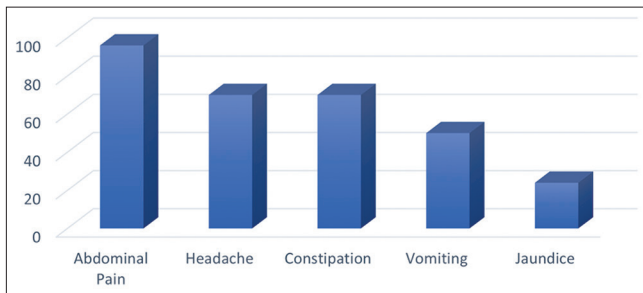

Figure 4: The most common presenting symptoms

The most common physical signs found in our patients were: Relative Bradycardia which was noticed in 66% of our patients, Splenomegaly was found in 64% of our patients while hepatomegaly was found in 30% only, lymphadenopathy was found on clinical examination in 14% of the cases (Figure 5).
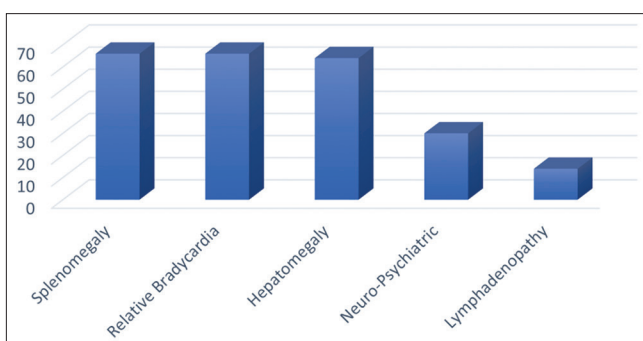

Figure 5: The most common signs

Laboratory investigations

Leukopenia was found in 80% of our cases, anemia was found in 88%, thrombocytopenia was found in 62%, and pancytopenia was found in 62%. Regarding the white blood cells differential count the predominant feature was lymphopenia in 90% and Eosinopenia in 76% of the cases. Elevated liver enzymes were found in 68% of our patients and elevated renal indices not attributed to other causes were found in 10%. Laboratory findings are described in Figure 6.
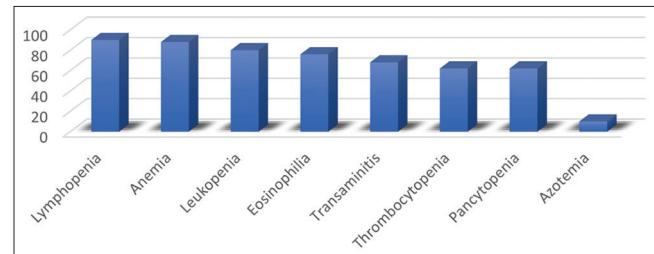
Figure 6: Laboratory investigations results of our study

Imaging

Ultrasound findings included splenomegaly in 88%, hepatomegaly in 74% and lymphadenopathy (mesenteric or paraaortic or both) in 44% while echocardiography revealed pericardial effusion in 6.5% of the patients as shown in Figure 7.

Figure 7: Ultrasound findings in our patients

Complications

Figure 8 and Table 1 describe the most common complications of typhoid fever in our patients:

Figure 8: Complications of typhoid fever according to the system involved

Table 1: Complications of typhoid fever

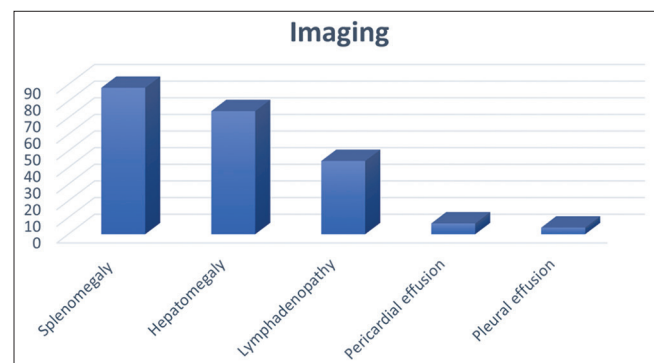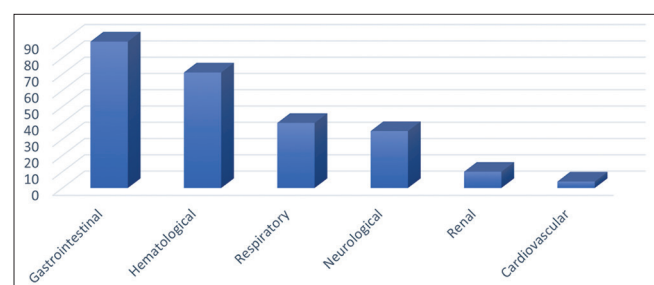| Complication	Percentage
Hepatitis	68
Pancytopenia	62
Mesenteric adenitis	44
Bronchitis	40
Encephalopathy	30
GI bleeding	24
Pleural effusion	6
DIC	6
Relapse	6
GI perforation	4
Pericardial effusion	4
Nephritis	4
Pancreatitis	2
Splenic abscess	2
Cholecystitis	2
Myelitis	2
Myocarditis	2
Death	2

Gastrointestinal complications were noticed to be the most common complications (90%) including gastrointestinal bleeding in the form of melena in 24%, hepatitis either clinical (jaundice, hepatomegaly, and raised liver enzymes) or subclinical (elevated liver enzymes only) in 68%. One case was complicated by acute acalculous cholecystitis, one case was complicated by splenic abscess, one case was complicated by acute pancreatitis, and two cases were complicated by gastrointestinal perforation that required surgical intervention. Hematological complications occurred in 71% of cases, pancytopenia occurred in 62% of the cases while 6% were complicated by DIC. CNS complications were seen in 16% of the patients including confusion, drowsiness, meningism, apathy, ataxia, delusions, and neuropsychiatric manifestations. One case was complicated by weakness and clonus that proved to be myelitis by magnetic resonance imaging (MRI). Respiratory complications were seen in 40% of the cases, bronchitis was the dominant manifestation followed by bronchopneumonia, pleurisy, lobar pneumonia and pleural effusion. Cardiac complications were seen in 6% of the cases. About 2% presented with myocarditis and 6% with pericardial effusion. Renal complications were found in 10% of our patients.

Logistic regression model was designed to identify factors associated with the complications (Table 2). It revealed that complications of typhoid fever was significantly associated with the degree of thrombocytopenia (p <0.001, confidence interval 95% 2.07 –10.02), anemia (p < 0.001, confidence interval 95% 0.196 –2.156), lymphopenia (p < 0.001, confidence

Table 2: Factors associated with complications, results of logistic regression model using SPSS

Variable	Odd ratio	95% Confidence interval	p-value
Age	0.95	1.797–1.823	0.185
Gender	1.116	0.67–1.86	0.674
Duration of fever before admission	0.796	0.487–1.105	0.728
Thrombocytopenia	4.554	2.07–10.02	<0.001
Anemia	0.98	0.196–2.156	<0.001
Lymphopenia	0.956	0.188–1.724	<0.001
Eosinopenia	0.956	0.197–1.733	<0.001
Hospital stay	1.191	1.1–1.29	<0.001
Time to defeverence	0.68	0.675–0.685	0.05
Resistance to ciprofloxacin	0.951	0.848–1.054	<0.001
Resistance to azithromycin	0.951	0.848–1.054	<0.001
Resistance to cephalosporin	0.99	0.75–2.73	<0.001

Table 3: Spectrum of antibiotics susceptibility according to blood culture and sensitivity results

Antibiotic	Sensitive (%)	Moderate (%)	Resistant (%)	Non-sensitive (%)
Amikacin	80	20		
Augmentin	92		8	
Ampicillin	6		56	38
Cefazolin	6		60	34
Ceftriaxone	4		40	56
Cefotaxime	4		40	56
Ceftazidime	4		32	64
Ciprofloxacin	44		56	
Gentamicin	80	20		
Imipenem	84	16		
Levofloxacin	60	12	4	24
Meropenem	76	12	12	
Moxifloxacin	82		18	
Tazocin	84	14	2	
Piperacillin			94	6
Tetracycline				
TMP-SMX	94		6	
Nalidixic acid			28	72
Chloramphenicol	90	10		
Azithromycin	44		56	
Ofloxacin	70	10	20	
Doxycycline	80	16	4	

interval 95% 0.188–1.724), and Eosinopenia (p < 0.001, confidence interval 95% 0.197–1.733).

Significant association between the complications and the resistance patterns of the blood culture results mainly resistance to ciprofloxacin (p < 0.001, confidence interval 95% 0.848–1.054), ceftriaxone (p < 0.001, confidence interval 95% 0.75–2.73), and azithromycin (p < 0.001, confidence interval 95% 0.848.–1.054).

Spectrum of antibiotics susceptibility

In our study, only 4% of *S.* Typhi isolates were sensitive to third generation cephalosporins, 56% were resistant to ciprofloxacin. Meropenem sensitivity was 76%, Azithromycin sensitivity was 44%, and Trimethoprim sensitivity was 94%. Table 3 illustrates the spectrum of antibiotics sensitivity in our studies group.

Discussion

Typhoid fever is an endemic disease in Iraq and in 2020 it still represents a serious health problem; however, data on clinical profile, antibiotics sensitivity, and factors related to the development of complications is still limited.

The results of our study showed predominance of male (60%) over female (40%) which is similar to the findings of many other studies including study of kakaria, study of Dimitrov and study of crump J.A [13], [14], [15]. This is probably due to the fact that men tend to go out more and consume food and beverages from outdoor sources.

Although enteric fever affects patients from all age groups, the highest isolation was seen in younger age groups. Median age of presentation was

24.7 years and the majority of patients fell in the range of 15–20 years. Similar results were observed in other studies [15], [16].

Typhoid fever may occur throughout the year but it does follow a seasonal pattern with highest incidence in April-May (40%) followed by September-October(36%). This can be attributed to the fact that in endemic areas, infrastructural deficiencies could be susceptible to climatic events such as monsoon and floods also *S.* typhi tend to grow more in warmer temperatures especially on food with mean temperature of 20–25°C. A large 2007 surveillance study in Kolkata, India [17], found that communities' clean water sources are put in jeopardy during this period, also shown by recent studies in Morocco and Chile [18], [19].

All our patients presented with fever 40% of them presented in the 3rd week. The stepladder pattern which was the hallmark of typhoid fever was noticed in only 40% and it was as few as 16% in a study by Kukaria [13] and 12% in a study by Gupta [20]. In the most contemporary presentations of typhoid fever, the fever had a steady insidious onset and continuous high-grade pattern.

The incidence of jaundice in typhoid fever patients varies from (4 to 17%) and it was reported to be as few as 1% in a study by Joshi [1], [21], [22]. In our study, jaundice was seen in 24%. This wide range may be due to difference in the severity of the disease as a result of delay in presentation and diagnosis. Jaundice occurs as a result of typhoid hepatitis, septicemia, or liver abscesses and it improved with recovery of the patient [21].

Relative Bradycardia was noticed in 66% of our patients; therefore, it still represents a good diagnostic clue for typhoid fever, similar to a study conducted by Joshi [21].

Splenomegaly was found in 64% of our patients while hepatomegaly was found in 30% only, and lymphadenopathy was found on the clinical examination in 14% of the cases. Hepatomegaly was detected in 18% and splenomegaly was present in 36% of patients in a study done by Saha [23].

Leukopenia was found in 80%, anemia was found in 88%, thrombocytopenia was found in 62%, and pancytopenia was found in 62% which are similar results to studies conducted by Alshok and Alamidi and khosla *et al*. [24], [25]. Leukopenia is a result of altered adhesiveness of WBC to the endothelial lining of the blood vessels and pancytopenia can be attributed to myeloid maturation arrest and increased phagocytic activity of histiocytes in the bone marrow. Absolute eosinopenia is an important finding that should help timely diagnosis and early treatment initiation of enteric fever as shown by a study done by Aliasgar in United Arab Emirates [26]. In a study you Hanna, 18% of typhoid fever patients had marked thrombocytopenia [27].

Forty-two patients presented with complicated typhoid which constitute 84% of the cases, the mortality was 2% due to DIC, the incidence of complications during the course of the disease was studied with the revelation of the following results:

Gastrointestinal complications

Were noticed to be the most common complications occurred in (90%) of case and included gastrointestinal bleeding in the form of melena resulting from erosion of a necrotic Peyer's patches in 24% of patients, hepatitis either clinical (jaundice, hepatomegaly, and raised liver enzymes) or subclinical (elevated liver enzymes only) in 68% of patients which more common in our study than a study by Joshi (24% vs. 68%) [22]. Similar results were found in a study by Parry [3].

Hematological complications

It occurred in 71%. These findings are similar to a study conducted in Bangladesh by Khatun *et al*. [28]. Typhoid fever is characterized by activation of coagulation system with prolongation of prothrombin time (PT) and activated partial thromboplastin time (APTT) and consumption of platelets [25].

CNS complications

CNS complications were seen in 16% of the patients. Dipika sur found a relatively smaller percentage of neuropsychiatric manifestations which was 3% only [29].

Respiratory complications

Respiratory complications were seen in 40% our cases. Analysis of the pleural fluid revealed exudative lymphocytosis with complete improvement after treatment. This was similar to studies done by Sharma [30].

Elevated renal indices

Elevated renal indices were noticed in 10% of the cases, microscopic albuminuria and microscopic hematuria were detected in GUE suggesting acute nephritis which recovered completely after treatment. Studies suggest that typhoid nephritis is due to immune mediated glomerular damage and renal failure may be part of septicemia or shock [31].

Logistic regression model was designed to identify factors associated with the complications (Table 1). It revealed that complications of typhoid fever were significantly associated with the degree of thrombocytopenia, anemia, lymphopenia, and

eosinopenia. Thrombocytopenia was found to be an independent factor associated with the development of complications in studies done by khan *et al.* and Khatun *et al.* [28], [32]. Recent studies are suggesting that thrombocytopenia is an early manifestation of subclinical DIC and is correlated with the severity of the illness, potential development of complications especially intestinal perforation and bleeding [27], [33]. Anemia and leukopenia have been observed to be risk factors for intestinal perforation and other complications in the previous studies. In a study conducted by Hosuglu, leukopenia (95% CI: 1.46, 10.33; p = 0.04) was considered an independent risk factor for complications [34].

No significant association between the development of complications and the age or the gender of the patients was found unlike the results of a study done by Hosuglu which found a significant relation between the male gender and the development of complications in patients with typhoid fever [34].

We could not establish a significant association between the complications and the duration of the fever which contradicts the classical teaching that classify the complications according to the weeks of fever. Some studies explain this finding that the complications might be related to the virulence of the microorganism itself rather than to the duration of bacteremia [35]. Parry speculated that the genotype of the relevant *S. Typhi* organisms may play an important role in regulating disease severity and suggested an association between bacterial haplotype and the virulence of the strain [36]. A further study of *S. Typhi* isolates from in New Guinea, showed that only a narrow spectrum of PFGE types were found in patients with severe or fatal disease [37].

Significant association between the complications and the resistance patterns of the blood culture results mainly resistance to ciprofloxacin, ceftriaxone, and azithromycin. We hypothesized that the observed association between the complications and resistance to ciprofloxacin may because those patients had a longer duration of illness before hospital admission due to ineffective treatment; however, genetic factors have been proposed in recent studies that have shown an association between the ciprofloxacin intermediate and resistance phenotype with H58 haplotype [38].

In this study, *S.* typhi showed universal resistance to third generation cephalosporins ceftriaxone and cefotaxime (96% of isolates were resistant), similar outbreak of cephalosporins resistant *S.* typhi took place in Pakistan in 2016. The strain was studied and found to acquire an extended spectrum beta lactamase ESBL. Third generation cephalosporins were effective and widely used antibiotics against *S.* typhi until recently when reports on increased resistance from Bangladesh, India, Pakistan, and Nepal were published [12], [20], [29], [30], [39], [40], [41].

Resistance to ciprofloxacin was documented in 56% of the cases. Nalidixic acid resistance was found in 100% of isolates. *S.* typhi with decreased susceptibility to ciprofloxacin increased to 23% in the U.K [42], 59% in USA [43] ciprofloxacin resistance of 18.4% in a study by chitnis s in Bengaluru in India [44], and 22% in a study done by Kakaria in Mumbai [13]. Interestingly, fluoroquinolone and third-generation cephalosporin resistance are still low in Africa (<1%) [45]. Unlike resistance to cephalosporins, resistance to fluoroquinolones was mediated through conformational changes in DNA gyrase a, the main sites of fluoroquinolone action. A recent WHO report suggested that nalidixic acid sensitivity was a good indicator of fluoroquinolone sensitivity [45]. Kakaria recommended that all *S.* typhi isolates should be screened for nalidixic acid resistance and that patients with nalidixic acid-resistant strains should be treated with higher doses of ciprofloxacin [2], [13]. It was also noticed that time to defervescence in cases with *in vitro* sensitivity to ciprofloxacin was prolonged to a mean of 9 days with high rates of clinical therapeutic failure.

Levofloxacin, on the other hand, showed sensitivity of 60% with better clinical response and shorter time to defervescence (mean of 4 days) than ciprofloxacin and moxifloxacin sensitivity was 82%.

Azithromycin showed 56% resistance with mean time to defeverence of 7 days. Recent studies in India showed that MIC of azithromycin is creeping toward resistance [46], [47].

Meropenem sensitivity was 76%, mean time to defeverence was 5 days suggesting that it can be used as first-line drug especially complicated hood requiring hospital admission; however, cost remains a problem in resource limited areas.

Noteworthy observation that chloramphenicol and trimethoprim which were once the drugs of choice for typhoid fever but were no longer used due to resistance, regained sensitivity against *S.* typhi (90% and 94%, respectively). Mean time to defervescence on trimethoprim was 4 days.

MDR *S.* typhi (MDR) defined as resistance to ampicillin, trimethoprim, and chloramphenicol was not detected in any of our patients probably due to infrequent use of chloramphenicol and trimethoprim [45]. Resistance to ampicillin alone was found in 94% of isolates.

Aminoglycosides show good *in vitro* sensitivity of 80% for both Amikacin and Gentamicin but they were not used because aminoglycosides show poor activity against intracellular bacteria and show poor *in vivo* activity against *S.* typhi [48].

In the majority of recent studies, rollback of sensitivity to the classical first line agents has been observed due to their restricted use in the 1990s. Similar trends have been noticed in other studies [45], [49].

Conclusions

From our study, we concluded the following:

1. There is a concerning increase in resistance toward cephalosporins, ciprofloxacin, and azithromycin while meropenem and trimethoprim are emerging as effective drugs
2. High incidence of complications was found (84%)
3. Lymphopenia, anemia, eosinopenia, and thrombocytopenia are independent risk factors for the development of complications of typhoid fever.

Recommendations

1. Meropenem is preferred to be used in the complicated cases that require hospital admission while trimethoprim is a reasonable choice for uncomplicated outpatient cases
2. Improvement in the provision of clean water supply, effective sewage disposal, hygienic food preparation, and infrastructure
3. Typhoid vaccination program especially for school children with the introduction of the conjugate Vi vaccine as part of extended program of vaccination should be considered
4. All patients treated for typhoid fever should be followed to assess fecal excretion of *S.* typhi (three negative stool cultures are required) and carriers should be treated.

Limitations

1. Relatively small number of the cases and short duration of the study
2. Failure to perform antibiotic susceptibility testing on all recommended drugs
3. In hospital setting of the study prevented us from studying outpatient cases for interpretation of general population results
4. It is a single center study so collaboration with other centers is needed to come up with a national guideline for typhoid fever treatment.

References

1. Pegues DA, Salmonellosis SI. Harrison Principles of Internal Medicine. 20th ed. New York: McGraw Hill Education; 2018. p. 1173-80.
2. Paul UK, Bandyopadhyay A. Typhoid fever: A review. Int J Adv Med. 2017;4(2):300-6.
3. Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. N Engl J Med. 2002;347(22):1770-82. https://doi.org/10.1056/NEJMra020201

PMid:12456854

4. World Health Organizations. Background Document: The Diagnosis, Treatment and Prevention of Typhoid Fever. Department of Vaccines and Biologicals. Geneva: World Health Organizations; 2003. p. 1.
5. World Health Organization. Typhoid Vaccines, Who Position Paper, March 2018: Recommendations. Vaccine 2018 April 13. Geneva: World Health Organization; 2018.
6. Dockrell DH, Sundar S, Angus BJ, Hopson RP. Typhoid and paratyphoid (enteric) fever. In: Davidson's Principles and Practice of Medicine. 22nd ed. Edinburg: Churchill Livingstone, Elsevier; 2014. p. 339-40.
7. Richens J, Parry C. Typhoid and paratyphoid fever. In: Oxford Textbook of Medicine. Vol. 1. Oxford Academic; 2012. p. 503-7.
8. Gilman RH, Terminel M, Levine MM, Hernandez-Mendoza P, Hornick RB. Relative efficacy of blood, urine, rectal swab, bone-marrow, and rose-spot cultures for recovery of *Salmonella* Typhi in typhoid fever. Lancet. 1975;1(7918):1211-3. https://doi.org/10.1016/s0140-6736(75)92194-7

PMid:48834

9. Bhan MK, Bahl R, Bhatnagar S. Typhoid and paratyphoid fever. Lancet. 2005;366(9487):749-62. https://doi.org/10.1016/S0140-6736(05)67181-4

PMid:16125594

10. Lanata CF, Levine MM, Ristori C, Black RE, Jimenez L, Salcedo M, et al. Vi serology in detection of chronic *Salmonella* Typhi carriers in an endemic area. Lancet. 1983;2(8347):441-3. https://doi.org/10.1016/s0140-6736(83)90401-4

PMid:6192305

11. Olsen SJ, Pruckler J, Bibb W, Nguyen TM, Tran MT, Sivapalasingam S, et al. Evaluation of rapid diagnostic tests for typhoid fever. J Clin Microbiol. 2004;42(5):1885-9. https://doi.org/10.1128/JCM.42.5.1885-1889.2004

PMid:15131144

12. Andrews JR, Qamar FN, Charles RC, Ryan ET. Extensively drug-resistant typhoid are conjugate vaccines arriving just in time? N Engl J Med. 2018;379(16):1493-5. https://doi.org/10.1056/NEJMp1803926

PMid:30332569

13. Kakaria A, Asgaonkar D, Narkhede M. Clinical profile of enteric fever: A prospective study of fifty enteric fever patients. Int J Res Med Sci. 2014;2:1620-5.
14. Dimitrov T, Udo EE, Albaksami O, Alshehab S, Kilani A, Shehab M, et al. Clinical and microbiological investigations of typhoid fever in an infectious disease hospital in Kuwait. J Med Microbiol. 2007;56(Pt 4):538-44. https://doi.org/10.1099/jmm.0.46814-0

PMid:17374897

15. Crump JA, Mintz ED. Global trends in typhoid and paratyphoid fever. Clin Infect Dis. 2010;50(2):241-6. https://doi.org/10.1086/649541

PMid:20014951

16. Buch NA, Hassan MU, Kakaroo DK. Enteric fever- a changing sensitivity pattern, clinical profile and outcome. Indian Pediatr. 1994;31(8):981-5.

PMid:7883352

17. Sur D, Ali M, von Seidlein L, Manna B, Deen JL, Acosta CJ, et al. Comparisons of predictors for typhoid and paratyphoid fever in Kolkata, India. BMC Public Health. 2007;7:289. https://doi.org/10.1186/1471-2458-7-289

PMid:17935611

18. Cabello F, Springer AD. Typhoid fever in Chile 1977-1990: An emergent disease. Rev Med Chil. 1997;125(4):474-82.

PMid:9460291

19. Saad NJ, Lynch VD, Antillón M, Yang C, Crump JA, Pitzer VE. Seasonal dynamics of typhoid and paratyphoid fever. Sci Rep. 2018;8(1):6870. https://doi.org/10.1038/s41598-018-25234-w
    PMid:29720736

20. Gupta S, Handa A, Panda RC. Profile of culture positive enteric fever from Banlore. Med J Armed Forces India. 2009;65(4):328-31. https://doi.org/10.1016/S0377-1237(09)80093-2
    PMid:27408286

21. Ahmed A, Ahmed B. Jaundice in typhoid patients: Differentiation from other common causes of fever and jaundice in the tropics. Ann Afr Med. 2010;9(3):135-40. https://doi.org/10.4103/1596-3519.68361
    PMid:20710103

22. Joshi YK. Typhoid fever clinical features. J Indian Acad Clin Med. 2001;2(1-2):13-5.

23. Saha AK, Hassan MK, Kundera LC, Saha SK, Begum P. Study of clinical profile and antibiotic sensitivity in typhoid fever at Faridpur medical college hospital. Faridpur Med Coll J. 2017;12(1):2-4.

24. Alshok M, Alamidi B. Typhoid fever complications in Babylon. Med J Babylon 2004;1(2):149-54.

25. Khosla SN, Anand A, Singh U, Khosla A. Hematological profile in typhoid fever. Trop Doct. 1995;25(4):156-8. https://doi.org/10.1177/004947559502500404
    PMid:7502322

26. Lokhandwala A, Athar S, Turrin NP. Role of absolute eosinopenia as marker of enteric fever: Experience from a tertiary care hospital in the United Arab Emirates. Ibnosina J Med Biomed Sci. 2012;4(6):249-53.

27. de Jong HK, Parry CM, van der Vaart TW, Kager LM, van den Ende SJ, Maude RR, et al. Activation of coagulation and endothelium with concurrent impairment of anticoagulant mechanisms in patients with typhoid fever. J Infect. 2018;77(1):60-7. https://doi.org/10.1016/j.jinf.2018.03.008
    PMid:29746945

28. Khatun H, Islam SB, Naila NN, Islam SA, Nahar B, Alam NH, et al. Clinical profile, antibiotic susceptibility pattern of bacterial isolates and factors associated with complications in culture-proven typhoid patients admitted to an urban hospital in Bangladesh. Trop Med Int Health. 2018;23(4):359-66. https://doi.org/10.1111/tmi.13037
    PMid:29432657

29. Sur D, Barkume C, Mukhopadhyay B, Date K, Ganguly NK, Garrett D. A retrospective review of hospital-based data on enteric fever in India, 2014-2015. J Infect Dis. 2018;218(Suppl 4):S206-13. https://doi.org/10.1093/infdis/jiy502
    PMid:30307566

30. Sharma P, Dahiya S, Manral N, Kumari B, Kumar S, Pandey S, et al. Changing trends of culture positive typhoid fever and antimicrobial susceptibility in a tertiary care North Indian hospital over the last decade. Indian J Med Microbiol. 2018;36(1):70-6. https://doi.org/10.4103/ijmm.IJMM_17_412
    PMid:29735830

31. Matono T, Kato Y, Morita M, Izumiya H, Yamamoto K, Kutsuna S, et al. Case series of imported enteric fever at a referral center in Tokyo, Japan: Antibiotic susceptibility and risk factors for relapse. Am J Trop Med Hyg. 2016;95(1):19-25. https://doi.org/10.4269/ajtmh.15-0714
    PMid:27162265

32. Khan M, Coovadia Y, Connolly C, Sturm AW. Risk factors predicting complications in blood culture-proven typhoid fever in adults. Scand J Infect Dis. 2000:32(2):201-5. https://doi.org/10.1080/003655400750045349
    PMid:10826909

33. Pegues D, Miller S. *Salmonella* species, including *Salmonella* typhi. In: Mandell G, Bennet J, Dolin R, editors. Principles of Practice of Infectious Diseases. 7th ed. New York: Churchill-Livingston; 2010. p. 2887-903.

34. Hosoglu S, Aldemir M, Akalin S, Geyik MF, Tacyildiz IH, Loeb M. Risk factors for enteric perforation in patients with typhoid fever. Am J Epidemiol. 2004;160(1):46-50. https://doi.org/10.1093/aje/kwh172
    PMid:15229116

35. Everest P, Wain J, Roberts M, Rook G, Dougan G. The molecular mechanisms of severe typhoid fever. Trends Microbiol. 2001;9(7):316-20. https://doi.org/10.1016/s0966-842x(01)02067-4
    PMid:11435104

36. Parry CM, Thompson C, Vinh H, Chinh NT, Phuong LT, Ho VA, et al. Risk factors for the development of severe typhoid fever in Vietnam. BMC Infect Dis. 2014;14:73. https://doi.org/10.1186/1471-2334-14-73
    PMid:24512443

37. Thong KL, Goh YL, Yasin RM, Lau MG, Passey M, Winston G, et al. Increasing genetic diversity of *Salmonella enterica* serovar Typhi isolates from Papua New Guinea over the period from 1992 to 1999. J Clin Microbiol. 2002;40(11):4156-60. https://doi.org/10.1128/JCM.40.11.4156-4160.2002
    PMid:12409390

38. Holt KE, Phan MD, Baker S, Duy PT, Nga TV, Hinds J, et al. Emergence of a globally dominant IncHI1 plasmid type associated with multiple drug resistant typhoid. PLoS Negl Trop Dis. 2011;5:e1245.

39. Date KA, Newton AE, Medalla F, Blackstock A, Richardson L, McCullough A, et al. Changing patterns of enteric fever incidence and increasing antibiotic resistance of enteric fever isolate in the United States, 2008-2012. Clin Infect Dis. 2016;63(3):322-9. https://doi.org/10.1093/cid/ciw232
    PMid:27090993

40. Capoor MR, Nair D. Quinolones and cephalosporins resistance in enteric fever. J Glob Infect Dis. 2010;2(3):258-62. https://doi.org/10.4103/0974-777X.68529
    PMid:20927288

41. Hussain A, Satti L, Hanif F, Zehra NM, Nadeem S, Bangash TM, et al. Typhoidal *Salmonella* strains in Pakistan: An impending threat of extensively drug-resistant *Salmonella* Typhi. Eur J Clin Microbiol Infect Dis. 2019;38(11):2145-9. https://doi.org/10.1007/s10096-019-03658-0

42. Rowe B, Ward LR, Threlfall EJ. Ciprofloxacin-resistant *Salmonella* Typhi in the UK. Lancet. 1995;346(8985):1302. https://doi.org/10.1016/s0140-6736(95)91906-6
    PMid:7475749

43. Medalla F, Sjölund-Karlsson M, Shin S, Harvey E, Joyce K, Theobald L, et al. Ciprofloxacin-resistant *Salmonella enterica* Serotype Typhi, United States, 1999-2008. Emerg Infect Dis. 2011;17(6):1095-8. https://doi.org/10.3201/eid1706.100594
    PMid:21749779

44. Chitnis S, Chitnis V, Hemvani N, Chitnis DS. Ciprofloxacin therapy for typhoid fever needs reconsideration. J Infect Chemother. 2006;12:402-4. https://doi.org/10.1007/s10156-006-0472-9
    PMid:17235649

45. Britto CD, John J, Verghese VP, Pollard AJ. A systematic review of antimicrobial resistance of typhoidal *Salmonella* in India. Indian J Med Res. 2019;149(2):151-63. https://doi.org/10.4103/ijmr.IJMR_830_18
    PMid:31219079

46. Frenck RW Jr., Mansour A, Nakhla I, Sultan Y, Putnam S, Wierzba T, et al. Short-course azithromycin for the treatment of uncomplicated typhoid fever in children and adolescents. Clin

Infect Dis. 2004;38(7):951-7. https://doi.org/10.1086/382359

PMid:15034826

47. Misra R, Prasad KN. Antimicrobial susceptibility to azithromycin among *Salmonella enterica* Typhi and Paratyphi A isolates from India. J Med Microbiol. 2016:65(12):1536-9. https://doi.org/10.1099/jmm.0.000390

PMid:27902389

48. Lo J, Kulp SK, Chen C, Chiu H, Sensitization of intracellular *Salmonella enterica* serovar Typhimarinarum to aminoglycosides *in vitro* and *in vivo* by a host-targeted antimicrobial agent. Antimicrob Agents Chemother. 2014;58(12):7375-82. https://doi.org/10.1128/AAC.03778-14

PMid:25267669

49. Kumar A, Pundit V, Shetty S, Rao CR, Pattanshetty S, Samarasinghe CM. Study of clinical profile and antibiotic sensitivity in culture positive typhoid fever cases. Indian J Community Med. 2012;37(4):256-8. https://doi.org/10.4103/0970-0218.103475

PMid:23293441