

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.807.113>

Study of *Salmonella* Isolates and their Antimicrobial Susceptibility Pattern in a Tertiary Care Hospital of NE-India

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ABSTRACT

Enteric fever is a global health problem and is also endemic in India. The etiologic agents of enteric fever *i.e.* *S. typhi* and *S. paratyphi* occurs mostly through food-borne or waterborne transmission. Worldwide, however, there are an estimated 22 million cases of enteric fever annually, with 600,000 deaths and an estimate of the annual typhoid incidence rate of 493.5 per 100,000 person years was reported from India in 2008. The laboratory diagnosis of enteric fever is very important mainly in this post-antibiotic era. Isolation of *Salmonella* by blood culture is proof that the patient has a salmonella septicemia and still is the definitive diagnosis for a patient. Antibiotic therapy is the only effective treatment for enteric fever. Currently, fluoroquinolones and third generation cephalosporins are drugs of choice for treatment of typhoid fever though recent reports of decreased susceptibility to these agents and emergence of resistance to chloramphenicol, ampicillin and cotrimoxazole have led to the prospect of re-emergence of untreatable typhoid fever and an increasing global burden. A total of 110 nos. of blood samples were collected from patients (cases) clinically suspected of Enteric fever for a duration of one year from August'2016-July' 2017. *Salmonella* isolation was done by conventional methods of culture. Species identification was done by KBO11. Confirmed *Salmonella* isolates were tested by serotyping and antimicrobial susceptibility of isolated *Salmonella spp* were tested by modified Kirby-Bauer disc diffusion method as per the recommendation of Clinical and Laboratory Standard Institute (CLSI). Out of 110 blood culture samples, *Salmonella spp* were isolated and prevalence was 18.18% (20 cases), out of which *S.typhi* (18 cases-90 %) was most commonly isolated organism followed by *S. paratyphi* (2cases -10 %). 70% of the culture positive cases were males (14) and 30% were females (6), with a male and female ratio 2.33 : 1. The majority of the blood culture confirmed cases (55%) belong to the age group of 21-40 years. Though enteric fever cases occurred in all months throughout the year, maximum cases occurred during the rainy season from April-July followed by August-September. All 18 *S.Typhi* isolates showed sensitivity to Ceftriaxone (100%), Azithromycin (100 %) whereas sensitivity to Cotrimoxazole (94.44 %) and Chloramphenicol (88.88 %) was also high. 100% sensitivity was observed to third-generation cephalosporins. Least sensitivity was observed to Nalidixic acid. Though nowadays, rapid diagnostic procedures is being used to "suspect" enteric fever it is prudent to follow them up with blood culture confirmation. In-vitro antibiotic sensitivity should be routinely carried out in all patients of enteric fever for prompt and effective management which will minimise the indiscriminate use of antibiotics.

Keywords

Blood culture,
Salmonella,
Antimicrobial
sensitivity

Article Info

Accepted:
10 June 2019
Available Online:
10 July 2019

Introduction

Enteric fever is a global health problem and is also endemic in India. It is a systemic disease characterized by fever and abdominal pain and caused by dissemination of *S. typhi* or *S. paratyphi*. The disease was initially called typhoid fever because of its clinical similarity to typhus. However, 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.⁽¹⁾

The etiologic agents of enteric fever i.e. *S. typhi* and *S. paratyphi* serotypes A, B, and C—have no known hosts other than humans. Most commonly, food-borne or waterborne transmission results from fecal contamination by ill or asymptomatic chronic carriers. Health care workers occasionally acquire enteric fever after exposure to infected patients or during processing of clinical specimens and cultures.⁽¹⁾

With improvements in food handling and water/sewage treatment, enteric fever has become rare in developed nations. Worldwide, however, there are an estimated 22 million cases of enteric fever annually, with 600,000 deaths.⁽²⁾

The incidence is highest (>100 cases per 100,000 population per year) in south central and Southeast Asia; medium (10–100 cases per 100,000) in the rest of Asia, Africa, Latin America, and Oceania (excluding Australia and New Zealand); and low in other parts of the world.⁽¹⁾ An estimate of the annual typhoid incidence rate of 493.5 per 100,000 person years was reported from India in 2008 in the Bulletin of the World Health Organization.⁽³⁾

A high incidence of enteric fever correlates with poor sanitation and lack of access to

clean drinking water.⁽¹⁾ The highest incidence occurs in the regions where water supply serving large populations is contaminated with faeces.⁽⁴⁾

A recent outbreak of typhoid fever occurred in Jorhat town of Assam, India in the January'2014 which occurred due to *S. typhi* (46.15%) contaminating the water supply⁽⁶⁾.

The laboratory diagnosis of enteric fever is very important mainly because in post-antibiotic era most of the patients are treated empirically by the local medical practitioners and when the fever does not subside, these cases are labeled as pyrexia of unknown origin (PUO) and investigated for various causes of PUO including enteric fever. At this stage the typical signs and symptoms are hardly observed.⁽⁵⁾

The presence of *S. typhi* or *S. paratyphi* is detected either by culture of the organism or by the demonstration of specific antibodies or antigen in the serum.⁽³⁾ Isolation of a *Salmonella* by blood culture is proof that the patient has a *Salmonella* septicemia. Isolation from the feces is of less certain significance. Since *Salmonella* may be present in the faeces of carriers, it does not amount to proof of a causal role.⁽⁷⁾

There is an urgent need for the rational design and evaluation of effective and appropriate diagnostic tools for enteric fever which must include the emerging threat of *S. Paratyphi*.⁽⁸⁾

Antibiotic therapy is the only effective treatment for enteric fever. Although effective treatment with chloramphenicol was introduced in 1948, the emergence of resistance to chloramphenicol, ampicillin and cotrimoxazole has been of concern. Currently, fluoroquinolones and third generation cephalosporins are drugs of choice for treatment of typhoid fever. However, recent

reports of decreased susceptibility to these agents have led to the prospect of re-emergence of untreatable typhoid fever and an increasing global burden.⁽⁹⁾

Keeping in view all the above, the present study was undertaken at GMCH, Assam to study the prevalence of *Salmonella* species in clinically suspected cases of Enteric fever and to study the antibiotic susceptibility pattern of the isolates.

Materials and Methods

A total of 110 nos. of blood samples were collected from patients (cases) clinically suspected of Enteric fever for a duration of one year from August'2016-July' 2017.

The study was commenced with ethical approval and clearance certificate from the IEC, GMCH.

Salmonella isolation was done by conventional methods of culture. Species identification was done by KBO11 (HIMEDIA). Confirmed *Salmonella* isolates were tested by slide agglutination test using commercially prepared anti-sera using O typing sera for group identification and H typing sera for type identification. Antimicrobial susceptibility of isolated *Salmonella spp* were tested by modified Kirby-Bauer disc diffusion method as per the recommendation of Clinical and Laboratory Standard Institute (CLSI). *E. coli* 25922 ATCC were used as quality control strains.

Inclusion criteria

- 1) Patients attending outpatient department and in-patients of various departments clinically suspected to be suffering from enteric fever were included in the study.
- 2) Patients suspected with having enteric

(typhoid) fever may have the following signs and symptoms:

Gradual onset fever with headache, malaise, anorexia, a coated tongue and abdominal discomfort with either constipation or diarrhea.

Step-ladder pyrexia, with relative bradycardia and toxemia.

A soft, palpable spleen with hepatomegaly.

Rose-spots that fade on pressure (mainly appear during 2nd or 3rd week of enteric fever).

- 3) Individuals of both sexes representing all ages.

Exclusion criteria

- 1) Patients having no signs and symptoms of Enteric (typhoid) fever.
- 2) Fever with any obvious focus for other infection such as Urinary tract infection, Blood septicaemia, Fungal infection, Otitis media etc.
- 3) Patients with prior antibiotic administration and TAB vaccination.

Results and Discussion

Out of 110 cases, 75 cases were male and 35 were female. Majority of the cases (45.45%) belong to the age group of 21-40 years with overall male-female ratio 2.14: 1. Majority of the male cases 46.67% (35/75) and female cases 42.85% (15/35) belonged to age group of 21-40years.

Out of 110 cases, 18.18% (20 cases) were confirmed by blood culture. Similar observation was seen in study done by Patel *et al.*, (2013)⁽¹⁰⁾ and Bhattacharya *et al.*, (2017)⁽¹¹⁾. Relative low rate of isolation from blood culture has been attributed to delay in visiting the physician for diagnosis, wide

spread antibiotic use and the difficulties of obtaining large enough blood volumes. 70% of the culture positive cases were males (14) and 30% were females (6), with a male and female ratio 2.33:1 which correlates with the studies of Sood and Taneja⁽¹²⁾ and Jog *et al.*,

(2008)⁽¹³⁾. This might be due to our cultural background where male is more likely to report to hospital, at the same time more likely to contract infection due to more outdoor activities (Table 1–3).

Table.1 Age distribution of cases

Age (in yrs)	Male	Percentage	Female	Percentage	Total	Percentage
0-20	5	6.67 %	2	5.71 %	7	6.36 %
21-40	35	46.67 %	15	42.85 %	50	45.45 %
41-60	20	26.67 %	10	28.57 %	30	27.28 %
>60	15	20 %	8	22.85 %	23	20.9 %
Total	75/110	68.18%	35/110	31.82%	110	

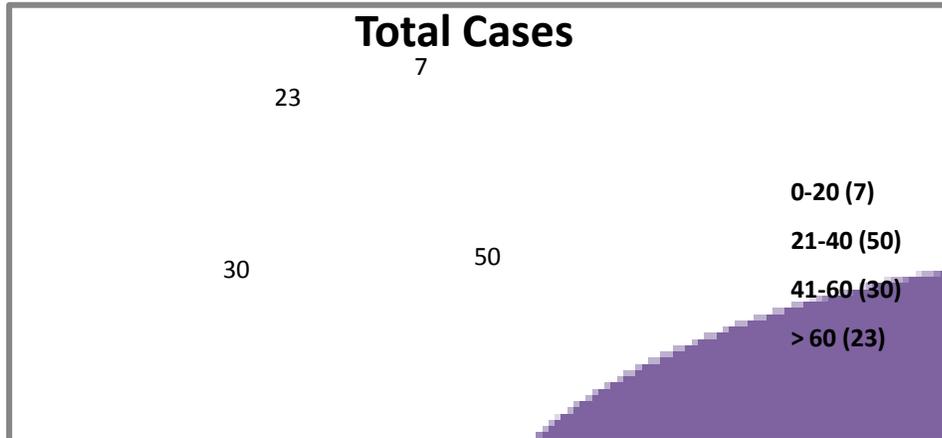
Table.2 Age and sex distribution of culture positive cases

Age (inyears)	Positive cases confirmed by Culture method			Percentages
	Male	Female	Total	
0-20	2	0	2	10
21-40	8	3	11	55%
41-60	2	1	3	15%
>60	2	2	4	20%
Total	14	6	20	
Percentage	70%	30%		

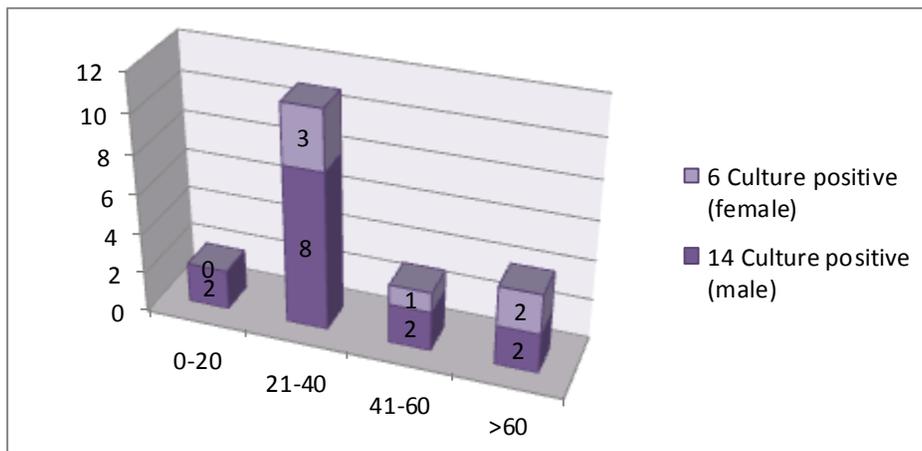
Table.3 AST of total 20 isolated *Salmonella* group of organisms

Antibiotic Discs	Abbreviation	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin	AMP	75 % (15)	-	25 % (5)
Cotrimoxazole (sulfamethoxazole+Trimethoprim)	COT	95 % (19)	-	5 % (1)
Ceftriaxone	CTR	100 % (20)	-	-
Ciprofloxacin	CIP	100 % (20)	-	-
Azithromycin	AZM	90 % (18)	-	10 % (2)
Chloramphenicol	C	85% (17)	-	15% (3)
Nalidixic acid	NA	60 % (12)	-	40 % (8)

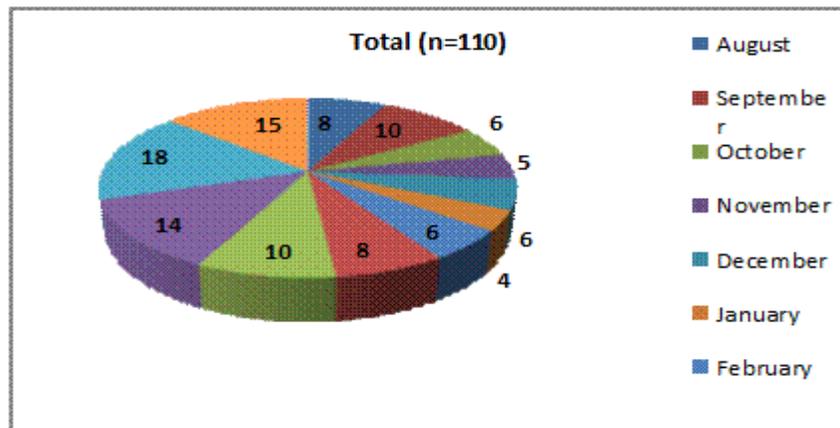
Graph.1 Age distribution of total cases



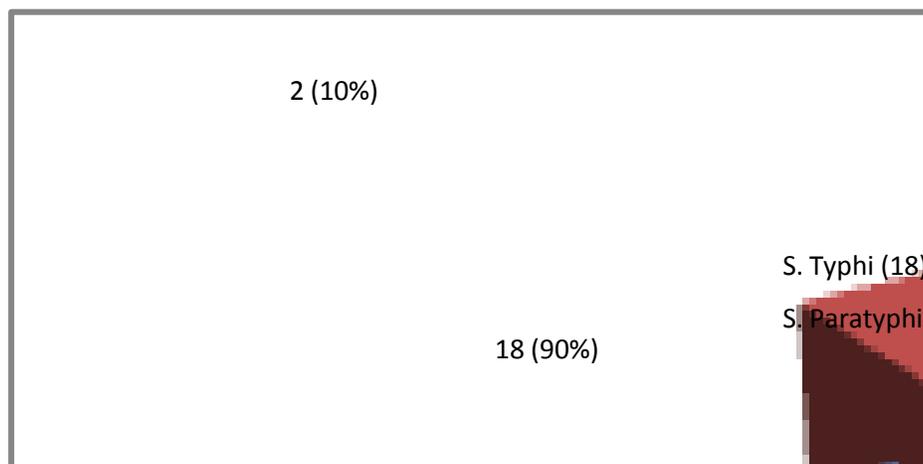
Graph.2 Age and sex distribution of culture positive cases



Graph.3 Seasonal variation of cases



Graph.4 Species distribution of 20 *Salmonella* isolates



The majority of the blood culture confirmed cases (55%) belong to the age group of 21-40 years which correlates with the study done by of J Sharma *et al.*, (2013)⁽¹⁴⁾. The possible causes for enteric fever being common in age group 21-40 yrs include their mobility, consumption of unhygienic food outside home and drinking of contaminated or unfiltered water in colleges and offices or at their jobs. Majority of Blood culture positive (75%) were detected in the 1st week of illness. In the present study, enteric fever cases occurred in all months throughout the year though maximum cases occurred during the rainy season from April-July followed by August-September. Similar observation seen in studies done by Singhal *et al.*, (2013)⁽¹⁵⁾ and J Sharma *et al.*, (2013)⁽¹⁴⁾. This occurs probably due to the higher chances of water contamination.

In this present study, out of positive blood cultures, *S. typhi* (18 cases-90 %) was most commonly isolated organism followed by *S. paratyphi* (2 cases -10 %). Similar observations are seen in study done by Manchanda *et al.*, (2006)⁽¹⁶⁾, Gupta *et al.*, (1985)⁽¹⁷⁾ and Prasad *et al.*, (2015)⁽¹⁸⁾

It is observed in our study that the 20 *Salmonella* isolates showed sensitivity to Ceftriaxone (100%) and Ciprofloxacin (100 %), followed by Azithromycin (90 %), Cotrimoxazole (90%), Chloramphenicol(85 %), Ampicillin (75 %) and Nalidixic acid (60%). Similar observation was seen in study done by Ashwini *et al.*, (2013)⁽¹⁹⁾ and Behl *et al.*, (2017)⁽²⁰⁾. Patel *et al.*, (2013)⁽¹⁰⁾ which reported 100% sensitivity to Azithromycin and Ofloxacin. S Dahiya *et al.*, (2017)⁽²²⁾ reported 100% sensitivity to third-generation cephalosporins which correlates with the present study.

All 18 *S. typhi* isolates showed sensitivity to Ceftriaxone (100%), Ciprofloxacin (100 %), Azithromycin (100 %) whereas some sensitivity to Cotrimoxazole (94.44 %), Chloramphenicol (88.88 %), Ampicillin (83.33 %) and Nalidixic acid (55.55 %) were also observed.

The present study correlates with studies done by Patel *et al.*, (2017)⁽²³⁾ who reported 98% *S. typhi* sensitivity to Chloramphenicol and least sensitivity to Nalidixic acid (23.4%) and Kumar Y *et al.*, (2011)⁽²¹⁾ which reported *S. typhi* isolates to be sensitive to

Chloramphenicol (95.3%) and Trimethoprim (94.5%).

100% of *S. paratyphi* B sensitive to Ciprofloxacin, Ceftriaxone and Cotrimoxazole. 50% of *S. paratyphi* were sensitive to Ampicillin, Chloramphenicol and Nalidixic acid. None of *S. paratyphi* were sensitive to Azithromycin.

In conclusion, after analyzing it was concluded that the clinical diagnosis of enteric fever is a true diagnostic challenge as it may simulate many fever causing infectious diseases. Definitive diagnosis is still the isolation of the causative organism from the patient. Though nowadays, rapid diagnostic procedures can be used to “suspect” enteric fever more strongly than on clinical grounds alone, it is prudent to follow them up with blood culture confirmation. In-vitro antibiotic sensitivity should be routinely carried out in all patients of enteric fever for prompt and effective management which will minimise the indiscriminate use of antibiotics, minimise occurrence of drug resistance and reduce the economic and morbidity burden in hospitals.

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How to cite this article:

Anjan Sarma and Dipa Barkataki. 2019. Study of *Salmonella* Isolates and their Antimicrobial Susceptibility Pattern in a Tertiary Care Hospital of NE-India. *Int.J.Curr.Microbiol.App.Sci*. 8(07): 939-946. doi: <https://doi.org/10.20546/ijemas.2019.807.113>