A Study on Incidence of Enteric Fever among Febrile Patients in a Tertiary Care Hospital of Eastern India with Special Emphasis on Comparative Evaluation of Blood Culture and Serodiagnosis along with Their Antimicrobial Susceptibility and Sociodemographic Profiles

Santanu Pramanik¹, Bipasa Chakraborty², Gadadhar Mitra³, Somnath Dasgupta⁴, Raja Ray⁵

¹The University of Burdwan, Burdwan, West Bengal, India. ²Department of Microbiology, R G Kar Medical College, Kolkata, West Bengal, India. ³Department of Microbiology, Burdwan Medical College, Burdwan, West Bengal, India.

⁴Department of Medicine, Burdwan Medical College, Burdwan, West Bengal, India. ⁵Department of Microbiology, Institute of Post Graduate Medical Education & Research (IPGMER), Kolkata, West Bengal, India.

ABSTRACT

BACKGROUND

Enteric fever is an important communicable disease, endemic in India and continues to be major cause of morbidity and mortality. This study was undertaken with the objectives of detecting the incidence of enteric fever, evaluating their socio-demographic profiles, comparative evaluation of sensitivity and specificity of diagnostic modalities like blood culture, Widal test, and Typhidot test in the laboratory diagnosis of enteric fever and to determine the antimicrobial susceptibility patterns.

METHODS

Blood samples were collected from 145 patients having fever for at least 5 days and symptoms suggestive of enteric fever. Blood culture and identification by standard conventional methods and antimicrobial susceptibility tests using modified Kirby-Bauer disc diffusion method were performed. Widal and Typhidot tests were done. Sociodemographic data and socioeconomic classification (updated BG Prasad scale) were collected from every patient by standard questionnaires at the time of sample collection. Data analysis was done using GraphPad Prism software.

RESULTS

Incidence of enteric fever among 145 suspected febrile patients was 36.55% of which laboratory confirmed culture positive incidence was 18.62% and probable cases of enteric fever based only on positive serodiagnosis was 17.93%. Isolation rate of *Salmonella Typhi* and *S. Paratyphi A* was 17.24% and 1.38% respectively. Resistance showed by these isolates were 55.55% to ampicillin, 29.63% to chloramphenicol, 44.44% to cotrimoxazole, 25.92% to ciprofloxacin and nalidixic acid, 7.41% to azithromycin, whereas they were 100% sensitive to ceftriaxone. Multidrug resistant (MDR) *Salmonella Typhi* was 22.22%. Widal test & Typhidot seropositivity were 35.86% and 26.89% respectively. The sensitivity, specificity, positive predictive value and negative predictive value of Widal test were 96.29%, 77.97%, 50% and 98.92% respectively and for Typhidot were 100%, 89.83%, 69.23% and 100% respectively. Though most of the febrile patients were between 13-24 years of age, the burden of enteric fever was maximum in under 12 years age group and it is the only significant risk factor ($p=0.0020$) associated with enteric fever. Socioeconomic status showed that majority of patients were in the lower and lower middle class (78.62%).

CONCLUSIONS

High rate of MDR *S. Typhi* is alarming and suggests need for better living standards, health education, correct diagnosis and proper treatment as control strategies.

KEY WORDS

Enteric Fever, Blood Culture, Widal Test, Typhidot, Antimicrobial Susceptibility, MDR *Salmonella Typhi*

Corresponding Author:

Dr. Bipasa Chakraborty,
Flat-1B, Anandam Apartment,
107, Garfa Main Road,
Kolkata-700075, West Bengal, India.
E-mail: bipasa_doc@yahoo.co.in

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BACKGROUND

Enteric fever is an important communicable disease prevalent in Eastern India. Advances in public health and hygiene have led to the virtual disappearance of enteric fever from most of the developed countries. It is a sporadic disease in developed countries with occasional point-source epidemics.¹ But the disease is still endemic in many developing countries like India where it continues to be major cause of morbidity and mortality.² Potential risk factors for enteric fever are taking contaminated food and water,^{3,4} having close contact with recent enteric fever patient,⁵ poor housing admixture of sewage and drinking water supplies during monsoon flooding due to inadequate and modernised sewage system and finally inadequate personal hygiene,⁶ and recent use of broad-spectrum antibiotics.⁷ With changing pattern in clinical presentation of enteric fever in different age groups and large number of culture negative cases due to early inadvertent use of antibiotics, diagnosis of enteric fever became more difficult. In 1948, entry of chloramphenicol revolutionised treatment of enteric fever transforming a severe and fatal disease into a treatable condition.⁸ But the emergence of resistance to chloramphenicol and other antimicrobial agents gradually became a major set back.⁹ However, rising antimicrobial resistance has been associated with increased severity of illness and related complications. Multidrug resistant (MDR) *Salmonella Typhi*, resistant to chloramphenicol, cotrimoxazole and ampicillin are prevalent in parts of Asia including India and Africa.¹⁰ Fluoroquinolone resistance is also widespread and now resistance to third generation cephalosporins and azithromycin sporadic cases have emerged.¹¹ Treating physician is also facing problem with increase in number of drug resistant strains of *Salmonella Typhi*. So, to improve our ability of the correct diagnosis, we have to give emphasis on diagnostic modalities of better sensitivity and specificity, along with proper validated antimicrobial susceptibility testing technique from the culture isolates.^{12,13,14}

So, this study was undertaken with the objectives to detect the incidence of enteric fever, to evaluate the socio-demographic profiles, to do comparative evaluation between blood culture, Widal test, and Typhidot test in the laboratory diagnosis of enteric fever and to determine the antimicrobial susceptibility patterns.

METHODS

This is a cross-sectional observational study done at Department of Microbiology, Burdwan Medical College and Hospital (BMCH), West Bengal, a tertiary care hospital giving service to patients coming from rural and urban areas. This study was done from March 2015 to August 2016 after obtaining approval from the Institutional Ethical Clearance Committee. Patients who had attended medicine OPD of BMCH with fever for at least 5 days with associated signs and symptoms suggestive of enteric fever were included in this study.

Blood samples were collected aseptically from 145 patients after taking proper informed consent. The required

sample size was calculated using the single proportion formula $N = (Z)^2 P (1-P)/d^2$ by considering expected prevalence (P) of 31%, 95% confidence interval ($Z = 1.96$) with a 5% margin of error or precision (d). Also, these 145 cases were selected based on inclusion criteria attending medicine OPD during the study period. Socio-demographic profile data like age, sex, urban-rural distribution, source of drinking water based on pipeline water supply, tube well or well were collected from every patient by standard questionnaires at time of sample collection. The socioeconomic status of every patient was determined based on modified updated BG Prasad scale.¹⁵ It is an updated income-based scale which is a measure of the social standing and an important factor affecting the health condition of an individual or a family in the society.¹⁵

For blood culture, blood was collected aseptically from two different sites half an hour apart and immediately introduced into sterile blood culture bottle with brain heart infusion (BHI) broth. For adult patients 5 ml blood was added in 50 ml of BHI broth and for paediatric patients 2 ml blood added in 20 ml BHI broth. The blood culture bottles were immediately sent to Microbiology laboratory and incubated aerobically at 37° C till there were signs of turbidity or growth positivity for up to 7 days. All blood culture bottles with turbidity were sub-cultured on xylose-lysine-deoxycholate agar (XLD), deoxycholate citrate agar (DCA) and MacConkey agar plate and incubated aerobically at 37° C for 24 hours. Characteristic growth on XLD and DCA were further identified by standard conventional phenotypic methods of identification using biochemical tests.¹⁶ Isolation of *S. Typhi* and *S. Paratyphi A* were confirmed serologically by slide agglutination test with polyvalent O *Salmonella* group antisera, and serotype specific antisera procured from Difco Laboratories, (Difco-Thermofisher Scientific) USA.

Antimicrobial susceptibility testing was performed on Muller Hinton agar plate using modified Kirby-Bauer disc diffusion method following standard methods.¹⁷ The antibiotic discs used were ampicillin (30 µg), ceftriaxone (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), tetracycline (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg) and azithromycin (15 µg), all procured from Himedia Laboratories Pvt Ltd. Interpretation was done as per Clinical and Laboratory Standards Institute (CLSI) guidelines.¹⁷

Widal tube agglutination test was done using *S. Typhi* TO and TH antigens and *S. Paratyphi* AH and BH antigens procured from Tulip diagnostics, Tydal test kit. Serum sample was serially diluted by using fresh 0.95% saline preparation from 1:20 to 1:640 dilution for anti O and anti H titre separately in Felix and Dreyers tubes. The test was performed following the manufacturer's instructions.

Typhidot test, a rapid dot-enzyme immune assay, which detects IgG and IgM antibodies against a specific 50 kD outer membrane protein (OMP) antigen of *Salmonella Typhi* was also done. The test was done on serum samples using *Salmonella Typhi* IgG/IgM Rapid Test (Sensit RAPID), manufactured by Ubio Biotechnology Systems Pvt Ltd. Following the manufacturer's instructions, the results were visually interpreted within 15 minutes.

We defined enteric fever with blood culture confirmed cases for *Salmonella Typhi* or *Salmonella Paratyphi A*, and with positive serodiagnosis based on interpretation of Widal

test and positive Typhidot IgM test. Widal test was considered positive, when titre was $\geq 1:80$ for TO and $\geq 1:160$ for TH/AH/BH or there was four fold rise in titre in paired sera taken after 10-14 days apart. WHO definition of probable/suspected enteric fever is given as fever of at least 3 days, but to increase the diagnostic sensitivity, fever for at least 5 days duration were included in our study.¹⁸

Statistical Analysis

Data analysis from questionnaires and laboratory investigations was done using Excel spread sheet (Microsoft Corporation), descriptive biostatistics, % prevalence, sensitivity, specificity, positive predictive value and negative predictive value and chi square test by Graph Pad Prism version 5.00 (GraphPad software, San Diego, CA, USA). All statistical tests by chi square test were considered significant if the P-value was ≤ 0.05 .

RESULTS

During the study period total 145 febrile patients with symptoms suggestive of Enteric fever were included. Incidence of enteric fever among these 145 patients was 36.55% (n=53), of which laboratory confirmed culture positive incidence was 18.62% (n=27) and probable cases of enteric fever based on positive serodiagnosis where culture showed no growth was 17.93% (n=26).

Blood Culture and Antimicrobial Susceptibility Test

From blood culture, 25 *Salmonella Typhi* and two *S. Paratyphi A* were isolated. (table-1) Overall resistance shown by these 27 isolates were 55.55% to ampicillin (n=15), 29.63% to chloramphenicol (n=8), 44.44% to cotrimoxazole (n=12), 25.92% to ciprofloxacin and nalidixic acid (n=7), 7.41% to azithromycin (n=2), whereas all 27 isolates (100%) were sensitive to ceftriaxone. (figure-1) Among these, six isolates were multidrug resistant (MDR) *Salmonella Typhi* (22.22% prevalence) with resistance to ampicillin, cotrimoxazole (trimethoprim/ sulfamethoxazole) and chloramphenicol.

Widal Tube Agglutination Test

The serological positivity by Widal test in this study was 35.86% (n=52/145). Whereas blood culture showed only 27 (18.62%) febrile patients as culture confirmed cases, with one Widal false negative test result. (table-1) The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Widal test were 96.29%, 77.97%, 50% and 98.92% respectively as compared with the gold standard test of blood culture. (table-2)

Typhidot Test

Serodiagnosis by Typhidot IgM was 26.89% (n=39/145). (table-1) The sensitivity, specificity, PPV and NPV for Typhidot IgM test were 100%, 89.83%, 69.23% and 100% respectively. (table-2)

Sociodemographic Profiles

Out of 145 patients, 76 (52.41%) were male and rest 69 (47.58%) were female. Age of the participants ranged from 5 years to 60 years and 60% study population were less than

25 years old (n=87/145) with mean age of 23.51 years. The results of enteric fever cases and its association with sociodemographic factors were shown in table 3.

Laboratory Investigations (n=145)	Organisms Identified	No. of Positive Results	% Incidence
Blood culture	<i>S. Typhi</i>	25	17.24%
	<i>S. Paratyphi A</i>	2	1.38%
Widal tube agglutination test	<i>S. Typhi</i>	48 TO ($\geq 1:80$) + TH ($\geq 1:160$) = 46, TO ($\geq 1:80$) = 2	33.10%
	<i>S. Paratyphi A</i>	4TO ($\geq 1:80$) + AH ($\geq 1:160$)	2.76%
Typhidot IgM test	<i>S. Typhi</i>	39	26.89%

Table 1. Comparison of Incidence of Enteric Fever by Laboratory Diagnosis by Blood Culture, Widal Test, and Typhidot Test

Parameters	Widal Test	Typhidot
Sensitivity	96.29%	100%
Specificity	77.97%	89.83%
Positive Predictive Value	50%	69.23%
Negative Predictive Value	98.92%	100%

Table 2. Comparative Evaluation of Widal Test and Typhidot Test as Compared with Blood Culture

Socio-Demographic Parameters Studied		Total Numbers Tested (n=145)	Enteric Fever Positive (n=53)	% Prevalence	X ²	p
Sex	Male	76	33	43.42%	3.250	0.0714
	Female	69	20	28.98%		
Age	≤ 12 yrs.	42	25	59.52%	14.76	0.0020
	13-24 yrs.	45	15	33.33%		
	25-40 yrs.	31	7	22.58%		
	>40 yrs.	27	6	22.22%		
Urban-rural distribution	Urban	33	11	33.33%	0.1908	0.6622
	Rural	112	42	37.50%		
Source of drinking water	Pipeline	61	21	34.43%	0.2924	0.8640
	Tube well	62	23	37.10%		
	Well	4	1	25%		
Socio-economic class (updated B G Prasad scale)	I (upper)	4	1	25%	2.478	0.6486
	II (upper middle)	6	3	50%		
	III (middle)	21	9	42.86%		
	IV (lower middle)	79	25	31.65%		
	V (lower)	35	15	42.85%		

Table 3. Socio-Demographic Data among Suspected Febrile Patients (n=145) and in Laboratory Diagnosed Enteric Fever Cases (n=53) and their Association

Figure 1. Antimicrobial Susceptibility Testing Profile of *S. Typhi* and *S. Paratyphi A* Isolated from Blood Cultures (n=27), Showing Overall Antimicrobial Sensitivity (Marked in Green) and Resistance Pattern (Marked in Red)

DISCUSSION

The present study detected higher seropositivity (36.55%) while lower prevalence from blood culture (18.62%) with detection of 22% MDR *S. Typhi*. MDR *S. Typhi* and even sporadic cases of azithromycin and quinolone-resistant *S. Typhi* strains have been a major public health concern specially in Pakistan, Sindh reported by Qamar FN et al¹¹ and in India¹⁹ reported by Chowta MN et al. Even there are reports of the emergence and spread of extensively drug-resistant (XDR) *S. Typhi* isolates resistant to chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones, and third-generation cephalosporins, in Pakistan, by Klemm EJ et al, leaving limited treatment options for enteric fever in our hand.²⁰ In our study no XDR strains had been isolated but higher rate of isolation of MDR strains was quite alarming. Because of the readily availability of over-the-counter antibiotics and subsequent resistance to these drugs in areas of endemicity, enteric fever is becoming harder to treat. Hence proper AST reporting for each and every case of culture confirmed enteric fever is the need of the hour.

The varied positivity rate between serodiagnosis and blood culture might be due to difference in time of sample collection, false positivity in Widal test and local endemicity of enteric fever with patients having raised antibody titre from old infections. Blood samples when collected beyond 7-10 days history of fever, had lesser chance to give blood culture positive report, while Widal seropositivity increased. Similarly Widal false negative report with blood culture and Typhidot IgM test positive report was because of duration of fever was 5-7 days. Widal false positive is also not uncommon and could exist because of infection with other Enterobacteriaceae with cross reacting antibody or Malaria. Moreover in our study, Widal test though commonly used as routine screening test, it had low specificity (77.97%) and PPV (50%) in our study indicating high false positive reporting. Similar studies by Chandrashekhar et al²¹ and Aziz T et al²² also showed lower sensitivity, specificity, PPV, NPV of Widal tests. Therefore, rapid tests with better sensitivity and specificity are needed for the correct diagnosis of enteric fever.

Typhidot IgM test is a rapid dot-enzyme immune assay that becomes positive in enteric fever as early as in the first week of fever. It had high sensitivity (100%) and specificity (89.83%) with better performance than Widal test in our study. Similar studies by Bhutta ZA et al,²³ Sanjeev H et al²⁴ and Jesudason MV et al²⁵ also showed that Typhidot-M was superior to Widal test in their diagnostic sensitivity and specificity.

Maximum febrile patients were under 13-24 years age group, but the burden of enteric fever was maximum under 12 yrs age group and it is the only significant risk factor associated with prevalence of enteric fever (by Chi-square test, $p=0.0020$, 95% CI-Confidence Interval). (table-3) Though no significant association between enteric fever and socioeconomic (SE) status was found in our study (by Chi-square test, $p=0.6486$, 95% CI), but majority of patients under this study belonged to lower (V) and lower middle (IV) class ($n=114/145$, 78.62%). Better living standards with good water source, improved sanitation and better hygiene measures along with correct diagnosis and proper treatment

may contribute to control endemicity of enteric fever by potentially eliminating faecal carriers and shedders from the population. Also increasing rate of MDR *S. Typhi* is alarming and suggests need for health education and effective control strategies.

CONCLUSIONS

High rate of MDR *S. Typhi* isolation suggests the need for proper AST reporting and judicious use of antibiotics for treating enteric fever. These measures are essential to prevent emergence & spread of XDR *S. Typhi*, again transforming enteric fever into a fatal and untreatable condition. Though blood culture is the gold standard test for its diagnosis, the number of culture confirmed enteric fever cases is low. So, we often depend on serodiagnosis. For correct diagnosis of enteric fever, rapid serological tests with better sensitivity and specificity are needed. Also, better living standards, health education, and correct treatment are required to control endemicity of enteric fever.

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