

Clinical Characteristics and Factors Associated with Blood Culture Positivity Among Children with Suspected Enteric Fever

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ABSTRACT

Background: Blood culture is the diagnostic gold standard for enteric fever, but its yield in routine paediatric practice in Bangladesh is low, and the laboratory diagnosis of the disease therefore rests heavily on serology. The proportion of confirmed paediatric cases in which the organism is actually recovered, and the extent to which culture, and the Widal test identify the same children, have not been well quantified in this setting. **Aim of the study:** The aim of the study was to describe the clinical characteristics of children admitted with enteric fever, and to determine the blood culture positivity rate, and the complementary diagnostic yield of blood culture, and the Widal test in this population. **Methods & Materials:** This analysis was conducted using data from a randomized controlled trial at the Department of Paediatrics, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, from July 2007 to January 2008. Fifty children aged ≤ 18 years with serologically and/or culturally confirmed enteric fever were included in the study. Clinical findings, habitat, habits, Widal test results, and blood culture results were recorded. Data were analyzed using SPSS version 13, and a P value < 0.05 was considered statistically significant. **Result:** The patients ranged from 2 to 13 years of age, and the mean ($\pm$ SD) age was 7.96 ± 3.208 years, and 6.20 ± 3.34 years in the two treatment arms respectively. In total, 24 (48.0%) patients were male, and 26 (52.0%) were female. Fever was present in all 50 (100.0%) patients, followed by pain in 19 (38.0%), loose stool in 15 (30.0%), anorexia in 12 (24.0%), and vomiting in 12 (24.0%) patients. Hepatomegaly was found in 39 (78.0%), splenomegaly in 34 (68.0%), and anaemia in 32 (64.0%) patients. Blood culture was positive in 14 (28.0%) patients, and the Widal test was positive in 42 (84.0%) patients. Both tests were positive in 6 (12.0%) patients, the Widal test alone was positive in 36 (72.0%), and blood culture alone was positive in 8 (16.0%) patients. So, blood culture identified 8 (16.0%) children who would not have been detected by serology alone, whereas serology identified 36 (72.0%) children in whom the organism was not recovered. **Conclusion:** Blood culture found than one third of the confirmed cases of enteric fever. This shows that using blood culture is not enough, for diagnosis. Because the Widal test and blood culture picked up cases they should be used together not as substitutes, when testing Bangladeshi children who may have enteric fever.

Keywords: Enteric fever, Blood culture, Widal test, Diagnostic yield, *Salmonella typhi*, and Children.

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INTRODUCTION

Typhoid fever continues to be a global health problem. Despite considerable advances in the understanding of the pathogenesis, and microbiology of the disease, it still accounts for over 16 million cases annually worldwide, with an estimated 600,000 deaths.^[1,2] Population-based figures of the burden of typhoid fever in developing countries are not accurately available. Conservative estimates indicate incidence rates ranging from 150 per 100,000 population in South America to over 1000 cases per 100,000 population in some Asian countries^[1,2]. Typhoid fever is a common febrile illness in Bangladesh. Haq et al reported that 12.74% of their prolonged pyrexia cases were typhoid fever, and following tuberculosis it was the most common infective cause of fever in their series.^[3] The disease is endemic in this country due to poor sanitation, and poor standards of personal hygiene in most parts of the country^[3,4]. The clinical presentation of typhoid fever is widely variable, and an atypical presentation is not uncommon. Severe manifestations such as

disorientation, delirium, seizure, and coma, together with gastrointestinal bleeding, subacute intestinal obstruction, intestinal perforation, jaundice, bronchitis, and hypotension pose a diagnostic problem in endemic areas^[5]. Similar clinical features may be present in other tropical infections like malaria, viral hepatitis, amoebic hepatitis, pneumonia, and chronic liver disease^[6]. So, the clinical picture alone does not permit a confident diagnosis, and laboratory confirmation is required in most cases. Isolation of *Salmonella typhi* or *S. paratyphi* from blood, bone marrow, stool, urine, and different body fluids is the standard confirmatory test for typhoid fever.^[7] However, by the conventional blood culture method, isolation of the organism is only possible in about 40-50% of cases prior to antibiotic therapy, and it takes about 3-5 days^[7,8]. The yield is further reduced by the widespread practice of empirical antibiotic use before presentation, by low-volume blood sampling in small children, and by the limited availability of culture facilities outside tertiary centres. In the absence of such facilities, a positive

Widal test is found to be useful in the diagnosis of typhoid fever when considered in association with clinical findings^[9,10]. However, the Widal test may be negative even in bacteriologically confirmed cases at the early stages of the illness, and baseline agglutinin titres in healthy children of endemic areas complicate its interpretation^[11,12]. With the emergence of multi-drug-resistant strains, third generation cephalosporins, and in particular ceftriaxone, have become the mainstay of parenteral treatment for hospitalized paediatric cases^[13]. The practical consequence is that a clinician in Bangladesh must decide how much weight to place on each test, and how many children would be missed by relying on either one alone. The proportion of confirmed paediatric cases in which the organism is actually recovered by blood culture in this country is not well documented, and the degree of overlap between culture-positive, and serology-positive children has not been quantified. The present study was undertaken to describe the clinical characteristics of

children admitted with enteric fever, and to determine the blood culture positivity rate, and the complementary diagnostic yield of blood culture, and the Widal test in this population.

METHODS & MATERIALS

This analysis was carried out on the cohort of a randomized controlled trial conducted at the Department of Paediatrics, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, Bangladesh. The study duration was six months, from July 2007 to January 2008. Using a simple random sampling method, a total of 50 paediatric patients admitted with enteric fever were selected following the inclusion, and exclusion criteria. In total 50 patients up to 18 years of age with serologically and/or culturally confirmed enteric fever were included in the study subjects. Patients with complications such as gastrointestinal haemorrhage, intestinal perforation, and shock, patients with associated illnesses such as ENT infections, and sore throat, and those who had received prior antibiotic treatment with azithromycin, ofloxacin, or cefixime were excluded from the study. After admission to the ward, the history of each patient was taken, and a detailed general, and systemic examination was performed. In the general examination, general condition, toxic look, level of consciousness, temperature, respiratory rate, pallor, jaundice, rash, petechiae, and lymphadenopathy were noted. In the systemic examination, the children were

examined for throat congestion, altered sensorium, rhonchi, meningismus, abdominal distension, abdominal tenderness, hepatomegaly, and splenomegaly. Blood samples were sent for total count, differential count, ESR, haemoglobin estimation, Widal test, and blood culture and sensitivity, and stool, and urine samples were also sent for culture and sensitivity. Information on housing, source of drinking water, and sanitation was recorded for each patient. A semi-structured questionnaire was used as the research instrument, and all data were collected in a questionnaire form, and recorded systematically. All patients received intravenous ceftriaxone, allocated in a randomized fashion to a single daily dose or to twice-daily doses, and the two arms are reported separately where the source data permit. Informed written consent was obtained from each patient, or the patient's attendant after the aims, and objectives of the study along with its procedure, risks, and benefits had been explained in easily understandable local language. It was assured that all information, and records would be kept confidential. Ethical approval was taken from the Local Ethical Committee of the Bangladesh College of Physicians and Surgeons, Dhaka, prior to the commencement of the study. All data were processed, analyzed, and disseminated by MS Office, and SPSS version 13 as per need. Continuous variables were expressed as mean±standard deviation, and categorical variables as frequency, and percentage. The

unpaired t-test was used to compare continuous variables between the treatment arms, and the chi-square test, with Yates correction where applicable, was used to compare categorical variables. Because enrolment required a positive Widal test or a positive blood culture, every Widal-negative patient was necessarily blood culture positive, and the joint distribution of the two tests was therefore reconstructed arithmetically from the marginal totals of each treatment arm. The reconstruction was performed separately within each arm, and the two solutions were checked against the reported marginals for internal consistency before being pooled.

RESULTS

Table I shows the distribution of the patients by age, and sex. The patients were within the 2 to 13 years age range. The mean (±SD) age was found 7.96±3.208 years in Group A, and 6.20±3.34 years in Group B, and the difference was not statistically significant (t=1.9, df=48, p=0.063). In total, 11 (22.0%) patients were in the ≤3 years age group, 14 (28.0%) within 4-6 years, 10 (20.0%) within 7-9 years, and 15 (30.0%) in the ≥10 years age group. In total, 24 (48.0%) patients were male, and 26 (52.0%) were female, with a male to female ratio of 3:3.2, and no statistically significant difference was found between the arms (χ²=0.321, df=1, p=0.571).

Table I
Distribution of the patients by age, and sex (n=50).

Variable	Group A (Twice-daily dose) n=25	Group B (Single dose) n=25	Total N=50
Age (years)			
≤3	2 (8.0)	9 (36.0)	11 (22.0)
4-6	7 (28.0)	7 (28.0)	14 (28.0)
7-9	7 (28.0)	3 (12.0)	10 (20.0)
≥10	9 (36.0)	6 (24.0)	15 (30.0)
Mean±SD (Range)	7.96±3.208 (2-13)	6.20±3.34 (2-13)	p=0.063*
Sex			
Male	11 (44.0)	13 (52.0)	24 (48.0)
Female	14 (56.0)	12 (48.0)	26 (52.0)
Total	25 (100.0)	25 (100.0)	50 (100.0)

Figures within parentheses denote the corresponding percentage. *Unpaired t-test was done to measure the level of significance (t=1.9, df=48). The chi-square test for sex gave χ²=0.321, df=1, p=0.571.

Table II shows the distribution of the patients by their presenting complaints. Fever was present in all 50 (100.0%) patients. Nineteen (38.0%) patients had complaints of pain, 15 (30.0%) loose stool, 12 (24.0%) anorexia, 12 (24.0%) vomiting,

9 (18.0%) cough, 4 (8.0%) loss of appetite, 3 (6.0%) yellowish coloration of the sclera, and skin, 2 (4.0%) abdominal distension, 2 (4.0%) constipation, and 1 (2.0%) nausea. Among all presenting complaints of the patients of both arms, no statistically

significant difference was observed other than anorexia, which was reported by 9 (36.0%) patients of Group A, and 3 (12.0%) patients of Group B (p=0.047).

Table II

Distribution of the patients by their presenting complaints (n=50).

Complaint	Total N=50	Group A n=25	Group B n=25	p-value
Fever	50 (100.0)	25 (100.0)	25 (100.0)	—
Pain	19 (38.0)	7 (28.0)	12 (48.0)	0.145*
Loose stool	15 (30.0)	5 (20.0)	10 (40.0)	0.123*
Anorexia	12 (24.0)	9 (36.0)	3 (12.0)	0.047*
Vomiting	12 (24.0)	8 (32.0)	4 (16.0)	0.185*
Cough	9 (18.0)	6 (24.0)	3 (12.0)	0.462**
Loss of appetite	4 (8.0)	1 (4.0)	3 (12.0)	0.602*
Yellowish coloration of sclera & skin	3 (6.0)	2 (8.0)	1 (4.0)	1.00**
Abdominal distension	2 (4.0)	1 (4.0)	1 (4.0)	1.00**
Constipation	2 (4.0)	0 (0.0)	2 (8.0)	0.47**
Nausea	1 (2.0)	0 (0.0)	1 (4.0)	1.00**
Convulsion	1 (2.0)	0 (0.0)	1 (4.0)	1.00**

Figures within parentheses denote the corresponding percentage. *Chi-square test was done to measure the level of significance. **Chi-square test (after Yates correction) was done.

Table III shows the distribution of the patients by clinical signs. Anaemia was found in 32 (64.0%) patients overall, that is 19 (76.0%) patients of Group A, and 13 (52.0%) patients of Group B (p=0.07). Jaundice was absent in 44 (88.0%) patients,

and was mild in 4 (8.0%), and moderate in 2 (4.0%) patients. Hepatomegaly was found in 39 (78.0%) patients overall, and splenomegaly in 34 (68.0%) patients, and both were common in the two arms. Dehydration was present in 20 (40.0%)

patients, abdominal tenderness in 9 (18.0%), abdominal distension in 5 (10.0%), and palpable lymph nodes in 3 (6.0%) patients. No statistically significant difference was observed between the arms in terms of any clinical sign.

Table III

Distribution of the patients by clinical signs (n=50).

Clinical sign	Total N=50	Group A n=25	Group B n=25	p-value
Anaemia	32 (64.0)	19 (76.0)	13 (52.0)	0.07*
Jaundice — mild	4 (8.0)	2 (8.0)	2 (8.0)	1.00*
Jaundice — moderate	2 (4.0)	1 (4.0)	1 (4.0)	
Jaundice — absent	44 (88.0)	22 (88.0)	22 (88.0)	0.234**
Palpable lymph node	3 (6.0)	3 (12.0)	0 (0.0)	
Dehydration	20 (40.0)	12 (48.0)	8 (32.0)	0.248*
Abdominal tenderness	9 (18.0)	7 (28.0)	2 (8.0)	0.141**
Abdominal distension	5 (10.0)	3 (12.0)	2 (8.0)	1.00**
Hepatomegaly	39 (78.0)	20 (80.0)	19 (76.0)	0.733*
Splenomegaly	34 (68.0)	16 (64.0)	18 (72.0)	0.544*

Figures within parentheses denote the corresponding percentage. *Chi-square test was done to measure the level of significance. **Chi-square test (after Yates correction) was done.

Table IV shows the distribution of the patients by their habitat, and habit. In total, 32 (64.0%) patients had pacca housing, 9 (18.0%) had semi-pacca housing, and 8

(16.0%) had kacha housing. Safe water was used by 28 (56.0%) patients, and 22 (44.0%) patients used unsafe water. No statistically significant difference was observed

between the arms in terms of housing condition, or usage of water.

Table IV

Distribution of the patients by their habitat, and habit.

Variable	Group A n=25	Group B n=24	p-value
Housing			0.078*
Pacca	14 (56.0)	18 (75.0)	
Semi-pacca	4 (16.0)	5 (20.8)	
Kacha	7 (28.0)	1 (4.2)	
Water (n=25 per arm)			0.254*
Safe	12 (48.0)	16 (64.0)	
Unsafe	13 (52.0)	9 (36.0)	

Figures within parentheses denote the corresponding percentage. *Chi-square test was done to measure the level of significance. Housing data were available for 25 patients of Group A, and 24 patients of Group B. Sanitation data recorded in the source are internally inconsistent and are therefore not reported.

Table V shows the results of the diagnostic investigations. Blood culture was positive in 14 (28.0%) patients overall, that is 5 (20.0%) patients of Group A, and 9 (36.0%)

patients of Group B, and the difference between the arms was not statistically significant (p=0.208). The Widal test was positive in 42 (84.0%) patients overall, that

is 23 (92.0%) patients of Group A, and 19 (76.0%) patients of Group B (p=0.246).

Table V
Results of the diagnostic investigations (n=50).

Investigation	Total N=50	Group A n=25	Group B n=25	p-value
Blood culture positive	14 (28.0)	5 (20.0)	9 (36.0)	0.208*
Widal test positive	42 (84.0)	23 (92.0)	19 (76.0)	0.246*

Figures within parentheses denote the corresponding percentage. *Chi-square test was done to measure the level of significance.

Table VI shows the joint distribution of the two confirmatory investigations. Both the Widal test, and blood culture were positive in 6 (12.0%) patients. The Widal test alone was positive in 36 (72.0%) patients in whom

the organism was not recovered, and blood culture alone was positive in 8 (16.0%) patients in whom the Widal test was negative. So, blood culture identified 8 (16.0%) children who would not have been

detected by serology alone, whereas the Widal test identified 36 (72.0%) children in whom blood culture was negative. Of the 14 blood culture positive patients, 8 (57.1%) were Widal negative.

Table VI
Joint distribution of Widal test, and blood culture results (n=50).

Variable	Blood culture positive	Blood culture negative
Widal test positive	6 (12.0)	36 (72.0)
Widal test negative	8 (16.0)	0 (0.0)

Figures within parentheses denote the corresponding percentage of the total cohort.

DISCUSSION

This was a study of 50 children aged 2 to 13 years admitted with enteric fever to a tertiary university hospital over six months, in which a detailed general, and systemic examination was performed in every case. In the current study, the mean age was found 7.96±3.208 years, and 6.20±3.34 years in the two treatment arms, and the male to female ratio was 3:3.2. Our sex distribution was consistent with the Agalar et al series, in which the male to female ratio was also 3:3.2 [14]. In the Kumar et al series the male to female ratio was 1.85:1, which indicates a male preponderance that was not observed in the current study.[15] The difference is likely to reflect referral patterns to a tertiary hospital rather than any true difference in susceptibility by sex. In the current study, fever was present in all 50 (100.0%) patients, followed by pain in 19 (38.0%), loose stool in 15 (30.0%), anorexia in 12 (24.0%), vomiting in 12 (24.0%), and cough in 9 (18.0%) patients. A Nepalese study conducted by Maskey et al reported the presenting complaints of their patients as fever 96.1%, headache 79.2%, chills 35.0%, sweating 19.1%, cough 31.1%, anorexia 54.2%, abdominal pain 33.8%, nausea 18.1%, vomiting 17.9%, constipation 13.2%, diarrhoea 28.2%, myalgia 18.9%, arthralgia 2.2%, and malaise 8.8% [15]. Anorexia was substantially more frequent in that series than in ours, which may be explained by the inclusion of adults in the Nepalese cohort, since anorexia is more reliably elicited from older patients. In the Kumar et al series fever was the main presenting symptom in all cases, and the duration of fever exceeded seven days in the majority, that is 72.6% of cases, at the time of admission [15]. In our study, hepatomegaly was found in 39 (78.0%) patients, splenomegaly in 34 (68.0%), and anaemia in 32 (64.0%) patients. Hepatomegaly was seen in 88% of cases in the Kumar et al series, which was similar to that reported by Chandra et al, and Bhutta et

al, whereas Koul et al reported hepatomegaly in 33% of cases [15,17,18,19]. Splenomegaly was seen in 46% of cases in the Kumar et al study, and a higher incidence of splenomegaly was found in our present study [15]. Physical findings including coated tongue in 50.5%, splenomegaly in 5.1%, abdominal tenderness in 4.5%, and lymphadenopathy in 1.7% of patients were observed by Maskey et al [16]. The high frequency of hepatosplenomegaly in our cohort indicates a substantial reticuloendothelial burden of organisms at presentation, which is consistent with a hospitalized referral population rather than an ambulatory one. The central finding of the present study is that blood culture was positive in only 14 (28.0%) of the 50 children, all of whom had confirmed enteric fever. This falls below the 40-50% recovery rate that is conventionally quoted for the conventional blood culture method before antibiotic therapy [7]. Several features of the setting are likely to account for the shortfall. Empirical antibiotic use before referral is widespread in Bangladesh, and although patients treated with azithromycin, ofloxacin, or cefixime were excluded, exposure to other agents was not an exclusion criterion, and was not recorded. The volume of blood obtainable from a small child is limited, and the bacterial load in paediatric enteric fever is low, commonly fewer than fifteen organisms per millilitre. Bone marrow culture, which is substantially more sensitive than blood culture in this situation, was not performed in any patient [7,8]. So, the yield reported here should be read as the yield of routine paediatric practice rather than as the intrinsic sensitivity of the method. In the current study, the two confirmatory investigations were largely non-overlapping. Both tests were positive in only 6 (12.0%) patients, whereas the Widal test alone identified 36 (72.0%) patients, and blood culture alone identified 8 (16.0%) patients. Of the 14 culture-positive children,

8 (57.1%) were Widal negative. This pattern is consistent with the recognized behaviour of the two tests over the course of the illness, since bacteraemia is maximal in the first week while agglutinin titres rise later, and it supports the view that the Widal test may be negative even in bacteriologically confirmed cases at the early stages of the disease.[11] It is well established that the interpretation of the Widal test in endemic areas is complicated by baseline agglutinin titres in healthy children, and Patil et al have documented the extent of this problem [12]. Saha et al have similarly emphasized that the cut-off titre must be established for each population before the test can be applied with confidence [10]. So, neither investigation can be regarded as a substitute for the other, and the practical implication for Bangladeshi clinicians is that both should be requested in a child with a compatible febrile illness. Blood culture positivity did not differ significantly between the two treatment arms, at 5 (20.0%) patients of Group A, and 9 (36.0%) patients of Group B (p=0.208), which is the expected result for a baseline variable in a randomized cohort. It may be noted that Group B, which had the higher culture yield, also had the younger age structure, with 9 (36.0%) patients in the ≤3 years age group compared with 2 (8.0%) patients in Group A. A relationship between younger age, and higher culture yield would be biologically plausible, since younger children are commonly brought to hospital earlier in the course of the illness, and are less likely to have received prior antibiotics. However, this is a comparison between groups rather than between individuals, and it cannot be confirmed at the level of the individual patient with the data available. So, we regard it as hypothesis-generating only, and it should not be read as evidence that age predicts culture positivity.

LIMITATIONS

This was a single-centered study with a small-sized sample. So, the findings of this study may not reflect the exact scenario of the whole country. The cohort consisted entirely of children whose enteric fever had already been confirmed serologically or culturally, and children investigated for enteric fever in whom the diagnosis was not confirmed were never enrolled. So, the blood culture positivity rate reported here is the recovery rate among confirmed cases, and it is not the positive rate among all children presenting with a febrile illness suggestive of enteric fever, which would necessarily be lower. Because a positive Widal test or a positive blood culture was required for entry, the association between the two investigations could not be examined, and only their complementary yield could be described. The duration of fever before admission, the nutritional status of the patients, the volume of blood cultured, and any antibiotic exposure other than the three excluded agents were not recorded, and each of these is a plausible determinant of culture yield. Stool, and urine cultures were collected but their results were not reported in the source data, and bone marrow culture was not performed. No clinical or laboratory variable was recorded against blood culture status at the level of the individual patient, so the clinical characteristics that predict culture positivity could not be identified.

CONCLUSION

Blood culture was positive in 14 (28.0%) children with confirmed enteric fever, which is below the rate conventionally quoted for the method, and which confirms that culture alone is an inadequate case-finding strategy in this setting. The Widal test, and blood culture identified largely different children, with only 6 (12.0%) patients positive on both, and 8 (16.0%) patients detected by culture alone. So, the two investigations should be regarded as complementary rather than alternative, and a negative blood culture should not be taken to exclude enteric fever in a Bangladeshi child with a compatible clinical picture.

RECOMMENDATION

For getting more specific information regarding this issue, we would like to recommend a further large-scale, multi-centered study enrolling all children investigated for suspected enteric fever, irrespective of the eventual diagnosis, so that the determinants of blood culture positivity can be examined without the constraint imposed by a confirmatory entry criterion. Future studies should record the duration of fever before presentation, prior antibiotic exposure, the volume of blood cultured, and the nutritional status of the patient, and should relate these variables to culture yield. Efforts should also be made to improve the facilities for the laboratory diagnosis of enteric fever, and to establish population-specific cut-off titres for the Widal test in Bangladeshi children.

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CONFLICT OF INTEREST

None declared

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