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Cross sectional study to evaluate rapid diagnostic test (Typhidot-M) as a tool for early diagnosis of Typhoid fever keeping blood culture as gold standard.

Dr Neeraj Lata[1], Dr Vijay Gotwal[2], DR. Tanushree Joshi[3], DR. YogeshGupta[4], DR. D P Pande[5], Dr BrahmPrakash[6]

[1]Senior Resident, Department of Pediatrics, Government Medical College, Amritsar
[2]Junior Resident, Department of Chest and Tuberculosis, Government Medical College, Amritsar
[3]MD Pediatrics , ADMO (Pediatrics), Northern Railways Central Hospital Basant Lane, New Delhi
[4]DCH, MD Pediatrics, Head of Department of Pediatrics ,Northern Railways Central Hospital ,Basant Lane, New Delhi
[5]DCH,DNB Pediatrics, Chief Pediatrician Northern Railways Central Northern Railways Central Hospital , Basant Lane, New Delhi
[6]DCH,PG DIP HA,Phd(Med), Medical Director, Northern Railways Central Northern Railways Central Hospital , Basant Lane, New Delhi
Corresponding Author: **Dr Neeraj Lata**
E-mail: *neerajlatavijay@gmail.com*

Abstract

Introduction: Typhoid fever is highly prevalent in India with hight rate of morbidity and mortality. There is paucity of data from India for its rapid detection by Rapid diagnostic test like Typhidot-M . Thus this study has been taken up to study the use of rapid diagnostic test for typhoid fever keeping blood culture test as gold standard.

Materials and Methods : This cross sectional study was carried out in the Department of Pediatrics, Northern Railways Central Hospital, New Delhi. Blood samples of 100 patients with fever of more than 3 days duration were tested by Typhidot-M, Blood culture and S.Widal .S.Widal being done in second week of illness. Other tests for evaluation of other causes of fever were also done..

Results: Sensitivity and Specificity of Typhidot- M was found to be 97.8% and 46.3% .Positive and Negative predictive values for this test were 60.8%and 96.2%.Typhidot-M had better sensitivity and specificity than serum widal test.The association between blood culture positivity and Typhidot-M test was found to be highly significant statistically.

Conclusion: We concluded that there was significant association between blood culture positivity and Typhidot -M test in our study thus we can recommend Typhidot –M as the rapid, accurate and reliable tool for the early diagnosis of typhoid fever .

Keywords: Typhidot-M ,Typhoid fever, Blood culture

Introduction

Typhoid fever continues to be a global health problem, especially in tropics and subtropics.[1] Typhoid fever ,also called enteric fever is caused by the facultative intracellular organisms salmonella, enteric serotype typhi(S.typhi)and salmonella paratyphi.Human beings become infected with S.typhi through ingestion of faecal contaminated food, milk or water. Therefore global distribution of the disease is limited to areas with poor standards of hygiene and sanitation which facilitate its transmission.[2]

Confirmed case of typhoid fever is defined, according to the World Health Organization (WHO), as a patient with fever (> 38°C) that has lasted for at least three days, with a laboratory confirmed positive culture of S. typhi.[3]

Probable case of typhoid fever is a patient with fever (> 38°C) that has lasted for > 3 days, with a positive serodiagnosis or antigen detection test but without S. typhi isolation.[3]

Enteric fever is endemic in India with a rate of incidence ranging from 102 to 2219 per 100,000 in the population.[4]

An incidence of 980/100000 was recorded in late 1990's in a five year community based study of children in Delhi.[5]

According to the best global estimates there are at least 17 million new cases of typhoid fever each year, with 600,000 deaths.[7]Reported data by GOI for year2005shows 653580 cases and 417 deaths of typhoid fever .[2]

History, physical findings and fever pattern are suggestive but can neither confirm nor exclude typhoid.[4] One has to rely on serological diagnosis since many diagnostic laboratories in developing countries do not have facilities for blood culture.[6]

Blood culture is generally recognized as the most useful diagnostic test for detecting S.typhi. However, a single blood culture is estimated to be only 50% to 80% sensitive and the delay from specimen collection to diagnosis can be 5 to 7 days[7]. Moreover, blood culture is prohibitively expensive in most settings where typhoid fever is endemic.Serodiagnosis of typhoid fever has been attempted since 19th century when Widal and Sicard showed that serum of patients with typhoid fever agglutinate typhoid bacilli.[8]

Therefore, diagnosis and treatment of typhoid fever in endemic and resource-constrained settings is commonly done on the basis of clinical presentation or a positive Widal test, which has suboptimal sensitivity and specificity[9-11] .Isolation of serotype type from blood remains the method of choice for the laboratory diagnosis. Widal test is the mainstay in the diagnosis of typhoid fever in most laboratories but it has drawbacks.[9,10,12]

Thus there is a pressing need to develop more reliable user-friendly rapid diagnostic assays for typhoid fever.

As signs and symptoms of typhoid fever are nonspecific, the isolation of the organism from blood, bone marrow, or stool is required to confirm the diagnosis. Isolation of the organism from blood requires 5 to 10 mL of blood, 2 to 7 days' time, elaborate laboratory equipment, and a level of technical expertise, which may not be present in resource-poor laboratories. Even under the best conditions, there may be failure to isolate the organism, especially after antimicrobial treatment has been started. Culture of bone marrow is more sensitive, but the procedure is invasive [13]. Rapid dot enzyme immunoassays have been tested and are being used widely over the world from few years with varied result. Test is based on the presence of specific IgM antibodies to a specific 50 kD outer membrane protein (OMP) antigen on S.typhi and becomes positive as early as in the first week of the fever. The results can be interpreted visually and is available within three hours.[14,15]

Rapid accurate diagnosis and early treatment with suitable antimicrobials is essential for speedy recovery and for prevention of complications and deaths due to this disease.

The emergence of multidrug-resistant strains of salmonella typhi is known to be associated with significant morbidity and mortality. Its a well recognized fact that a delay in diagnosis and institution of appropriate therapy may significantly increase the risk of adverse outcome and mortality. [10]

The disease is predominantly a disease of school going children and young adults and is reported to be milder in infants and young children . [16]

This study has been taken up with the objective to evaluate the sensitivity and specificity of the Typhidot –M assay while keeping blood culture positivity as a gold standard. Panel of tests were done to rule out other causes of fever.

This study will help to diagnose typhoid fever at the earliest thus preventing the complications. Paucity of data in India on rapid diagnostic tests for detecting typhoid fever at earliest ,led us to study this issue in a cross section of population of patients attending Northern Railway Central Hospital.

Materials and Methods

The present study was a hospital based cross-sectional study. The study was carried out in the Department of Pediatrics, Northern Railways Central Hospital, New Delhi. Parents of patients were told about the nature of study and informed consent was taken. Ethics committee clearance was taken for study.

Inclusion criteria

1. Age:1-14yrs
2. Both sexes
3. Both Indoor and outdoor patients
4. Duration of fever of more >3days.
5. Fever>380C

Exclusion criteria

1. Previously antibiotic treated patients.
2. Proven localized infection.

Sample size:

100 patients from opd and indoor admission with fever of greater than 3 days duration were included in the study. Typhidot-M test,Serum Widal, Blood culture and other panel of tests for fever were sent. Other panel of tests like hemogram ,ESR,Periferal smear for malarial parasite ,Indirect coombs test for plasmodium vivax and falciparum, Liver function and kidney function tests were done. Serum widal was sent in 2nd week of fever by tube method.

Test details

1.Typhidot-M Test (ENTEROCHECK-WB by Zephyr Biomedicals)

Principle: This is a rapid, qualitative, sandwich immunoassay for the detection of IgM antibodies to S. typhi in human serum/plasma or whole blood specimen. Early rising antibodies to Lypopolysaccharide (LPS) O are predominantly IgM in nature. Detection of S. typhi specific IgM antibodies instead of IgG or both IgG & IgM (as measured by the Widal test) would serve as a marker for recent infection, qualitatively detects the presence of IgM class of Lypopolysaccharide (LPS) specific to S. typhi in human serum/plasma or whole blood specimens.It utilizes the principle of Immunochromatography, a unique two-site immunoassay on a nitrocellulose membrane.

Testing procedure and interpretation of results:

The kit components of device have to be brought to room temperature before testing. Once opened, the device must be used immediately. Place the testing device on a flat horizontal surface.Dispense 5µl of whole blood / serum / plasma into the specimen port ‘A’ using a micropipette or the sample loop provided. Dip the sample loop in the sample container and blot the sample in the sample port ‘A’. Add five drops of sample running buffer into the reagent port ‘B’. At the end of 15 minutes, read results as follows:

If IgM antibodies to S.typhi are not present, only one coloured band appears in the Control Window (C). If IgM antibodies to S.typhi are present, two coloured bands appear in the Test (T) and Control Windows (C). The test is invalid if the Control band is not visible at fifteen minutes.

2.Blood culture(BACT/ALERT- PF)

These culture bottles were used with BacT/ALERT Microbial Detection System.Its a quantitative procedure for enhanced recovery and detection of bacteria(aerobic and facultative anaerobic) from blood.

Principle of the test

The method utilizes a colorimetric sensor and reflected light to monitor the presence and production of carbondioxide dissolved in the culture medium.If microorganisms are present in the test, carbondioxide is produced as the organisms metabolize the substrates in the culture medium.When growth of the microorganisms produces CO2,the color of the gas permeable sensor installed in the bottom of the each culture bottle changes from blue green to yellow.The lighter color results in an increase of reflectance units monitored by the system. Bottle reflectance is monitored and recorded by the instrument every 10 minutes.

Specimen collection and preparation

Proper skin disinfection is an essential requirement to reduce the incidence of contamination. Upto 4 ml of sample obtained and transferred to the bottle under aseptic precautions.In the laboratory blood culture bottles were incubated at 37^0 C and checked for growth at1,2,3, and 7 days.For days1,2and 3,only bottles showing signs of positive growth were cultured on agar plates.On day 7 all bottles were subcultured before being discarded as negative.[24]

After bact-alert (blood culture)positivity for growth of bacteria,subcultures were done. [21]On blood agar,S.typhi and S.paratyphi produce non hemolytic smooth white colonies.On MacConkey agar,Salmonella produces lactose non fermenting smooth colonies..Biochemical tests were done to diagnose growth of salmonella typhi by recommended standard protocol.

Aims and Objectives

To determine the sensitivity and specificity of rapid diagnostic test (Typhidot-M) as a tool for early diagnosis of typhoid fever keeping Blood Culture as gold standard.

Results

Demographic, clinical data, laboratoryParameter details were noted and analysed using SPSSsoftware version 17(SPSS Inc., Chicago, IL, USA).

In our study Male to female ratio is 1.3:1 . Males were more affected than females.

Patients in age group 5-8yrs were maximally affected by typhoid fever followed by 9-12 yr age group.

In our study age ranged from 1.5 yrs to14 yrs with mean age of 8.2 yrs +/-3.3SD.

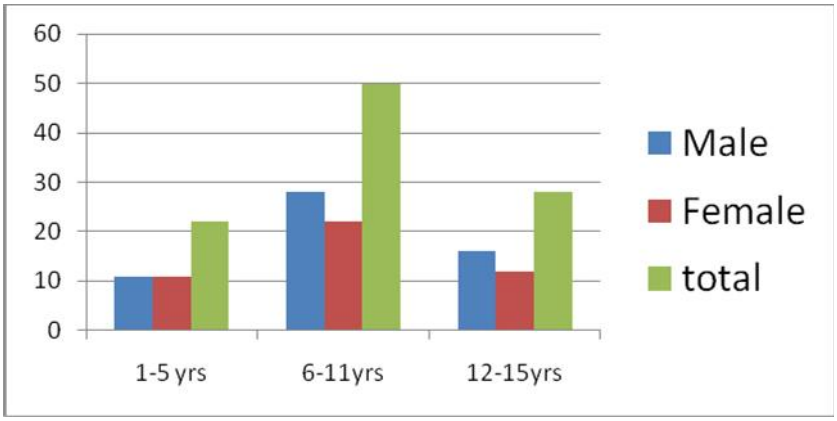

Fig 1.Age distribution of cases.

Age group 6-11 years was affected most. 50% of patients belonged to this group .In this age group males were affected more than the females

In our study population of 100 patients, 46 were having blood culture positivity for S.typhi .When we compared blood culture results in both genders , blood culture positivity in male gender was found to be statistically significant($p<0.05$)

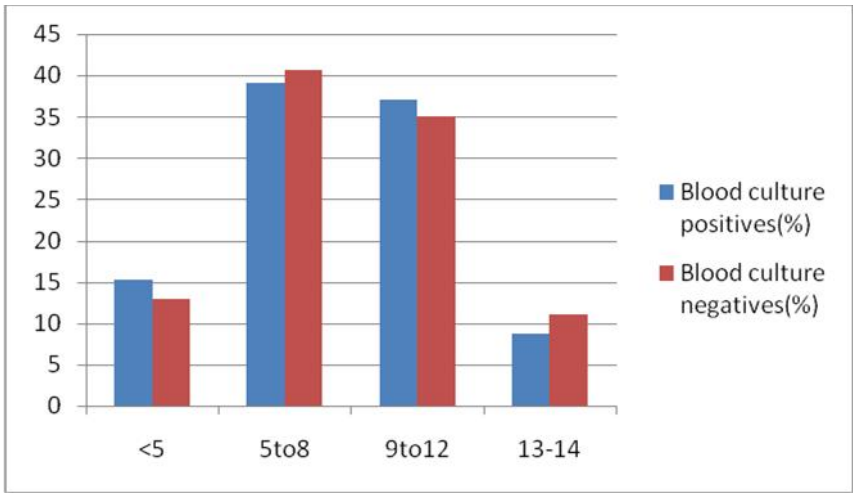

Fig 2. Distribution of Blood culture(%) results in various age groups.

When we plotted the distribution of Blood culture results in various age groups , maximum positivity was seen in age group 5-8yrs followed by 9-12yrs.

Comparison of blood culture results in various age groups found that there was no statistical significance($p>.05$).

Blood culture positivity ranged from 1.5 yrs to 14 yrs with mean age 7.880+-3.24SD and its association with age distribution was found to be statistically not significant($p>0.05$)

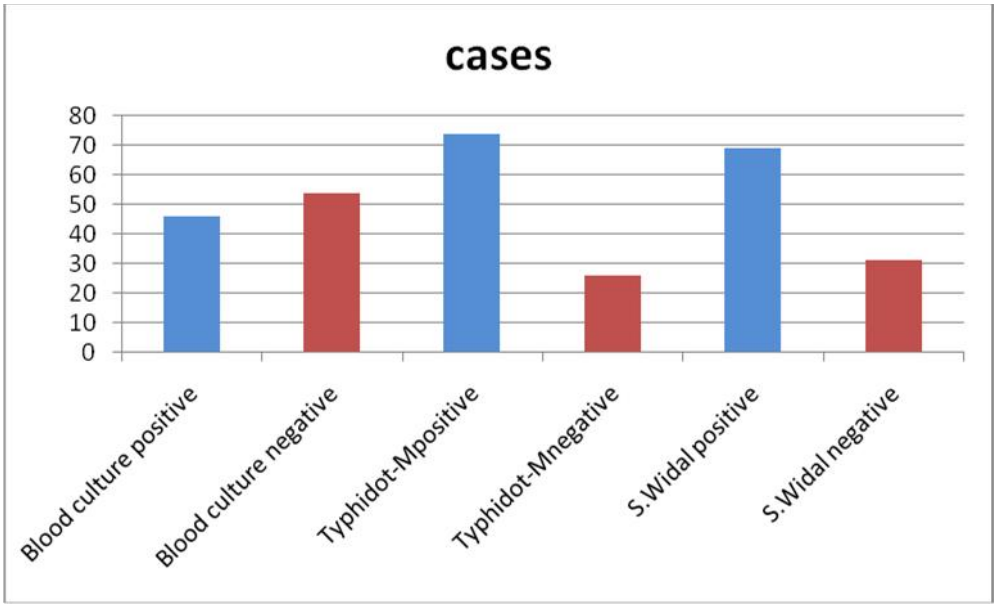

Fig 3. Results in % of three tests.

In our study 74% cases are positive for Typhidot-M test followed by 69% positive for S.Widal test.46% cases were positive for Blood culture test

Table1. Typhidot-M test and Blood culture test. 2x2 Contingency table and Calculation of S,Sp, PPV,NPV.

			BLOOD CULTURE		
			POSITIVE	NEGATIVE	Total
TYPHIDOT-M	POSITIVE	Count	45	29	74
		% within TYPHIDOT-M	**PP+60.8%**	39.2%	100.0%
		% within BLOOD CULTURE	**Se=97.8%**	53.7%	74.0%
		% of Total	**45.0%**	29.0%	74.0%
	NEGATIVE	Count	1	25	26
		% within TYPHIDOT-M	3.8%	**NP-96.2%**	100.0%
		% within BLOOD CULTURE	2.2%	**Sp=46.3%**	26.0%
		% of Total	1.0%	**25.0%**	26.0%
Total		Count	46	54	100
		% within TYPHIDOT-M	46.0%	54.0%	100.0%
		% within BLOOD CULTURE	100.0%	100.0%	100.0%
		% of Total	46.0%	54.0%	100.0%

Sensitivity(S) of Typhidot-M test - 97.8%
Specificity(Sp) of Typhidot-M test - 46.3%
Positive predictive value(PPV) - 60.8%
Negative predictive value (NPV) - 46.3%
When calculation for p value was done then we found that p value is less than 0.001 .So there was significant association between Typhidot-M and Blood culture tests.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	25.134[a]	1	.000		
Continuity Correction[b]	22.893	1	.000		
Likelihood Ratio	30.413	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	24.883	1	.000		
N of Valid Cases[b]	100				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 11.96.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	25.134[a]	1	.000		
Continuity Correction[b]	22.893	1	.000		
Likelihood Ratio	30.413	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	24.883	1	.000		
N of Valid Cases[b]	100				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 11.96.

b. Computed only for a 2x2 table

Symmetric Measures

	Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement Kappa	.422	.074	5.013	.000
N of Valid Cases	100			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Table 2. Calculation of p value for Typhidot-M test and blood culture(gold standard).

			BLOOD CULTURE		
			POSITIVE	NEGATIVE	Total
S WIDAL	POSITIVE	Count	22	47	69
		% within S WIDAL	**PP+=31.9%**	68.1%	100.0%
		% within BLOOD CULTURE	**Se=47.8%**	87.0%	69.0%
		% of Total	**22.0%**	47.0%	69.0%
	NEGATIVE	Count	24	7	31
		% within S WIDAL	77.4%	**NP-=22.6%**	100.0%
		% within BLOOD CULTURE	52.2%	**Sp=13.0%**	31.0%
		% of Total	24.0%	7.0%	31.0%
Total		Count	46	54	100
		% within S WIDAL	46.0%	54.0%	100.0%
		% within BLOOD CULTURE	100.0%	100.0%	100.0%
		% of Total	46.0%	54.0%	100.0%

Table 3. Widal and Blood culture test 2x2 Contingency table

Sensitivity of S.Widal test - 47.8%
Specificity of S.Widal test - 13%
Positive predictive value - 39.1%
Negative predictive value - 22.6%

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	17.855[a]	1	.000		
Continuity Correction[b]	16.069	1	.000		
Likelihood Ratio	18.484	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	17.676	1	.000		
N of Valid Cases[b]	100				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 14.26.

b. Computed only for a 2x2 table

Sensitivity of S.Widal test - 47.8%
Specificity of S.Widal test - 13%
Positive predictive value - 39.1%
Negative predictive value - 22.6%

Symmetric Measures

	Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement Kappa	-.378	.087	-4.225	.000
N of Valid Cases	100			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Table 4. Calculation of p value for S.Widal test and Blood culture (gold standard).

When calculation for p value is done then we found that p value < 0.001,So there is significant association between S.Widal and Blood culture tests.

Comparison of sensitivity and specificity, PPV and NPV of Typhidot-M and S.Widal test shows that Typhidot-M test has better results.

Discussion

This study was carried out to study the Rapid diagnostic test Typhidot-M.We calculated sensitivity, specificity, positive predictive and negative predictive value of Typhidot-M and S.Widal test by keeping blood culture positive cases as gold standard.

The sensitivity and specificity of Typhidot-M test were 97.8% and 46.3%.Positive and Negative predictive values were 60.8%and 96.2%.p value for Typhidot-M test was <0.001 ie. highly significant statistically.

The sensitivity and specificity of S.Widal test were 47.8% and 13%.Positive and Negative

predictive values were 31%and 22.6%. p value for S.Widal test was <0.001 ie. significant statistically.Typhidot-M test was evaluated by other workers who also found that this test is superior to Widal test in sensitivity and specificity.

Table 5. Comparison of our result with various studies done for evaluation of Typhidot-M test.

Author	Year of study	Test	S	Sp	PPV	NPV
Our Result	2012	Typhidot-M	97.8	46.3	60.8	96.2
Choo et al.[25]	1994 Malasia	Typhidot	95	75		96
Bhutta et al[19]	1999 karachi	Tyhidot-M	94	77	88	87
Jesudason et al[17]	2002-2003 Vellore	Typhidot	92.3	98.8	85.7	99.4
Oslen et al18	2000-2002Vietnam	Typhidot	79	89	96	59
D Narayanapa et al[11]	2008Mysore	TyphidotM	92.6	37.5	48.7	88.8
Beig et al[23]	2010 Aligarh	Typhidot-M	90	100	100	92.1

Various studies which had evaluated typhidot-M test showed sensitivity and specificity which ranged from 47-94% and 37.5-100% as shown in the above chart. In our study the sensitivity and specificity of Typhidot-M is 97.8% and 46.3% with PPV- 60.8 and NPV 96.2, which is comparable to the studies done by Bhutta et al, Narayanappa et al and Beig et al. Typhidot-M meets one of the criteria of an ideal diagnostic test as it usually doesn't miss the diagnosis when compared to blood culture.Only one case which was positive for blood culture was negative for Typhidot-M Test in our study.This patient had fever duration of more than 10 days .Probably decreasing levels of IgM against outer membrane protein of cell wall and masking of IgM by IgG in the 2nd week may be the reason for Typhidot-M negativity. There were 29 cases which were apparently false positive by Typhidot-M out of 54 blood culture negative cases. As Typhidot-M test detects IgM antibodies in 2nd week when blood culture positivity declines thus more cases were picked up by this test when duration of fever was more . Out of 46 blood culture positive cases, Typhidot-M test was positive in 97.8 % and Widal test was positive in 47.8 %. In our study Sensitivity and Specificity of S.Widal test were 47.8% and 13% .Positive predictive value and Negative predictive values were 31.9% and 22.6%. Comparison of sensitivity and specificity, PPV and NPV of Typhidot-M and S.Widal test shows that Typhidot-M test had better results. Thus Typhidot-M was significantly more sensitive than the Widal test, although the sensitivity and specificity of S.Widal test were lower than the other studies.[22-25] In contrast to findings from other parts of Asia, [27,28] our data support the contention that the Widal test had poor diagnostic value in children with typhoid fever.[29,30] This indicates that Typhidot-M test can also be effectively used for early diagnosis of typhoid fever as also reported earlier. It is optimal to evaluate the rising IgM titers as these titers rise during early phase of typhoid fever. Hence it is recommended to do this test for the early diagnosis and appropriate treatment of typhoid fever. This test does not require special equipment and technical training of staff but the instructions are to be stringently followed. It uses a small volume of serum and the result can be interpreted in an hour. Blood culture was positive in 46% of cases which is comparable to other studies by Bhutta et al [19], Dheer et al [26], Mishra et al [31] and this may be attributable to the difficulties of obtaining large enough blood volumes for cultures from children and it is a low bacteremic illness.

It must be emphasized that although cultures are associated with a lag period of at least 48 hr for preliminary confirmation of infection, with the recent emergence of drug resistance among S. typhi, they remain an essential investigation. In many circumstances, especially among partially treated cases presenting to health facilities, combining cultures with a rapid serologic test may reduce the diagnostic difficulty in typhoid fever. Our data indicates that combining the blood culture with a Typhidot-M test will significantly improve the diagnostic yield of these

investigations among children. We do not believe that our data support the use of either the Widal or Typhidot tests as a substitute for cultures in typhoid fever. The Typhidot-M offers an additional advantage among second- line serologic diagnostic tests for typhoid fever in that the test strips do not require an ELISA reader for evaluation. Also, only minimal operator training is required. Nevertheless, the 3–4-fold higher cost of the test in comparison with the Widal test, as well as cold-storage requirements for test strips, are additional impediments in using this test in developing countries.

Conclusion

To Conclude: Typhidot –M test helps to identify cases of typhoid fever at the earliest before the other test results like s.widal test and blood culture are available. Thus it can prove to be of help in early diagnosis of typhoid fever which further will help in early institution of antibiotics for its treatment.

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