

Original Research Article

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Comparison of Blood Clot Culture with Conventional Blood Culture and Biphasic Blood Culture for the Diagnosis of Enteric Fever

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ABSTRACT

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Diagnosis of enteric fever based on clinical presentation is difficult as signs and symptoms are often non-specific. Isolation of enteric fever pathogens remains the gold standard in diagnosis. Removal of serum with antibacterial activity in clot culture, may enhance the growth of typhoid bacilli. We would like to evaluate clot blood culture system over conventional blood culture and Biphasic blood culture for diagnostic accuracy. Blood cultures by conventional blood culture, Biphasic blood culture and clot culture were performed for 100 consecutive patients for whom enteric fever is suspected. The sensitivity and specificity of whole blood culture and Biphasic medium was found to be 72% and 100%. Both systems were of equal sensitivity. Clot culture showed 100% sensitivity. From our study, we strongly recommend clot bile culture be used for routine diagnostic purpose in areas where enteric fever is endemic. Major advantage of practicing clot culture is we utilize what is usually considered as leftover, avoiding the need for additional volume of blood and found to have increased potential for isolation.

Introduction

Enteric fever remains as an important cause of morbidity despite advances in diagnostic technology and public health strategy. According to the most recent estimates by WHO, approximately 2,00,000 people die every year worldwide.

The bulk of the burden being borne by Asian countries as it accounts for 90% of this mortality (Amicizia, 2017). Enteric fever is caused by *Salmonella enterica* serovar Typhi (*S. typhi*), *S. paratyphi* A and, less commonly,

by *S. paratyphi* B (Schotmulleri) and *S. paratyphi* C (Hirschfeldii).

Diagnosis of enteric fever based on clinical presentation is difficult as signs and symptoms are often non-specific. The most reliable means of confirming an infection in an endemic area is isolation of serotype Typhi from blood, urine, or stool.

There are technical and practical limitations for bone marrow culture which is still considered to be the gold standard for definite diagnosis (Christopher, 2011).

Delay in diagnosis and inappropriate treatment may result in the emergence of drug resistance *Salmonella* Typhi. Resistance to common first line antibiotics and quinolones by *Salmonella* Spp., has complicated the management of typhoid fever and heightened the need for accurate at the same time, prompt diagnosis of enteric fever.

Accurate diagnosis can be made by isolating the organism by the Blood culture, the most standard diagnostic method. (Lee, 2004)

In our hospital settings, isolation can be done by conventional blood culture method, clot culture and Biphasic blood culture.

In clot culture, serum with bactericidal activity has been removed well before culturing clot. This would probably enhance the growth of typhoid bacilli.

It was reported to be more sensitive than other blood culture system. We considered clot culture as gold standard and compared it with other systems. Biphasic culture medium has an advantage that subculture could be done by tilting, reducing the risk of contamination.

We would like to evaluate clot blood culture system over conventional blood culture and Biphasic blood culture for diagnostic accuracy.

Materials and Methods

The present study was aimed to evaluate the performance of blood clot culture over whole blood culture in BHI broth and in BHI Broth in Trypticase Soy Agar

Biphasic Medium. The study was carried out in the department of Microbiology, in a teaching tertiary care Hospital, Chennai. Institutional Ethical Committee clearance was obtained.

Inclusion criteria

100 consecutive patients clinically suspected for enteric fever presenting with duration of fever less than a week, were included in this study. Informed consent was obtained before collecting the blood specimen.

Exclusion criteria

Patients with known cause of fever, like UTI, Tuberculosis, Malaria, AIDS were excluded.

History of fever more than a week.

Under strict aseptic precaution, 20 ml of venous blood was collected in the first week of clinically suspected cases of enteric fever before starting the antibiotics and processed in the following methods.

About 20ml of blood sample is collected under strict aseptic precautions in the first week of clinically suspected cases of enteric fever before starting the antibiotics and processed in the following methods.

Conventional blood culture method with BHI broth

About 10 ml of blood is inoculated into 50 ml of BHI broth (1: 5 dilution) and incubated at 37⁰ C. After 48 hours, subculture onto MacConkey agar, blood agar and Nutrient agar and incubated at 37⁰ C. If no growth occurs, subculture everyday till seven days.

Clot culture method

About 5ml of blood is collected in a sterile container and left at room temperature for two hours. After separating the serum aseptically, the clot was lysed with sterile glass rod and inoculated in Ox bile broth and incubated at 37⁰ C. After 48 hours subcultured onto MacConkey agar, blood agar, and Nutrient

agar and incubated 37⁰ C. If no growth occurs, subcultured everyday till seven days.

Biphasic blood culture method with BHI broth and Trypticase soy agar

About 5ml of blood is inoculated into BHI broth in Trypticase Soy Agar biphasic medium and incubated at 37⁰ C.

Tilt the biphasic medium container in such a way that the broth tilted over the slant and incubated further in erect position and observe for growth daily. If no growth occurs, observe everyday till seven days.

The date of appearance of growth in both culture systems was recorded for comparison of growth rates.

Following isolation of *Salmonella* spp., they were identified by standard microbiological methods. Antibiotic susceptibility test was performed in accordance with CLSI guidelines using Ampicillin, Ciprofloxacin, Chloramphenicol, Cotrimoxazole, Tetracycline and Ceftriaxone discs (CLSI, 2017).

Statistical analysis: Data were entered into an Excel spreadsheet. Accuracy of conventional blood culture and Biphasic medium was determined in terms of Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) using 2x2 table. Results of these two systems were compared with Clot culture.

Results and Discussion

Blood culture by all three techniques were performed and 11 cultures were positive by one or the other method. Out of 11, 7 (63.63%) and 4 (36.36%) were found to be *S. typhi* and *S. paratyphi* respectively (Fig. 1). Clot culture was positive in all 11 cases, there

were no single specimen where clot was negative but other system was positive for growth. Overall, culture positivity for Clot, Blood and Biphasic was 100%, 72.72% and 72.72% (Table 1). Isolation time for majority of the isolate (90.90%) was found to be 48 hrs by clot culture whereas only 54.54% of isolates were grown by other two systems at the same incubation time (Table 2). We considered clot culture as gold standard and compared it over whole blood and biphasic medium. Sensitivity and Specificity of whole blood culture and Biphasic medium was found to be 72.73% and 100% (Table 3–5).

Enteric fever, a systemic infection caused by *Salmonella enterica* serotype Typhi (*S. Typhi*) and *S. enterica* serotype Paratyphi (*S. paratyphi*) A, B, and C is an important cause of avoidable mortality. Our data indicate that clot bile culture is excellent in primary isolation of *Salmonellae*.

In our study, isolation rate of *S. Typhi* and *S. Paratyphi A* was 63.6 % and 36.36% respectively. This is in accordance with the findings of Krishnan *et al.*, (2009), who had reported 70% and 30% of isolates were *S. typhi* and *S. paratyphi*. In a similar study at Chennai, 76% and 24% were observed to be *S. typhi* and *S. paratyphi* which was also in accordance with our findings (Renu Mathew *et al.*, 2013).

Among the 100 patients studied, clot bile culture confirmed 11 patients as enteric fever. Hence, culture positivity was 11%. This observation is slightly higher than prevalence of 5.5% as reported by Peerepur *et al.*, in his similar study.

In contrast, high prevalence of 34% was observed by Renu Mathew *et al.*, (2013) Geographical and time based variations may probably responsible for this wide difference in prevalence.

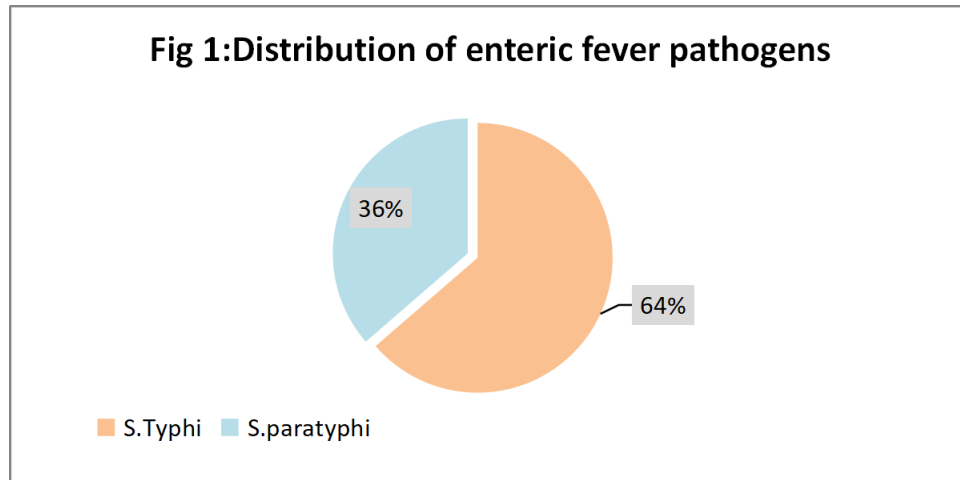


Table.1 Isolation rate of enteric fever *Salmonella* spp from each of three blood culture systems

Organisms isolated	No of specimens' positive (%)		
	BHI	Biphasic - TSA	Clot culture
S. Typhi	5(45.45)	6(54.54)	7(63.63)
S. paratyphi	3(27.27)	2(18.18)	4(36.36)
Total	8(72.72)	8(72.72)	11(100)

Table.2 Recovery rate of enteric fever pathogens at various incubation period

S.no	Incubation time	BHI	Biphasic with TSA	Clot culture
1	Within 24 hrs	0	0	0
2	24 -48 hrs	6	6	10
3	> 48 hrs	2	2	1

Table.3 Clot culture vs Conventional BHI blood culture

		Clot culture		Total
		Positive	Negative	
Conventional BHI blood culture	Positive	8	0	8
	Negative	3	89	92
Total		11	89	100

Table.4 Clot culture vs Biphasic medium with TSA

		Clot culture		Total
		Positive	Negative	
Biphasic medium with TSA	Positive	8	0	8
	Negative	3	89	92
Total		11	89	100

Table.5 Parameters of interest to compare biphasic and conventional blood culture medium

Parameters	Biphasic medium	Conventional blood culture
Sensitivity	72.73%	72.73%
Specificity	100%	100%
Positive predictive value(PPV)	100%	100%
Negative predictive value(NPV)	96.74%	96.74%

Whole blood culture and Biphasic culture was positive in 8 cases whereas clot bile culture was positive in 11 cases. Bile effectively inactivates anti-*Salmonella* activity of blood. Hence, several studies reported it to be superior to enriched non selective broths for isolation of enteric fever *Salmonella* spp. from whole blood (Escamilla *et al.*, 1986 and Watson *et al.*, 1954).

The data from our study are in agreement with this finding, showing that clot bile system is more sensitive in isolation compared to other systems. As 3 cases isolated only by clot bile remained negative by other systems even after 2 weeks of subculture.

90.90% (10) of total isolates of clot bile culture grew within 48hrs. In contrast, 54.54% (6) of whole blood and Biphasic medium isolates had grown at the same incubation period. Bacterial growth in clot bile culture was faster than other culture systems this was in accordance with other study (Mantur *et al.*, 2007).

The sensitivity and specificity of whole blood culture and Biphasic medium was found to be 72.73% and 100%. Both system were of equal sensitivity. Clot culture showed 100% sensitivity, it was observed to be more sensitive than other systems.

In a similar study by Mantur *et al.*, (2007) clot culture was found to be much more sensitive than whole blood. The results of the above study correlated well with our study.

From our study, we strongly recommend clot bile culture be used for routine diagnostic purpose in areas where enteric fever is endemic. Major advantage of practicing clot culture is we utilize what is usually considered as leftover, avoiding the need for additional volume of blood and found to have increased potential for isolation.

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