

**Evaluation of Blood Culture, Clot Culture and Diazo test in
Diagnosis of Typhoid fever and Antibiotic Sensitivity Pattern of
Salmonella Typhi in a tertiary care hospital, Bhavnagar, Gujarat**

Dr. Urmi Shah¹, Dr. Jatin Sarvaiya², Dr. Kairavi Desai³

ABSTRACT

Introduction: Typhoid fever is a life threatening systemic infection occurring more frequently in developing countries where over-crowding and poor sanitation are prevalent and is a major public health problem. **Aims & Objectives:** Purpose of our study is to evaluate efficacy of Diazo test, blood culture and clot culture in diagnosis of Enteric fever in patients of Sir Takhtsinhji General Hospital, Bhavnagar. **Materials and Method:** From May 2013 to July 2014, total of 125 patients suspected of having enteric fever came to Sir Takhtsinhji General Hospital, were included in the study. Urine & blood sample of patients were collected aseptically before administration of antibiotics to be used for blood culture, clot culture and Diazo test. **Results:** Out of 125 suspected enteric fever patients, 89 (71.20%) patients were positive for Diazo test. As far as blood culture was concerned total 23 (18.40%) cases were reported positive out of 125 patients. In case of clot culture, 33 (26.40%) samples were shown growth suggestive of *S. typhi*. **Conclusion:** In our study, Diazo test had more sensitivity than blood culture and clot culture but less specificity. So for the quick diagnosis of enteric fever cases, as a bedside screening method, it can be used as an adjuvant testing method to conventional blood culture and clot culture method for diagnosis of enteric fever.

Key words: Blood culture, Clot culture, Diazo test, Hatrley's broth

¹ 3rd year resident, ² Tutor, ³ Professor and Head

Dept. of Microbiology, Govt. Medical College, Bhavnagar, Gujarat, India

Corresponding author mail: urmishah2014@gmail.com

Conflict of interest: Nil

INTRODUCTION

Typhoid fever is a life threatening systemic infection occurring more frequently in developing countries where over-crowding and poor sanitation are prevalent and is a major public health problem.^{1,2} In 1829, Pierre Louis first named the *Salmonella bacillus* as “typhoide”, meaning “typhus-like”, derived from the Greek word “typhos” meaning “smoke” which refers to the apathy and confusion associated with the fully developed clinical syndrome of typhoid fever.¹ It is a systemic prolonged febrile illness caused by *Salmonella typhi*. Sixteen million cases are reported worldwide every year with six lakh deaths, of which, Asia accounts for 13 million cases.^{1,2} Typhoid fever is endemic, and one of the commonest major infectious disease prevalent in India, with reported data for the year 2005 showing 6,53,580 cases and 417 deaths.³ The annual incidence is as high as 980/100,000 in New Delhi.¹ The emergence of multidrug resistance

in *serotype typhi* strains has further complicated the situation.⁴

MATERIALS AND METHOD:

The study was conducted from May 2013 to July 2014 in Sir Takhtsinhji General Hospital, Bhavnagar, Gujarat. Over a period of 14 months about 125 febrile patients whose clinical symptoms were similar with typhoid fever were included in this study.

Before specimen collection, patients were interrogated thoroughly to obtain information regarding clinical features and complications. The details of clinical symptoms and signs were recorded in the proforma and examination of the patient was done to look for the presence of positive signs. The sterile gloves were worn and the venepuncture site on the patient's skin was disinfected by applying 70% isopropyl alcohol in water with povidone iodine for at least 1 min and allowed to dry. Around 15 mL of blood sample was drawn from each adult patient and around 7 mL of blood from children younger than 12 years who

were clinically suspected of typhoid fever. Blood is dispensed into the culture bottle containing 50ml of Hartely's broth for 24 hours overnight. The bottles were subcultured on blood agar and MacConkey's agar and incubated overnight at 37° C. After overnight incubation positive cultures were processed further while Negative broth cultures were incubated until 5- 7 days and sub cultured before reported negative. At the same time 5 ml of blood is collected from a patient and dispensed into the sterile test tube and allowed to clot. The serum is pipette off an the clot is broken up with sterile glass rod and added to a blood culture bottle containing hartely's broth. Diazo

test was performed on early morning urine samples of all cases which were included in the study. Diazo reagent is prepared from two stock solutions 'A' and 'B'. Their compositions are as below:

a) Stock solution A: Sulphanilic acid- 0.5 gm, Concentrated HCL- 5 mL, Distilled water- 100 mL.

b) Stock solution B: Sodium nitrite- 0.5 gm, Distilled water- 100 mL.

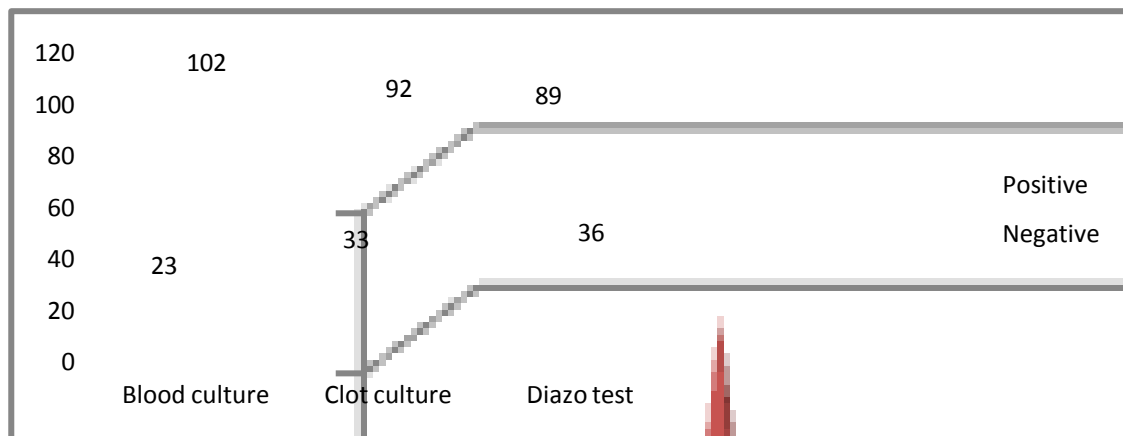
RESULTS:

Total of 125 patients attending Sir T Hospital, Bhavnagar, Gujarat from May 2013 to July 2014 who were suspected of having typhoid fever, were included in study. Following are the observation from the study.

Table – 1: Result of blood culture, clot culture & Diazo test in cases

Result	Blood culture		Clot culture		Diazo test	
	No.	%	No.	%	No.	%
Positive	23	18.4	33	26.4	89	71.2
Negative	102	81.6	92	73.6	36	28.8
Total	125	100	125	100	125	100

Chart – 1: Result of blood culture, clot culture & Diazo test in selected cases

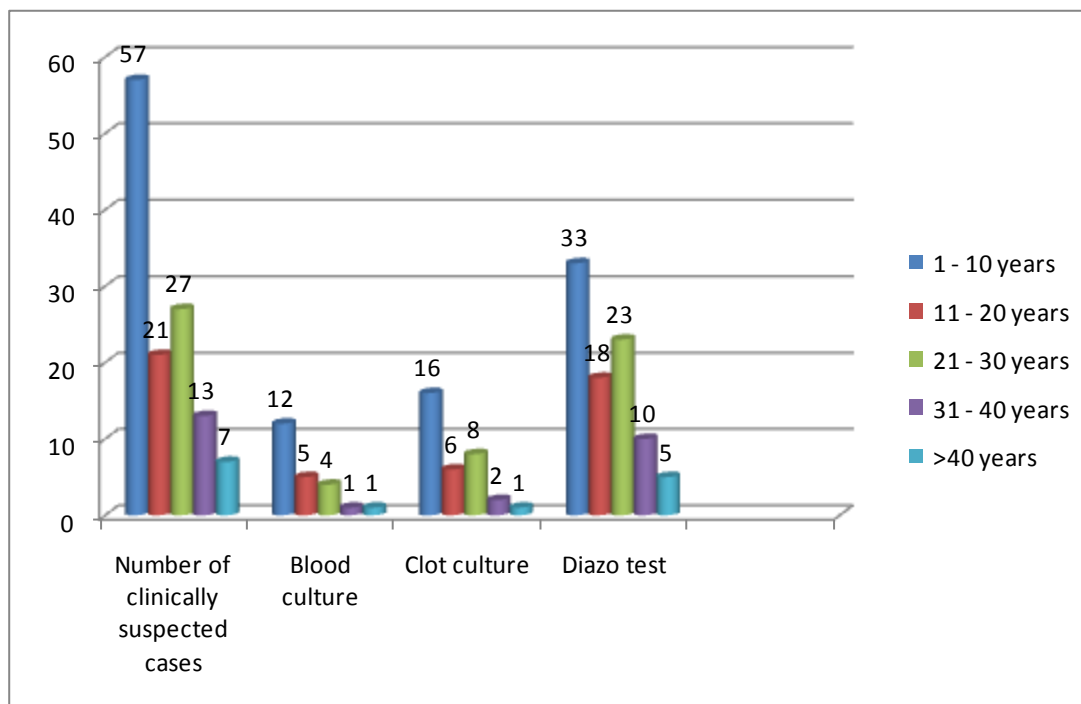


Out of 125 cases, we got 23 cases has shown growth in blood culture, 33 cases has shown growth in clot culture and 89 patients were positive for Diazo test.

Table – 2: Blood culture, clot culture and Diazo test positive results pattern in different age group amongst selected cases

Age (years)	Number of clinically suspected cases		Blood culture		Clot culture		Diazo test	
	No.	%	No.	%	No.	%	No.	%
1 – 10	57	45.6	12	21.05	16	28.07	33	57.89
11 – 20	21	16.8	5	23.80	6	28.57	18	85.71
21 – 30	27	21.6	4	14.81	8	29.62	23	85.18
31 – 40	13	10.4	1	7.69	2	15.38	10	76.92
>40	7	5.6	1	14.28	1	14.28	5	71.42

Chart – 2: Blood culture, clot culture and Diazo test positive results pattern in different age group amongst selected cases



As shown in table – 4 and chart – 4, out of 57 clinically suspected cases of typhoid fever in 1 – 10 years of age group, there were 12 (21.05%) cases who were shown positive growth for *S. typhi* in blood culture, 16 (28.07%) cases were shown positive growth for *S. typhi* in clot culture and 33 (57.89%) cases gave positive Diazo test. In age group 11 – 20 years, out of 21 clinically suspected cases, 5 (23.80%) cases were shown positive growth in blood culture, 6 (28.57%) cases were

shown positive growth in clot culture and 18 (85.71%) gave positive Diazo test.

In age group 21 – 30 years, out of 27 clinically suspected cases, 4 (14.81%) cases were shown positive growth in blood culture, 8 (29.62%) were shown positive growth in clot culture and 23 (85.18%) gave positive Diazo test. In age group 31 – 40 years, out of 13 clinically suspected cases, only 1 patient has shown positive growth for *S. typhi* in blood culture, 2

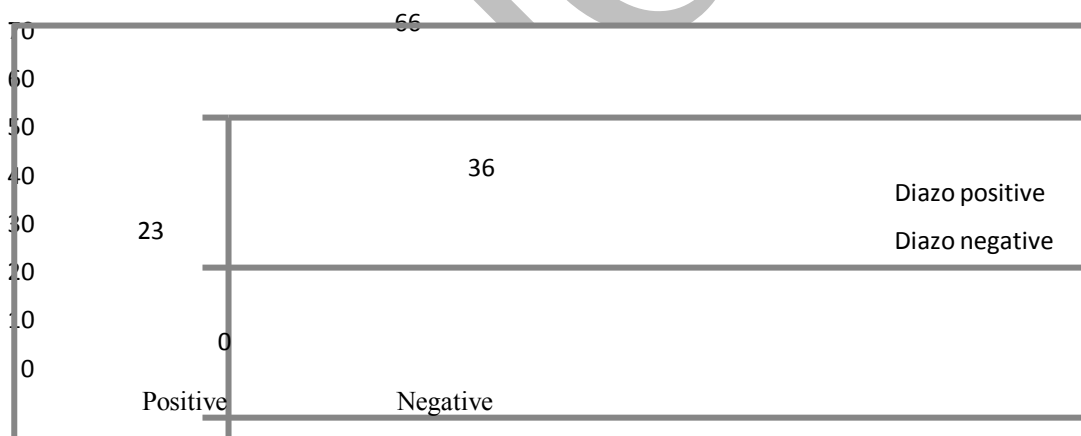
(15.38%) were shown positive growth in clot culture and 10 (76.92%) gave positive Diazo test. In last group of >40 years of age, out of only 7 clinically suspected cases, 1 (14.28%)

patients which was in both blood culture and clot culture were shown positive growth for *S. typhi* and 5 (71.42%) gave positive Diazo test.

Table – 3: Comparison of Diazo test with blood culture test

		Blood culture		Total
		Positive	Negative	
Diazo test	Positive	23	66	89
	Negative	0	36	36
Total		23	102	125

Chart – 3: Comparison of Blood culture with Diazo test

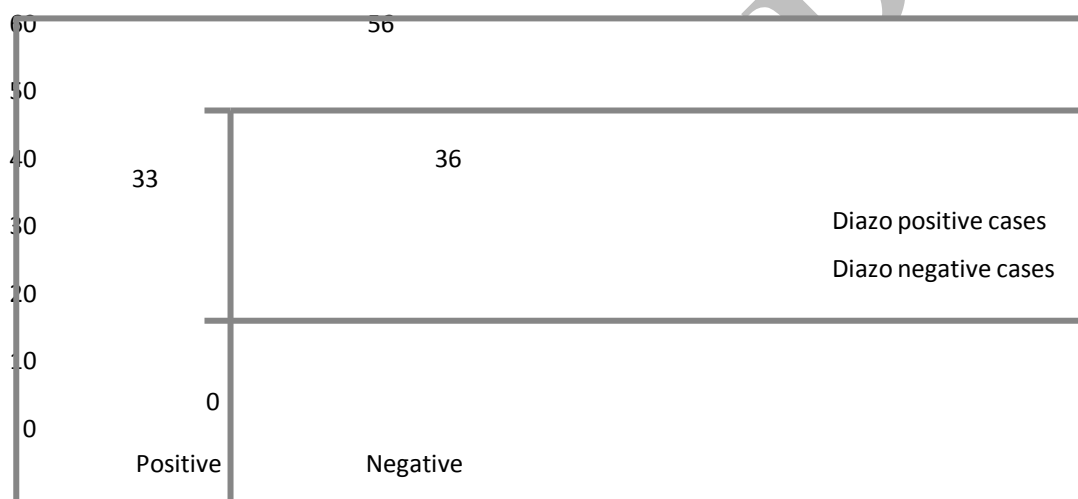


Out of 23 blood culture positive patients, all 23 Diazo tests were positive in all urine samples. The remaining 66 (out of 89) Diazo test were positive in blood culture negative cases. Sensitivity of Diazo test is 100% whereas specificity is 35%. Positive predictive value of Diazo test is 26% and negative predictive value is 100%.

Table – 4: Comparison of clot culture with Diazo test

		Clot culture		Total
		Positive	Negative	
Diazo test	Positive	33	56	89
	Negative	0	36	36
Total		33	92	125

Chart – 4: Comparison of clot culture with Diazo test



Out of 33 clot culture positive patients, all 33 Diazo tests were positive in all urine samples. The remaining 56 (out of 89) Diazo test were positive in clot culture negative cases. In this case also sensitivity of Diazo test in clot culture positive cases is 100% whereas specificity is 39%. Positive predictive value of Diazo test is 37% and negative predictive value is

100%.

Antibiotic sensitivity pattern of culture positive cases:

In our study, all isolates were tested for susceptibility to ampicillin, ceftriaxone, nalidixic acid, cefixime, chloramphenicol, ciprofloxacin, ofloxacin, levofloxacin, gentamycin and Trimethoprim-Sulphomethoxazole. All isolates are sensitive to above

mentioned drugs. No any single isolates are found which is resistant to any of above mentioned drug.

DISCUSSION

Salmonella infections occur worldwide in both developed and developing countries⁵. The contamination of food products with Salmonella generates serious health and economic consequences. Salmonella is frequently isolated from environmental sources that play a major role in its spread between different hosts. Although typhoid fever

is not common in industrialized countries, it remains an important and persistent health problem in developing nations. In India, typhoid fever is most prevalent in urban areas, usually affecting children below 15 years of age. The possible causes for typhoid fever being common in this age group include their mobility and consumption of unhygienic food and water. An increase incidence of typhoid fever in the summer – monsoon season are because of more food and water contamination.⁶

Table – 5: Blood culture isolates in various studies

Study series	Percentage positivity
Yaramis A et al. (2001) ⁷	21%
Retnosari S et al. (2001) ⁸	26%
Pena AC et al. (2002) ⁹	6.8%
Present study	18.4%

In the present study, 18.4% of the cases were positive by blood culture, which is more near with the study of Yaramis A et al.⁷

Table – 6: Diazo test positive in various studies

Study series	Percentage of positivity
Raman et al, 1994 ¹⁰	92%
Shivpuri et al, 2003 ¹¹	81%
Tanyigna et al, 2008 ¹²	27.3%
Present study	71.2%

In present study, 71.2% of the cases were positive by Diazo test, which is comparable to Shivpuri et al.¹¹ Diazo test can be used as a rapid bedside screening test in early diagnosis of typhoid fever as it has relatively high sensitivity.

Table – 7: Clot culture positive in various studies

Study series	Percentage of positivity
Renu et al ¹³	34%
Mantur B G et al ¹⁴	76%
Krishnan P et al ¹⁵	70%
Present study	26.4%

In our study, out of all suspected cases for typhoid fever, 26.4% cases are positive for Clot culture method which is comparable to Renu et al.¹³

Table – 8: Showing the sensitivity and specificity of Diazo test in culture proven cases in various studies

Study	Standard for diagnosis	Sensitivity of Diazo test	Specificity of Diazo test
Raman et al, 1994 ¹⁰	Blood culture	92%	83%
Shivpuri et al, 2003 ¹¹	Blood culture & Widal test	81%	90%
Tanyigna et al, 2008 ¹²	Widal test	27.3%	-
Present study	Blood culture, Clot culture	100%	35%

Sensitivity of Diazo test in our study is 100% in culture proven cases, which is in consistence with Raman et al.¹⁰ Specificity of Diazo test in our study is only 35% by which we can say that results of this test should be confirmed by either culture or other serological tests.

Blood culture is generally regarded as 'Gold standard' for diagnosis of enteric fever. But the delay involved in getting the culture report and the feasibility of submitting blood for culture in the first week of illness before antibiotics have been started, along with its poor sensitivity, makes blood culture not a very ideal choice in developing countries. This study shows that Diazo test has a high degree of sensitivity with lower specificity. False positive reactions may be found in pulmonary tuberculosis, measles and typhus.¹⁶

So this simple and quick test is a useful diagnostic tool in the early diagnosis of typhoid fever especially in areas with limited resources. More

importantly, prior administration of antibiotics does not interfere with the test. An important attribute of clot culture is that it utilizes what is usually considered the left over material, giving it the potential of increasing Salmonella species isolation without requiring additional blood from patients and also the sera after removal can be used for different serological assay. Clot culture technique in our experience has proved superior to the blood culture because of removal of serum which contains anti-bacterial activity.

CONCLUSION

We conclude from our study that Diazo test can be used as a simple, noninvasive and inexpensive bedside method for early diagnosis of typhoid fever but it should not replace conventional culture methods like blood culture and clot culture which are still the gold standard methods for the diagnosis of typhoid fever.

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