Original Article

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Rapid Diagnostic Test for the Diagnosis of Enteric Fever: A Cross-sectional Diagnostic Study

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Abstract

Introduction: In the present era, the rapid diagnosis of infectious disease is becoming a challenge to the medical field. At this setup, the most common infectious diseases are considered for rapid, accurate diagnosis. *Salmonella* is one of the most common needed infectious agents to be identified as early as possible.

Aim: To identify a specific and sensitive technique for early diagnosis of enteric fever thereby to reduce morbidity and mortality associated with the disease.

Materials and Methods: A total of 100 clinically suspected enteric fever cases were tested by blood culture, co-agglutination method, Widal test, and polymerase chain reaction (PCR). For co-agglutination method, blood culture supernatant, urine, and serum were used.

Results: Culture positive for *Salmonella* species was found to be 7.5%. *Salmonella typhi* was the predominant isolate (86%). *S. paratyphi* A (14%). Co-agglutination method showed positivity rate of 22.5%. Among the three types of samples, blood culture supernatant showed maximum positivity. 27% positivity was observed with the Widal test. PCR detected (10.8%) of cases.

Conclusion: Rapid diagnostic test evaluations to date have used blood culture primarily as the reference standard for typhoid fever diagnosis. Culture provides definitive evidence of infection, but it fails to detect all cases, due to low numbers of the pathogen in the bloodstream and/or prior exposure to antibiotics. Thus, it is evident that PCR is highly specific, and co-agglutination test is highly sensitive for the diagnosis of enteric fever.

Key words: Blood culture, Co-agglutination, Enteric fever, Polymerase chain reaction, Widal test

INTRODUCTION

Typhoid fever is rare in developed countries but remains as one of the most prevalent acute infectious diseases of the developing world.[1] It is endemic in India. Enteric fever is a septicemic disease caused by members of certain *Salmonella* serotypes: *Salmonella typhi*, *Salmonella paratyphi* A, and *S. paratyphi* B. The disease remains an important public health problem in developing countries.

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In 2000, it was estimated that over 2.16 million episodes of typhoid occurred worldwide resulting in 2,16,000 deaths and that more than 90% of this morbidity and mortality occurred in Asia.[2] In many areas, where the disease is endemic, laboratory capability is limited. Recent advances in molecular immunology have led to the identification of sensitive and specific markers for typhoid fever and technology to manufacture practical and inexpensive kits for their rapid detection. However, their limitation paves the way to continue to search for the ideal rapid test to diagnose acute typhoid fever. Blood culture is generally recognized as the best procedure-gold standard for definitive diagnosis of early typhoid fever. However, positivity is generally obtained only in about 45-50% patients even in well-equipped laboratories. Because of these problems, a number of newer tests such as passive hemagglutination, counter current immunoelectrophoresis, co-agglutination,

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latex agglutination, fluorescent antibody test, enzyme-linked immunosorbent assay, and polymerase chain reaction (PCR) have been tried for the rapid diagnosis of typhoid fever. The definitive diagnosis of the disease requires the isolation of *S. typhi* from blood, bone marrow, feces, urine, and other fluids.[3] In patients treated with antibiotics, antigen detection may be the one method available for diagnosis. Co-agglutination of Cowan-1 staphylococcal cells coated with specific antiserum, in the presence of antigen is one of the rapid methods to detect antigen.[4] Co-agglutination test can be used to detect soluble antigens of *S. typhi* in serum, urine, and blood culture supernatant. PCR is another new diagnostic test, which helps in the early detection of *S. typhi* from specimens such as blood, feces, and blood culture supernatant. In this study, blood culture, Widal, co-agglutination, and PCR were done, and the relative advantages of each test were evaluated.

MATERIALS AND METHODS

This cross-sectional diagnostic study was conducted in a tertiary care hospital for a period of 1-year. Ethics Committee Approval, patient's informed consent were obtained. The study group included 100 clinically suspected cases of typhoid fever between the age group of 5-65 years. Patients, with fever for more than 5 days, absence of any other signs and symptoms suggestive of other fevers were included. Patients taking drug for fever more than 1 day were excluded from the study. Blood cultures, Widal reaction, PCR, and co-agglutination were performed in study patients.

RESULTS

Seven cases excluded from the study due to inadequate material for PCR. 93 Samples collected from both gender in the age group of 5-65 years were subjected to blood culture, Widal, co-agglutination, and PCR (Figure 1).

Out of the 93 samples, blood culture positivity rate was 7.5%. *S. typhi* was the predominant isolate - 6 (86%) followed by *S. para typhi* A - 1 (14% Figure 2).

Comparing the results of three samples Serum, Urine and blood culture supernatant. Blood culture supernatant shown high positivity than other sample. (Table 1)

Of total 93 samples, one sample was positive after 24 h from brain heart infusion (BHI) broth. No positives were seen in 2 h and 4 h samples. However, 9 samples were positive after 24 h, three samples were positive after 4 h, and one sample was positive after 2 h of inoculation in the buffered peptone water (BPW).

Blood culture with co-agglutination test from blood culture supernatant and widal test have a fair agreement. (Table 3 and 4)

This shows a fair agreement between blood culture and PCR from BHI broth (Table 6).

Thus, from this study, it is evident that PCR is highly specific for the diagnosis of typhoid fever.

DISCUSSION

India being in the high incidence zone of enteric fever it is mandatory for medical personnel to know the appropriate method for diagnosis. This study focused on four methods: Two often used methods - blood culture and Widal; and two new methods - co-agglutination and PCR.

Out of 93 cases, seven were culture positive (7.5%). Of these, the predominant isolate was *S. typhi*[5,6] - 6/7 (86%). One isolate was *S. paratyphi* A - 1/7 (14%). Thus, this study, which shows an isolation rate of 7.5% correlates well with the study conducted by Rao, from India which showed positivity of 7.6%.[7] The isolation rate of this study was higher than the isolation rate given by Andualem *et al.*[8] 4.1% from conventional media. In contrary, studies by Jesudasan and Sivakumar[9] and Saha *et al.*,[10] showed higher positivity. This may be attributed to the fact that the above said

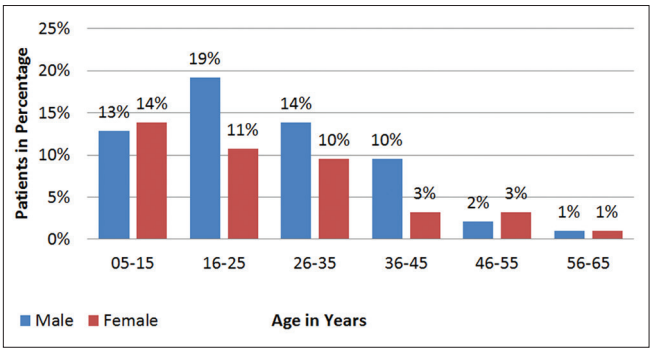

Figure 1: Distribution of cases as per age and sex

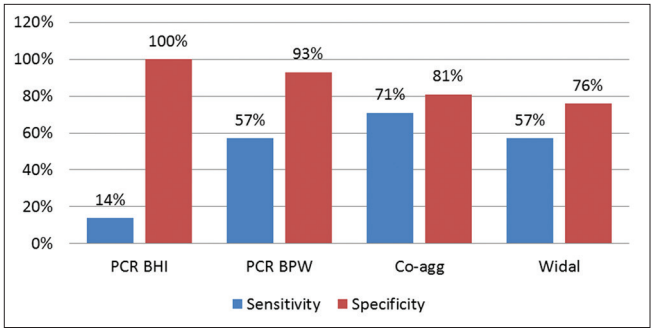

Figure 2: Comparison of all four methods

organization being a reference center, the cases admitted there were more selective in nature and hence the high isolation rate.

In this study, invA and invE genes were used as primers for detecting *Salmonella* species. Both BHI and BPW were used for PCR. Out of 93 samples, 10 (10.8%) were positive for PCR (Table 2). Of these, five samples were positive by blood culture also. Of the 10 positive samples, one was from BHI broth and 9 from BPW. One isolate was positive within 2 h of incubation (Table 5). This study used invA gene as probe for PCR, this is in concordance with the study of Maloney *et al.*[11] Multicenter validation of the analytical accuracy of the *Salmonella* PCR and International Standard concludes that the invA PCR assay using the primer set 139-141 that was originally published by Rahn *et al.*, by nested PCR demonstrated inclusivity for a wide range of *Salmonella* serotypes including all subspecies and exclusivity for other species and genera. In this study, PCR was able to detect all the *S. typhi* isolates except the *S. paratyphi* A. This isolate may also have been detected if nested PCR was used.[12] Use of PCR has increased the rapidity and sensitivity of diagnosing infectious diseases. However, laborious procedures are needed to eliminate inhibitory substances in clinical samples during DNA extraction. This study uses a simple boiling procedure to extract DNA which has been described by Lin. Combination of PCR technology with an enrichment procedure dilutes the PCR inhibitors and yields more number of *Salmonella* organisms as stated by Stone *et al.*[13] In this study, one isolate was positive within 2 h of incubation. Thus, this study proves that detection of *Salmonella* organisms as early as 2 h is possible by this combination method. The current study has a fair correlation with the study conducted by Rao.[7]

In this study, using co-agglutination method showed positivity rate of 22.5%. Mishra *et al.*, have reported (33.05%)[14] from blood culture supernatant. Co-agglutination method has been described to be more rapid and sensitive

Table 1: Results of co-agglutination (*n*=93)

Results	Serum (24 h)				Urine (24 h)				BL culture supernatant (48 h)			
	O	H	AH	BH	O	H	AH	BH	O	H	AH	BH
Positive	14	15	1	Nil	5	13	1	Nil	21	18	5	Nil
Negative	79	78	92	93	88	80	92	93	72	75	88	93

O: *S. typhi O antigen*, H: *S. typhi* H antigen, AH: *S. paratyphi* AH antigen, BH: *S. paratyphi B* antigen. *S. typhi*: *Salmonella typhi, S. paratyphi*: *Salmonella paratyphi*

Table 2: Results of PCR (*n*=93)

Results	BHI (h)			BPW (h)		
	2	4	24	2	4	24
Positive	0	0	1	1	3	9
Negative	93	93	92	92	90	84

BHI: Brain heart infusion broth, BPW: Buffered peptone water, PCR: Polymerase chain reaction

Table 3: Cross tabulation between co-agglutination from blood culture supernatant and blood culture

Co-agglutination from blood culture supernatant	Blood culture	
	Positive	Negative
Positive	5	16
Negative	2	70

McNemar test - *P*=0.001

	Estimate (%)	95% CI (%)
Sensitivity	71	29-96
Specificity	81	72-89
Correct classifications	81	71-88
Positive predictive value	24	8-47
Negative predictive value	97	90-100
Kappa agreement (K)	0.28	0.001

CI: Confidence interval

Table 4: Cross tabulation between blood culture and Widal test

Widal	Blood culture	
	Positive	Negative
Positive	4	21
Negative	3	65

McNemar test - *P*<0.0001

	Estimate (%)	95% CI (%)
Sensitivity	57	18-90
Specificity	76	65-84
Correct classifications	74	64-83
Positive predictive value	16	4-36
Negative predictive value	96	88-99
Kappa agreement (K)	0.15	*P*=0.001

CI: Confidence interval

Table 5: Comparison between blood culture and PCR from BPW broth

PCR BPW	Blood culture	
	Positive	Negative
Positive	4	6
Negative	3	80

McNemar test - *P*=0.508. PCR: Polymerase chain reaction, BPW: Buffered peptone water

	Estimate (%)	95% CI (%)
Sensitivity	57	18-90
Specificity	93	85-98
Correct classifications	90	82-95
Positive predictive value	40	12-75
Negative predictive value	96	90-99
Kappa agreement (K)	0.42	*P*=0.001

CI: Confidence interval

Table 6: Cross tabulation between blood culture and PCR from BHI broth

PCR	Blood culture	
	Positive	Negative
Positive	1	0
Negative	6	86

McNemar test - *P*=0.031. PCR: Polymerase chain reaction, BHI: Brain heart infusion broth

	Estimate (%)	95% CI (%)
Sensitivity	14	0-58
Specificity	100	0-95
Correct classifications	94	86-98
Positive predictive value	100	2-100
Negative predictive value	92	2-100
Kappa agreement (K)	0.23	0.001

CI: Confidence interval

method. In this study, co-agglutination test from blood culture supernatant after 48 h of incubation showed the maximum positive results (Table 3). This study showed a sensitivity of 71% and specificity of 81% and a fair correlation with the study conducted by Mathai and Jesudason[15] and Rao.[7] This study showed 81% agreement of co-agglutination with blood culture which is similar to the study by Mukherjee *et al.*,[16] which reported 95% agreement. The current study showed 27% positivity by Widal test which has a fair correlation with the studies by Gopalakrishnan *et al.*,[17] and Andualem *et al.*,[8] who had reported 34.7% and 32.6% positivity, respectively. This study showed less specificity which is similar to that of Chart *et al.*[18]

Thus, from this study, it is evident that PCR is highly specific and co-agglutination test highly sensitive among the four tests for the diagnosis of enteric fever.

CONCLUSION

Public health authorities should largely make use of the available rapid, simple and reliable diagnostic tools for typhoid fever, especially in health units where culture technique is unavailable. Co-agglutination is highly sensitive for the diagnosis of enteric fever and can be used for screening purpose. Widal is less specific and sensitive for diagnosis of enteric fever though it is the most widely used test. PCR proved to be highly specific for the diagnosis of enteric fever and can be used in patients who are culture negative.

REFERENCES

1. WHO, IRIS. In: Crump JA, Luby SP, Mintz ED, editors. The Global Burden of Typhoid Fever. Apps.who.int; 2004. Available from: http://www.apps.who.int/iris/handle/10665/72411. [Last cited on 2016 Apr 13].
2. Crump JA. The Global Burden of Typhoid Fever. 2004. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15298225. [Last cited on 2016 Apr 13].
3. WHO. The World Health Report 1996 - Fighting Disease, Fostering Development. Who.int. 1996. Available from: http://www.who.int/whr/1996/en/. [Last cited on 2016 Apr 13].
4. Sarvamangala Devi JN, Shivananda PG. Coagglutination for diagnosis of enteric fever. Indian J Pathol Microbiol 1985;28:349-53.
5. Prajapati B, Rai GK, Rai SK, Upreti HC, Thapa M, Singh G, *et al.* Prevalence of *Salmonella* Typhi and paratyphi infection in children: A hospital based study. Nepal Med Coll J 2008;10:238-41.
6. Aggarwal A, Vij AS. Three-year retrospective study on the prevalence, drug susceptibility pattern, and phage types of *Salmonella enterica* subspecies Typhi and paratyphi in Christian Med College and Hospital, Ludhiana, Punjab. Indian Acad Clin Med. 2007;8:32-5.
7. Rao S. Rapid diagnosis of typhoid fever by serum Vi Antigen by slide co-agglutination test. Ind J Path Micro 2004;40:287.
8. Andualem G, Abebe T, Kebede N, Gebre-Selassie S, Mihret A, Alemayehu H. A comparative study of Widal test with blood culture in the diagnosis of typhoid fever in febrile patients. BMC Res Notes 2014;7:653.
9. Jesudason MV, Sivakumar S. Prospective evaluation of a rapid diagnostic test Typhidot for typhoid fever. Indian J Med Res 2006;123:513-6.
10. Saha MR, Palit A, Chatterjee NS, Dutta P, Mitra U, Bhatacharya SK. National institute of cholera and enteric diseases, (ICMR) Kolkata, India. Indian J Med Res 2003;117:201-4.
11. Maloney B, Hoorfar J, Bunge C, Helmuth R. Multicenter validation of the analytical accuracy of *Salmonella* PCR: Towards an International Standard. Appl Environ Microbiol 2003;69:290-6.
12. Pratap CB, Kumar G, Patel SK, Shukla VK, Kumar K, Singh TB, *et al.* Mix-infection of S. Typhi and paratyphi A in Typhoid Fever and Chronic Typhoid Carriers: A Nested PCR Based Study in North India. J Clin Diagn Res 2014;8:DC09-14.
13. Stone GG, Oberst RD, Hays MP, McVey S, Chengappa MM. Detection of *Salmonella* serovars from clinical samples by enrichment broth cultivation-PCR procedure. J Clin Microbiol 1994;358:1742-9.
14. Mishra M, Thakar YS, Chande C, Tankhiwale NS, Saoji AM. Rapid detection of enteric fever by coagglutination and countercurrent immunoelectrophoresis. Indian J Pathol Microbiol 1998;41:391-6.
15. Mathai E, Jesudason MV. Coagglutination test in the diagnosis of typhoid fever. Indian J Med Res 1989;89:287-9.
16. Mukherjee C, Malik A, Khan HM, Malik A. Rapid diagnosis of typhoid fever by co-agglutination in an Indian hospital. J Med Microbiol 1993;39:74-7.
17. Gopalakrishnan V. Typhoid fever in Kuala Lumpur and a comparative evaluation of two commercial diagnostic kits for the detection of antibodies to *Salmonella typhi* was carried out by keywords: *Salmonella typhi*, ELISA, immunodot blot. Widal Singapore Med J 2002;43:354-8.
18. Chart H, Ward LR, Rowe B. Serological response of patients with clinical typhoid. Serol Immunother Infect Dis 1995;7:30-3.

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