

Utility of Enterocheck as a Rapid Diagnostic Tool for Typhoid Fever in Children

K.C. R¹, Karn SL², Gupta MM¹, Mahaseth S¹

ABSTRACT

Introduction: Typhoid is a major cause of morbidity and mortality worldwide. The annual incidence in the Indian subcontinent including Nepal has approximately 6 million cases each year. Blood culture is the gold standard for diagnosis but it requires well-equipped laboratory and trained staffs. Enterocheck is a rapid immunoassay which detects IgM antibodies to lipopolysaccharide specific to *S. typhi* in human blood. It is an alternative test which detects early infection. **Aims:** To study the sensitivity and specificity of Enterocheck for diagnosing typhoid. **Methods:** The cross-sectional study was carried out in the department of Pediatrics and Microbiology at Nepalgunj Medical College Teaching Hospital, Kohalpur, Banke, from August 2023 to February 2024, on 145 children, above 6 months- 15 years of age, who presented with fever for more than 72 hours and other features (e.g. headache, abdominal pain, vomiting, diarrhea or constipation, hepatomegaly, splenomegaly) suggestive of typhoid fever. Blood culture was done by aseptic standard method and Enterocheck was done according to the manufacturer's instructions. **Results:** Of the 145 cases, 17 (11.7%) had blood cultures positive for *S. typhi* and 29 (20%) were positive using Enterocheck. Male: female ratio was 2.15:1 and was most common in the age group 5 to 10 years (51.03%). The sensitivity and specificity of Enterocheck was 88.23% and 89.06% respectively (p -value<0.01). **Conclusion:** Enterocheck test was easy to perform and did not require special equipment or trained staffs. It had high sensitivity and specificity. Thus, it can be used as an alternative to blood culture for diagnosis of typhoid fever.

Keywords: Blood culture, Enterocheck, Sensitivity, Specificity, Typhoid fever

Authors:

1. Dr. Rajesh K.C.
2. Dr. Subhash Lal Karn
3. Dr. Murli Manohar Gupta
4. Dr. Sameer Mahaseth

¹Department of Pediatrics, Nepalgunj Medical College and Teaching Hospital, Kohalpur, Banke

²Department of Microbiology, Nepalgunj Medical College and Teaching Hospital, Kohalpur, Banke

Address for Correspondence:

Dr. Rajesh K.C.
Assistant Professor
Department of Pediatrics
Nepalgunj Medical College and Teaching Hospital
Kohalpur, Banke
Email: rajeshkc75@gmail.com

INTRODUCTION

Typhoid fever (TF) is a systemic febrile illness caused by *Salmonella* serotypes *S. typhi*, *S. paratyphi* A, B and C.¹ It is a major cause of morbidity and mortality worldwide, causing about 21 million cases with >600,000 deaths each year, according to World Health Organization.^{2,3} The disease occurs in all age group with highest incidence among children aged 5 to <15 years.⁴ The annual incidence in South Asia is 976 per 100,000 people and the Indian subcontinent including Nepal has approximately 6 million cases each year.^{5,6} It is transmitted faeco-orally and more cases are seen in areas with increased urbanization, limited safe water and health systems.³ Signs and symptoms of TF are non-specific. Therefore, laboratory tests are essential for accurate diagnosis.^{7,8} Blood culture is the gold standard for diagnosis with sensitivity of approximately 40%-60%.⁹ However, it requires well-equipped laboratory and trained staffs, that are significantly deficient in rural areas of

developing world.^{8,9} Widal test has been traditionally used as a rapid serologic test but it shows moderate sensitivity and specificity. Therefore, a rapid diagnostic method, which is reliable, easy to perform, with good sensitivity and specificity, without need for sophisticated equipment, is needed. Enterocheck is a rapid immunoassay which works on the principle of immunochromatography for detection of IgM antibodies to lipopolysaccharide specific to *S. typhi* in human blood.^{9,10} Detection of IgM antibodies serve as a good early marker of recent infection of disease. This study was undertaken to evaluate the role of Enterocheck test in diagnosis of typhoid fever.

METHODS

The cross-sectional study was carried out in the department of Pediatrics and Microbiology at Nepalgunj Medical College Teaching Hospital, Kohalpur, Banke from August 2023 to February 2024. Ethical clearance was obtained from the Insti

tutional Review Committee, Nepalgunj Medical College and Teaching Hospital. The study included 145 children, above 6 months to 15 years of age, who presented with fever for more than 72 hours and other features (e.g. headache, abdominal pain, vomiting, diarrhea or constipation, hepatomegaly, splenomegaly) suggestive of typhoid fever. Selection of cases was done by the convenient sampling method. After obtaining written informed consent, detailed relevant history and clinical examination, a blood sample by vein puncture was collected using aseptic techniques for blood culture and the Enterocheck test.

Blood culture: About 2 ml of blood was collected from the peripheral vein, inoculated into brain heart infusion (BHI) broth with 0.5 % SPS (sodium polyanethol sulphonate) bottles in 1:10 dilution and incubated at 37°C for 1 week. The formation of turbidity and hemolysis indicated the growth of bacteria. Once growth was seen on liquid medium, it was then sub-cultured after 1, 3, 7 days on blood agar and MacConkey agar. The isolated bacteria were identified to the species level by standard microbiological tests (Colony characteristics on different culture media, Gram stain findings and results of different biochemical tests (indole, triple sugar iron agar, citrate, urease and oxidase). Inoculation and sub-culturing was done by on-duty lab technician and reporting was done by microbiologist as per standard protocol.

Enterocheck test: was conducted on serum samples according to the manufacturer's instructions. The kit components of the device were brought to room temperature before testing. 5µl of serum was dispensed into the specimen port 'A' using a micropipette, and five drops of sample running buffer were dispensed into the buffer port 'B'. At the end of 15 minutes, results were interpreted according to leaflet, by on-duty technician.

The results were tabulated and statistically analyzed using SPSS version 25. Using blood culture as the gold standard, the sensitivity, specificity, positive and negative predictive values of the Enterocheck test were calculated and Chi square test was applied to calculate p-value.

RESULTS

A total of 145 patients, clinically suspected of typhoid fever, were investigated by both blood culture and Enterocheck. Among them, 17 (11.7%) patients had blood cultures positive for *S. typhi* and 29 (20%) were positive using Enterocheck. In the study, 99 (68.27%) were males with a male: female ratio of 2.15:1, as shown in the Figure 1. Most of the cases were, aged between 5 to 10 years, 74 (51.03%), with the median age of 10 years. 39 (26.89%) cases were of 6 months to 5 years, and 32 (22.06%) were aged 10-15 years (Figure 2).

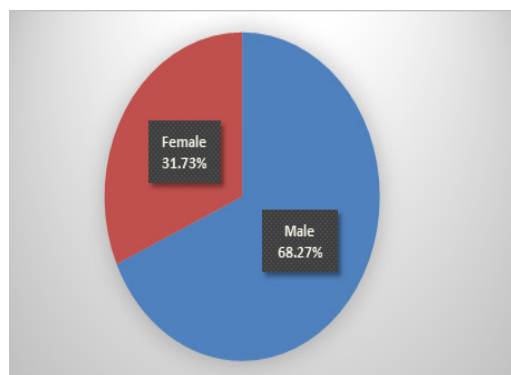


Figure 1: Sex distribution of the study population

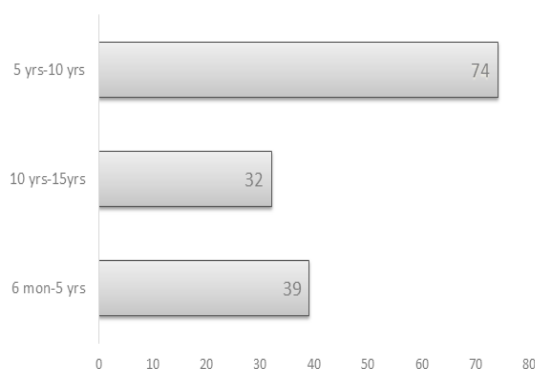


Figure 2: Age distribution of the study population

Of the 145 patients studied, 31 were positive for *S. typhi* by Enterocheck or blood culture or both. Both tests were positive in 15 of the 31 cases. In 14 patients, Enterocheck was positive but blood culture was negative and in 2 Enterocheck negative but blood culture was positive, as shown in Table I. Using blood culture as the gold standard, the sensitivity of Enterocheck was 88.23%, specificity 89.06%, negative predictive value 98.27%, positive predictive value 51.72% and there was statistically significant association ($p < 0.01$).

Enterocheck	Blood Culture		Total
	Positive	Negative	
Positive	15	14	29
Negative	2	114	116
Total	17	128	145

Table I: Comparison of results of Enterocheck with blood culture

Sensitivity- 88.23%, negative predictive value- 98.27%, specificity- 89.06%, positive predictive value- 51.72%, p-value < 0.01 .

DISCUSSION

Typhoid fever is a systemic illness with a significant morbidity and mortality in developing countries.¹¹ Any delay in diagnosis and inappropriate therapy increases the risk of outcome.¹² Blood culture has remained the gold standard for diagnosis of typhoid fever. However, a study from Delhi found it of limited

value when undertaken during the early phase of illness when isolation of the organism is difficult.¹¹ The sensitivity of blood culture in typhoid fever is also limited by the low bacterial count in blood and prior antimicrobial therapy.¹²

In the present study blood culture positivity among clinically suspected typhoid cases was in 17 cases (11.70%). Similar to the study by Sreenivasa B et al in India has positivity of 14%.¹³ In a study by Devaranavadagi RA et al blood culture was positive in 20% of cases¹⁴ which is contrast to our study. The relative low rate of positivity in the present study might be due to widespread and irrational use of antibiotics and low volume of blood obtained for cultures among children. Enterocheck test was positive in 20.48% in a study by Anagha K et al¹⁵ which is similar to the present study where positivity of Enterocheck test was 20%. The result is also consistent with the study by Sreenivasa B et al where test was positive in 27.5% cases.¹³

In present study, male predominance was seen 68.27%. Similar results were reported in studies by Jog S et al¹⁶ and Ganesh R et al.¹⁷ Commonest age group of typhoid fever in the present study was 5 to 10 years (51.03%) similar to the study by Devaranavadagi RA et al.¹⁴ A study done by R Modi et al also reported maximum incidence of typhoid in the age group 6 to 10 year.¹⁸ These results were in concordance with the concept that typhoid fever is common in school age children. They are at high risk of consuming contaminated drinking water and street foods which make them more vulnerable to exposure to typhoid bacilli.

The sensitivity of Enterocheck in the present study was 88.23% and specificity was 89.06%. Similar to the study by Anusha R et al where the sensitivity and specificity of Enterocheck was 85.5% and 88.6% respectively.¹⁹ Whereas in a study by Narayanappa D et al sensitivity and specificity of Typhidot-M was 92.6% and 37.5% respectively.²⁰ This difference might be due to genomic diversity among *S.typhi* isolates and differences in antigenic epitopes, various stages of the illness and the rate of increase of IgG antibodies, which might interfere with IgM antibodies. The present study showed statistically significant association between Enterocheck and blood culture (p -value <0.01), similar to the study by Maheshwari V et al²¹ and Udayakumar S et al.²² Two patients in the present study were positive by blood culture but negative by Enterocheck. The false negative results by latter might be due to test's failure to detect low levels of antibodies.² False positive results might be because of prior treatment with antibiotics or low bacterial counts in blood.²³

LIMITATIONS

The sample size was small with convenient sampling method. Blood culture, used as the gold standard, itself has limited sensitivity, which might have affected the result. Cross-reactivity of Enterocheck with non-typhoidal *Salmonella* or other febrile illnesses, prior antibiotics use and variable timing of illness during sample collection in different patients might also have altered the specificity.

CONCLUSION

Enterocheck is a practical alternative to other tests for rapid diagnosis of typhoid fever, where traditional culture facilities are not available. It has high sensitivity and specificity, can detect cases early, and is easy to perform with minimal infrastructure and availability of results within about 15 minutes.

REFERENCES

1. Willke A, Ergonul O, Bayar B. Widal test in diagnosis of typhoid fever in Turkey. *Clin Diagn Lab Immunol.* 2002; 9(4):938–41.
2. Olsen SJ, Pruckler J, Bibb W, Thanh NT, Trinh TM, Minh NT, et al. Evaluation of rapid diagnostic tests for typhoid fever. *J Clin Microbiol.* 2004; 42(5):1885-9.
3. Crump JA, Luby SP, Mintz ED: The global burden of typhoid fever. *Bull World Health Organ.* 2004; 82(5):346-53.
4. World Health Organization. Typhoid vaccines: WHO position paper. 2018. Available at https://www.who.int/immunization/policy/position_papers/typhoid/en/
5. Buckle GC, Walker CLF, Black RE. Typhoid fever and paratyphoid fever: Systematic review to estimate global morbidity and mortality for 2010. *J Global Health.*2012;2(1):1-9.
6. Divyashree S, Nabarro L, Veeraraghavan B, Rupali P. Enteric fever in India: Current scenario and future directions. *Trop Med Internat Health.* 2016; 21(10):1255–62.
7. Dutta S, Sur D, Manna B, Sen B, Deb AK, Deen JL, et al. Evaluation of new-generation serologic tests for the diagnosis of typhoid fever: data from a community-based surveillance in Calcutta, India. *Diag Microbiol Infect Dis.* 2006; 56(4):359-65.
8. Krishna S, Desai S, Anjana VK, Paranthaaman RG. Typhidot (IgM) as a reliable and rapid diagnostic test for typhoid fever. *Ann Trop Med Public Health.* 2011; 4:42-4.
9. Wijedoru L, Mallett S, Parry CM. Rapid diagnostic tests for typhoid and paratyphoid (enteric) fever. *Cochr Database Systematic Rev.* 2017; 26(5).
10. Ghimire JP, Upadhyay RR. Prevalance of typhoid fever in Kathmandu valley and its rapid diagnosis by detection of IgM antibodies. *J Nepal Assoc Med Lab Sciences.* 2008; 9:7–10.
11. Sherwal BL, Dhamija RK, Randhawa VS, Jais M, Kaintura A, Kumar M. A comparative study of Typhidot and Widal test in patients of Typhoid fever. *J Indian Acad Clin Med.* 2004; 5:244-6.
12. Begum Z, Hossain MA, Shamsuzzaman AK, Ahsan MM, Musa AK, Mahmud MC, et al. Evalutaion of Typhidot (IgM) for early diagnosis of typhoid fever. *Bangladesh J Med Microbiol.* 2009; 3:10-13.
13. Sreenivasa B, Kumar GV. Comparative Study of Widal and Typhidot Test in the Diagnosis of Typhoid Fever in Children. *J Nepal Paediatr Soc.* 2016; 36(2):131-5.
14. Devaranavadagi RA, Srinivasa S. A study on clinical profile of typhoid fever in children. *Int J Contemp Pediatr.* 2017;4(3):1067-73.
15. Anagha K, Deepika B, Shahriar R, Sanjeev K. The easy and early diagnosis of typhoid fever. *J Clin Diagn Res.* 2012; 6(2):198-9.

16. Jog S, Soman R, Singhal T, Rodrigues C, Mehta A, Dastur FD. Enteric fever in Mumbai—clinical profile, sensitivity patterns and response to antimicrobials. *JAPI*. 2008;56:237-40.
17. Ganesh R, Janakiraman L, Vasanthi T, Sathiyasekeran M. Profile of typhoid fever in children from a tertiary care hospital in Chennai-South India. *Indian J Pediatr*. 2010; 77(10):1089-92.
18. Modi R. Clinical profile and treatment outcome of typhoid fever in children at a teaching hospital, Ahmedabad, Gujarat, India. *Int J Med Sci Public Health*. 2016; 5:212-6.
19. Anusha R, Ganesh R, Lalitha J. Comparison of a rapid commercial test, Enterocheck WB®, with automated blood culture for diagnosis of typhoid fever. *Ann Trop Pediatr*. 2011; 31(3):231-4.
20. Narayanappa D, Sripathi R, Jagdishkumar K, Rajani HS. Comparative study of dot enzyme immunoassay (Typhidot-M) and Widal test in the diagnosis of typhoid fever. *Indian Pediatr*. 2010; 47:331-3.
21. Maheshwari V, Kaore NM, Ramnani VK, Sarda S. A comparative evaluation of different diagnostic modalities in the diagnosis of typhoid fever using a composite reference standard: A tertiary hospital based study in Central India. *JCDR*. 2016;10(10):1-4.
22. Udayakumar S, Pushpalatha K, Sagar HN, Swathi M, Yoganand R, Sushma C. Comparative study of Typhidot-M with Widal and blood culture in diagnosis of enteric fever. *Indian Journal of Child Health*. 2017;4(1):64-7.
23. Mohanty SK, Ramana KV. Single and unpaired sera tube Widal agglutination test in enteric fever. *Saudi J Gastroenterol*. 2007; 13(4):213.