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Comparative evaluation of Blood culture, Immunochromatographic test, Widal and Polymerase chain reaction for rapid diagnosis of Enteric fever

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ABSTRACT

Salmonella enterica serovar Typhi, the human-specific, causative agent of typhoid fever, causes an estimated 21 million new cases and 216,000 deaths every year. Accurate diagnosis to differentiate typhoid fever from other conditions is often difficult. Isolation of the causative organism remains the most effective diagnostic method in suspected typhoid fever. PCR based tests for detecting the causative pathogens of enteric fever have developed rapidly over the last decade. The present study was conducted to evaluate the different diagnostic methods like Blood culture, Immucheck antibody test widal and Polymerase chain reaction (PCR). Out of 104 patients 12 (11.5%) were blood culture positive. All the culture positive and three other cases 15 (14.4%) were reactive by Immucheck typhoid antibody test. In our study total 15(14.42%) cases were positive for the widal test on the basis of the set endemic titre for the Aligarh district. *dH flagellin* gene was detected in 27(25.9%) cases. *dH Flagellin* gene amplification in blood detected the highest number of cases. Considering the polymerase chain reaction as gold standard, sensitivity and specificity of blood culture was 44.44% and 100% respectively. Polymerase chain reaction is most sensitive and specific for the diagnosis of enteric fever.

Keywords: polymerase chain reaction, *dh flagellin* gene, immunochromatogrphic test.

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INTRODUCTION

Salmonella enterica subspecies *enterica* serovar Typhi, the human-specific, causative agent of typhoid fever, causes an estimated 21 million new cases and 216,000 deaths every year (Crump *et al.,* 2004).[1] Typhoid fever is one of the most common infectious diseases in developing countries including India. The clinical diagnosis of enteric fever is not always accurate because of a wide range of other common fever-causing infections like malaria, dengue fever, leptospirosis, hepatitis, melioidosis and rickettsioses endemic in India. Accurate diagnosis differentiating typhoid fever from these conditions is often difficult, both in the clinics and laboratory, but is imperative for effective treatment selection. Even in highly-resourced western countries, physicians often start typhoid treatment empirically whilst awaiting confirmation of the diagnosis. Isolation of the causative organism remains the most confirmatory method in suspected typhoid fever and blood has been the main sample for culture for *Salmonella* serovar Typhi since 1900 (Wain and Hosuglu 2008).[2] The sensitivity of blood culture is highest in the first week of the illness and reduces with advancing illness (Kundu *et al.,* 2006).[3] Serological tests, predominantly the Widal test, are available but have very low sensitivity and specificity, and no practical value in endemic areas despite their continued use (Levine *et al.,* 1978).[4] Various immunochromatographic test like TyphiDot, Typhifast, TUBEX, Multi-Test Dip-S-Ticks which detects IgM have been evaluated for the rapid diagnosis of typhoid fever. Molecular tests for detecting the causative pathogens of enteric fever have developed rapidly over the last decade; however questions regarding the clinical utility and standardization of tests still remains. Detection of bacterial DNA in whole blood by PCR assay is able to substantially decrease the turnaround time without bias from the inhibitory effect of antibiotics, yet the published PCR assays for diagnosis of enteric fever are in limited use. There is no uniform consensus regarding the use of any diagnostic test for enteric fever. Therefore, this study was done to evaluate the the diagnostic accuracy of blood culture, Immucheck Typhoid IgG/IgM test, widal and polymerase chain reaction for the diagnosis of enteric fever in an endemic region.

MATERIALS AND METHOD

The present study was conducted in the department of Microbiology, J.N Medical College, AMU, Aligarh, UP, India over a period of two and a half years from September 2011 to February 2014.

Case definition:

The cases included patients attending the paediatric and medicine OPD or IPD with clinical features suggestive of typhoid like fever with abdominal discomfort, headache, anorexia,

nausea, and vomiting, abdominal discomfort with diarrhoea, soft enlarged spleen, coated tongue, toxic look and relative bradycardia.

Exclusion criteria:

Patients with any other obvious focus of fever like malaria, urinary tract infection, otitis media, leptospirosis etc and patients with prior antibiotic therapy were excluded from the study.

Controls:

10 healthy persons with no clinical evidence suggestive of enteric fever matched with age and sex were selected as controls.

Blood culture:

Blood for culture was collected taking all sterile precautions by veni- puncture. A total of 5 ml of peripheral blood from adults and 2 ml from paediatric patients was collected and transferred to brain heart infusion broth. Subcultures were done after 24 hours, 48 hours and 7 days on 5% sheep blood agar and Teepol lactose agar. All isolates were identified by standard biochemical procedures and serotyping was done as per Kauffnamm white scheme (Collee, JG et el 2006).[5]

Immunochromatographic test:

Immucheck Typhoid IgG/IgM rapid test was done for all the serum samples as per the manufacturer's instructions. The Immucheck Typhoid IgG/IgM Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of anti-*Salmonella* Typhi (*S.* Typhi) IgG and IgM in human serum or plasma.(Immucheck Typhoid IgG/IgM Rapid Test from Immunoshop).

Widal Test:

Agglutinins against "O" (somatic) and "H" (flagellar) antigens of Salmonella group of organisms were detected quantitatively employing killed suspension of appropriate organisms using the reagents obtained from Span diagnostic limited using standard methods and guidelines.

Polymerase chain reaction for detection of *S.* Typhi from blood sample:

DNA extraction was performed by using QIAamp DNA investigator kit (Qiagen). PCR mixture consisted of 50 µl master mix containing 1µl sample, 10X buffer, primer mixture, dNTPs, and taq polymerase. The primers were designed based on published *dH flagellin* gene sequence using oligo computer program (Frankel G et al 1979).[6] A 486 base pair region was amplified using the following primers RK1 (5 TGG GCG ACG ATT TCT ATG CC 3), RK2 (5 TTT CGC GAA CCT GGT TAG CC 3) Cycling parameters of PCR were set as follows: hot start 94°C for 4 min followed by 30 cycles of melting at 94°C for 45s, annealing at 50°C

for 45s, and extension at 72°C for 1 min. Analysis of amplified products was done by gel electrophoresis. Amplicons of 486 bp were consistent with *dH* flagellin gene amplification.

Statistical analysis:

To evaluate the diagnostic accuracy of Blood culture, Immunocheck Typhoid IgG/IgM rapid test, widal and PCR, the various parameters like sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy were calculated.

Diagnostic accuracy was calculated using Youden's index. The statistical analysis of results was also done to establish the test of significance for clear and confirmatory statements. For all statistical tests, a P value less than 0.05 was taken to indicate a significant difference.

Ethical clearance:

Ethical clearance was obtained from institutional Ethics committee J N Medical college, AMU, Aligarh, held on 29/6/2012. A written consent was taken from all cases and healthy donors for testing their blood for enteric fever.

RESULTS AND DISCUSSION

In our study total no. of patients sample showed positive Band for the *dh flagellin* gene on PCR were 27(25.96%), while 77 (74.04%) samples showed negative (no bands for *dh flagellin* gene seen) result on PCR . while in control groups all 10 (100%) were negative by PCR .

Out of 104 patients suspected of enteric fever clinically, only 12 (11.5%) were blood culture positive and 90 (86.53%) were negative including 2 (1.94%) were contaminated. all the 10 controls were negative. Considering PCR as gold standard, Sensitivity of blood culture was found 44.44% while specificity was 100%. Positive predictive value and negative predictive value was 100% and 83.69% ($p < 0.05$). Diagnostic accuracy was 44.00% .

All the culture positive and three other cases making a total of 15 (14.4%) were reactive by Immucheck typhoid antibody test (Figure 1). Of these 15, 13 were reactive for both IgG & IgM and two were positive for IgM only. Out of 10 controls tested, one was positive by Immucheck however it was negative for *dh flagellin* gene and was considered as false positive. Sensitivity of Rapid test was 51.85% and specificity was 98.70%. Positive predictive value and negative predictive value was 93.33% and 85.69 % ($p < 0.05$), and diagnostic accuracy was 50.55%.

In our study total 15(14.42%) cases were positive for the widal test on the basis of the set endemic titre for the Aligarh district, which is ≥100 for d antigen and ≥200 for 9,12 antigen, and 89 (85.58%) cases were declared negative. In the control groups 2 (20%) were positive by widal test and 8(80%) were negative. The two control case showing raised antibody titre in widal did not shoe *dh flagellin* gene on PCR.

Sensitivity and specificity of widal test were found to be 40.74%, 94.80%, and Positive predictive value and negative predictive value and diagnostic accuracy was 73.33% , 82.02 % ($p < 0.05$) and 35.54% respectively.

DISCUSSION

Typhoid fever is one of the most common infectious diseases in developing countries including India. Enteric fever is a diagnostic challenge for the clinicians as well the microbiologists with a number of other tropical infections mimicking the clinical presentations.

dH Flagellin gene amplification in blood detected the highest number of cases (Figure 2&3). Only quarter of all the clinically suspected cases were found positive for enteric fever by all the methods used for diagnosing the same. This may be because of the non specificity of symptoms, which overlap with other endemic diseases prevalent in this region like malaria, viral hepatitis, dengue, leptospirosis, chikungunya etc.

Sensitivity of blood culture was found 44.44% while specificity was 100%. Positive predictive value and negative predictive value was 100% and 83.69% ($p < 0.05$). Low concentrations of *S.* Typhi in the blood of patients with typhoid fever (< 15 bacteria/ml) contribute to the moderate sensitivity of blood culture (Rubin *et al.*, 1989, Rockhill *et al.*, 1980).[7,8] Our is a tertiary care centre, where most of the patients had already taken antibiotic treatment from quacks before attending the hospital may be a reason for low sensitivity of blood culture. The volume of blood taken and the laboratory methods used for isolation are also important factors determining the yields from blood culture (Wain *et al.*, 2008).[9] This also explains the lower diagnostic accuracy of blood culture as compared to rapid test which is 50.55%.

Immunocheck Typhoid IgG/IgM rapid test had sensitivity of 51.85% and specificity 98.70%. Positive predictive value and negative predictive value were 93.33% and 85.69 % respectively ($p < 0.05$), Considering PCR as gold standard. A similar study carried out in the southern part of India reported typhidot of having a sensitivity of 100% and a specificity of 80% and was recommended for its utility in conjunction with widal test for an early diagnosis of typhoid fever (jesudason et el 2002).[10] In Vietnam, it was 87% sensitive among blood culture positive patients and 76% sensitive among hospitalized patients with fever (House et al 2001).[11] Diagnostic accuracy of Immucheck rapid test is higher than that of blood culture because rapid tests are more sensitive than the blood culture.

Results of all the studies done to evaluate typhidot test in developing countries have consistently shown similar and comparable results.

Sensitivity and specificity of widal test were found to be 40.74%, 94.80%, and Positive predictive value and negative predictive value was 73.33% and 82.02 % ($p < 0.05$) respectively. In a study conducted by Olsen et al. for the evaluation of sero-diagnostic assay of acute enteric fever, the sensitivity and specificity of Widal test were similar to our findings (Olsen et al 2004).[12] Widal test is still the most widely used diagnostic method of typhoid fever in developing countries including India. But the role of Widal tests for the diagnosis of typhoid fever has been debated widely, because first; the sensitivity, specificity, and predictive values of Widal test vary considerably among geographical areas (Bhutta Z A et al, 2006, Khoharo et al 2010)[13,14], second the titre of agglutinins also depend on the level of infections due to other salmonella species and other infectious agent that have cross reacting antigens (Parry CM et al 2002).[15] So evaluating the result of Widal test in the area where it is used is necessary for the correct interpretation of the result. Diagnostic accuracy of widaal test is lower than the blood culture and Immucheck rapid test showing that the occurrence of false positive resuts are more common in case of widal test.

Table 1. Comparison of all tests considering PCR as gold standard test.

Diagnostic test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy	P value
Blood culture	44.44%	100%	100%	83.69%	44.00%	<0.05
Rapid test	51.85%	98.70%	93.33%	85.39%	50.55%	<0.05
widal	40.74%	94.80%	73.33%	82.02%	35.54%	<0.05

Figure. 1: Immucheck showing positive and negative result.

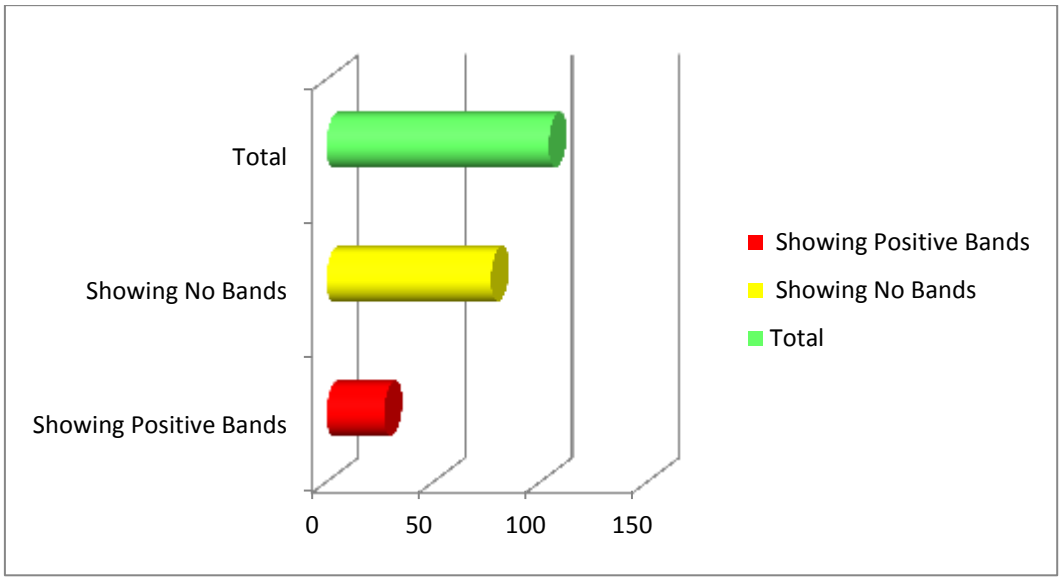

Figure.2: Result of PCR among total patients

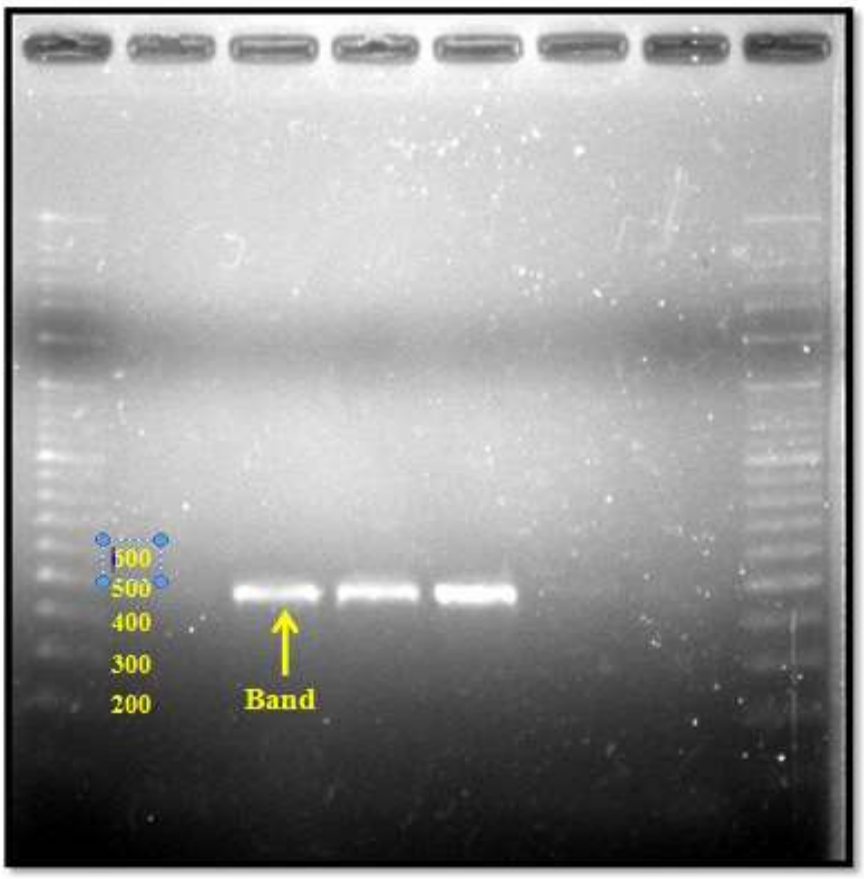

Figure.3: *dH flagellin* gene (486 bp) seen on gel electrophoresis.

CONCLUSION

Blood culture, with high specificity, is a traditional method of diagnosis during the first week of illness with the distinct advantage of bacterial identification with antimicrobial susceptibility, which plays an important role in judicious antimicrobial therapy and for epidemiological purposes. When diagnosis is required in the later part of illness,

amplification of *dH flagellin* gene is a good alternative, particularly in patients already on antibiotic treatment. In developing countries with low resources and for spot diagnosis immunochromatographic test is better option for managing the cases in remotely located set up in comparision to widal test which is time taking and requires technical skills. Alternatively Blood culture along with rapid test is better option for developing countries and Rapid test and PCR is better option for developed countries. Although a rapid, cheap and reliable single diagnostic method for enteric fever is urgently needed.

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