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Evaluation of Typhoid antigen test for qualitative and differential detection of *Salmonella* Typhi and *Salmonella* Paratyphi in human blood culture

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ABSTRACT

Introduction: Typhoid and Paratyphoid fever are enteric infections caused by the bacteria *Salmonella enterica serovar* Typhi (*S.* Typhi) and *S.* Paratyphi A, B and C respectively. This research evaluated an immunochromatographic rapid diagnostic tests (RDT) Typhisure ©, for detection of *S.* Typhi and *S.* Paratyphi antigen in blood culture.

Objectives: To test for qualitative and differential detection of *S.* Typhi and *S.* Paratyphi antigen in human blood culture specimen by Typhisure .

Methodology: A descriptive cross-sectional study was conducted in a tertiary care hospital from August- October, 2024 after obtaining ethical clearance. Blood sample from 758 patients were collected aseptically. Rapid diagnostic test was performed by Typhisure on blood culture positive specimen and in parallel, conventional subculture, isolate identification was done.

Results: Among 758 blood culture specimen, 100 blood culture broth showing evidence of growth were further evaluated. Among these, *Salmonella* Paratyphi A was identified from 4 patients and *Salmonella* Typhi from 6 patients. The Typhisure RDT by Koshibio Diagnostics showed a sensitivity of 100% and specificity of 100% in detection of *Salmonella* Typhi from blood culture broth. Similarly, in all 4 Blood culture positive *Salmonella* Paratyphi, antigen was detected by Typhisure (Sensitivity 100%). However, one test came as false positive. two tests were considered invalid as the control line failed to appear in the kit.

Conclusion: Conventional blood culture broth with the RDT for antigen detection could pave a pathway forward for early diagnosis of enteric fever in resource limited setting like ours where Bactec, biochemical tests and Serotyping is not available in primary healthcare settings.

Keywords: Blood culture; Enteric fever; Rapid diagnostic tests (RDTs); S. Typhi; S. Paratyphi

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INTRODUCTION

Enteric fever is a systemic infection caused by Salmonella enterica serovars Typhi and Paratyphi A, B, and C, with significant morbidity and mortality in resource-limited regions such as Asia, Africa, and South America due to poor sanitation and unsafe water.[1,2]

Clinical symptoms are non-specific, making laboratory diagnosis crucial but challenging.[3] Blood culture is the reference standard, yet it has variable sensitivity (40–80%), requires technical expertise, is costly, and takes

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2–3 days.[4] Many typhoid cases go undetected due to limited healthcare access or false negatives.[5]

Rapid diagnostic tests (RDTs) offer faster, simpler alternatives with lower costs and minimal training requirements.[6] However, a meta-analysis by Wijedoru et al. found that current RDTs, including TUBEX, Typhidot, and Test-it Typhoid, lack sufficient sensitivity and specificity to replace culture-based methods.[2] WHO recommends that ideal tests for typhoid should approach 100% sensitivity and specificity.[2] Thus, blood culture remains the gold standard for definitive diagnosis and antimicrobial susceptibility testing, despite its limitations.[7]

In this study, we evaluated the sensitivity and specificity of the Typhisure© test, a lateral flow immunoassay, which aims to improve detection of S. Typhi and S. Paratyphi antigens from blood culture samples.

METHODOLOGY

A descriptive cross-sectional study was done from a period of 6th August 2024 to 30th October 2024 in Department of Microbiology, after obtaining ethical clearance from Institutional Review Committee (IRC) of Kathmandu Medical College (Ref no.05082024/07). Convenient sampling method was used and the sample size was calculated using the Formula:

$N= z^2pq/e^2$

$(1.96)^2x5.4x94.6/5^2 = 78.49 = 79$

Where, z =1.96

Prevalence of enteric fever (p) = 5.4%[8]

q= 100-p (100-5.4) = 94.6

e (allowable error) = 5

All the positive blood culture bottles (n=100) from total sample of 758 patients received during the study period were further processed using standard microbiological methods for identification and characterization of the etiological agent. So the final sample size was 100. For rapid diagnostic testing by Typhisure, blood was drawn aseptically using sterile syringe from blood culture bottles which showed evidence of growth into fresh test tubes. The procedure was carried out in biosafety cabinets in Microbiology laboratory. 50-70 microliters of such specimen was added into the sample well of the test device. The broth used was BHI, according to our hospital's protocol and in lieu with kit's manual, which specifies any blood culture broth to be used for the test. Test result was read and interpreted within 15 minutes. A positive result was indicated by pink/purple lines at the test line regions "St" or "Spt," along with a line at the control line region "C." A negative result was indicated by the absence of lines at the test line regions and a line at the control line region "C." If the control line region "C" did not exhibit a pink/purple line, the test was considered invalid.

A structured Performa that included all pertinent information was used to gather the necessary data. Data analysis was done using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA.

RESULTS

Among total of 758 blood culture samples, 100 blood culture broth showing evidence of growth (eg. turbidity, pellicle formation) after 24 hrs of aerobic incubation at 37^{0}C were further evaluated. The culture bottle not showing evidence of growth at 24 hrs were further incubated and reevaluated at 48 hrs and 72 hrs. The highest no. of organisms isolated were *Klebsiella pneumoniae* (35) followed by *Acinetobacter baumanii* (24).

Salmonella Typhi was isolated from 6 patients and *Salmonella* Paratyphi A from 4 patients. Among them, 6 were male and 4 were female patients (Figure 1).

Typhisure RDT correctly identified *Salmonella* Typhi antigen in 6/6 (100%) blood culture samples, corresponding to a sensitivity of 100%. The specificity was 100% as none of the non- Salmonella isolates showed positive results (Table 1).

Similarly, in all 4 Blood culture positive *Salmonella* Paratyphi A, antigen was detected by Typhisure (Sensitivity 100%). However, 1 kit showed positive reaction in all 3 bands, i.e. for both *Salmonella* Typhi and *Salmonella* Paratyphi, however in repeated subcultures, only *Salmonella* Typhi was yielded (Specificity 90%). Moreover, 2 tests were invalid as the control line failed to appear after testing. On repeating the tests both cultures yielded negative results.

Table 1: Sensitivity and specificity of Typhisure in comparison with traditional culture and identification

Organism isoalted	Blood culture	Typhisure	Sensitivity/ Specificity
Salmonella Typhi	6	6	100%/ 100%
Salmonella Paratyphi	4	5	100%/ 90%
Invalid tests for typhisure: 2 (2%)			

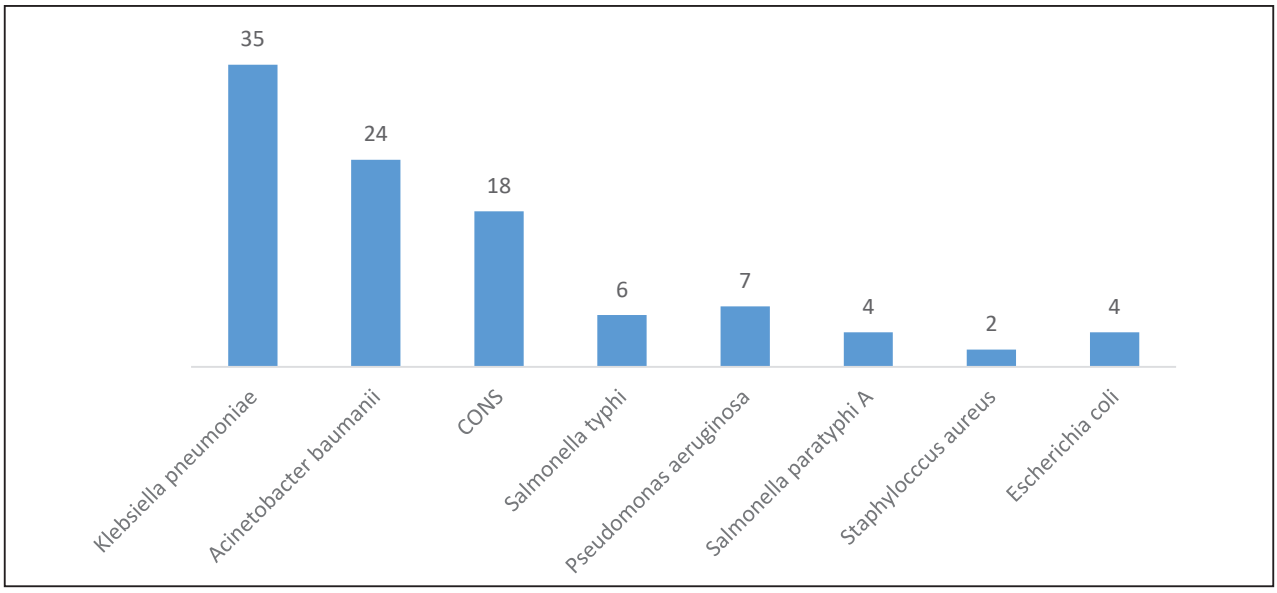

Figure 1: Organisms isolated form blood (n)

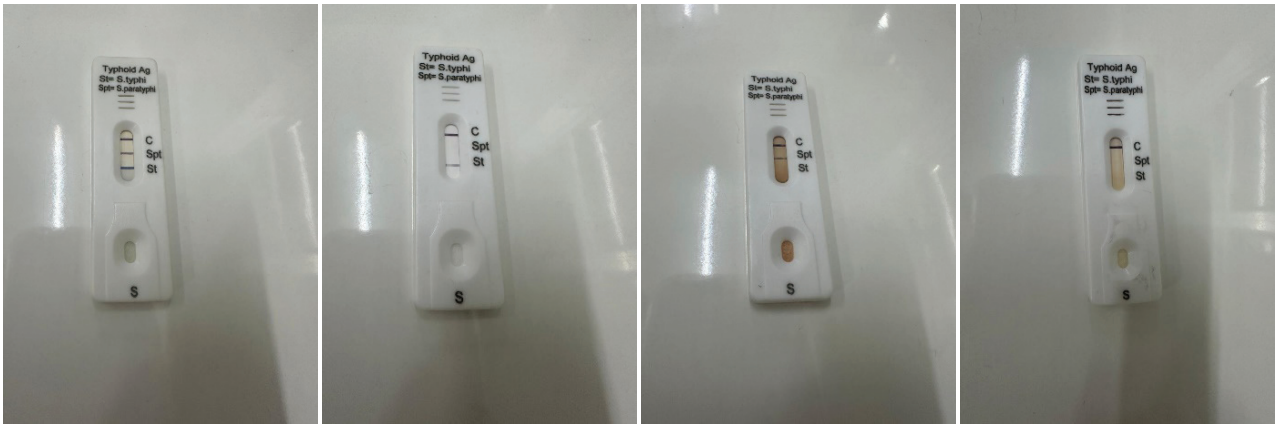

Figure 2: Photographs depicting both *Salmonella* Typhi and Paratyphi positive, *Salmonella* Typhi positive, *Salmonella* Paratyphi positive and Negative results respectively.

DISCUSSION

In this study, we evaluated the performance of lateral flow immunoassay for the qualitative and differential detection of *S.* Typhi and *S.* Paratyphi antigen in blood culture broth after aerobic incubation of 24 and 48 hrs at 37^{0}C.

Among total of 758 blood culture samples, 100 showed evidence of growth, which were further evaluated for the study. *Klebsiella pneumoniae* were isolated with the highest frequency (35) followed by *Acinetobacter baumanii* (24). The high specificity of Typhisure © for Salmonella Typhi (100%) was confirmed as all non-salmonella cases gave negative results. However, the test kit had 90% specificity for *S.* Paratyphi.

In this study, 6 (0.79%) *Salmonella* Typhi and 4 (0.52%) *Salmonella* Paratyphi A were isolated among 758 blood culture samples. Nepal is a low-income country with a population of 28 million where Typhoid is endemic.[9] In prospective surveillance at sites with blood culture capacity, only 4.1% of those clinically diagnosed with typhoid had positive blood cultures for typhoidal Salmonella, and this rate ranged between 0 and 2.8% in rural areas of Nepal.[10] In April 2022, a national campaign was launched to introduce typhoid conjugate vaccines, the success of which might have been reflected in our study as very less number of *Salmonella* Typhi were isolated.

The Typhi Sure RDT by Koshibio Diagnostics showed a sensitivity of 100% and specificity of 100% in detection

of *Salmonella* Typhi from blood culture broth. We did not find any false positive results. However, since we did not test Typhisure in all blood culture negative cases, we cannot accurately confirm its diagnostic ability in blood culture negative cases.

Similarly, in 4 Blood culture confirmed isolates, *Salmonella* Paratyphi antigen were detected (Sensitivity 100%). 1 kit showed positive reaction in all 3 bands, i.e. for both *Salmonella* Typhi and *Salmonella* Paratyphi, however in repeated subcultures, only *Salmonella* Typhi was yielded (Specificity 90%). This may be attributed to antigenic cross reaction between these 2 serovars. The modification of the kit may be warranted after further evaluation in different centres. Moreover, 2 (2%) tests were invalid as the control line failed to appear after testing. This may be due to manufacturer's defect, or the storage and shipment issues prior to arrival of the kit to the laboratory. The positive predictive value is one of the important character of a RDT. The positive predictive value of the Typhisure test was 100% as all culture positive salmonella tested positive when initially tested with the kit. Therefore, this assay can be helpful to the clinician for initiation of the therapy.

In a similar study for assessing dipstick assay for diagnosing typhoid fever in Indonesia, the sensitivity of the assay was found to be 69.8% and 86.5% when compared with bone marrow and blood cultures respectively. [11] A review by Wijedoru et al of 37 studies which evaluated the diagnostic accuracy of RDTs concluded that the RDTs evaluated are not sufficiently accurate to replace blood culture as a diagnostic test for enteric fever. [12]

The current study has limitations as the sample size of Salmonella positive blood culture were low.

Moreover, the kit does not differentiate between *Salmonella* Paratyphi A, B and C. The strength of the study was that such type of evaluation of Salmonella RDTs were not done previously in Nepal, which is endemic for typhoid. We evaluated conventional blood culture broth with the RDT which could pave a pathway forward for resource limited setting like ours where Bactec and Biochemical tests and Serotyping is not available in primary healthcare settings as diagnosis can be made earlier and thus therapeutic interventions could be started.

This was a descriptive study evaluating qualitative and differential detection of *S.* Typhi and *S.* Paratyphi antigen in human blood culture specimen by Typhisure©. A RCT evaluating different RDTs would have given comprehensive analysis on the kit's specificity and sensitivity in comaprison to others. Moreover, the Salmonella isolates were low in our study.

CONCLUSION

The test could be a reliable aid to conventional blood culture as in the time required for subculture and antimicrobial susceptibility results, presumptive diagnosis and treatment could be decided by the treating physician. However, more widespread trials of the kit has to be done for testing its efficacy.

Source of Support: Koshibio diagnostics provided the kit free of cost to conduct this study.
Conflict of Interest: None

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