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Blood Clot PCR For Diagnosis Of Enteric Fever

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Abstract:
Background*: Though several reports of the resistance to first-choice antimicrobial for enteric fever i.e. Ceftriaxone emerge, empirical use of the same antimicrobial without diagnostic confirmation continues. The gold standard, blood culture has poor sensitivity and lacks rapidity. Culturing bone marrow though definitive, is invasive. It is mostly used as last resort to solving undifferentiated fever cases. Serology cannot be used to expand its scope to give information on antimicrobial resistance. This leads us to molecular diagnosis as alternative. Hence, this study was aimed to evaluate performance of the multiplex Real-time PCR using novel targets for early diagnosis of enteric fever on blood clot samples at a tertiary care center.*
Materials and Methods*: In this retrospective non-randomized study, 116 patients with diagnosis of enteric fever were included and the DNA extracted from blood clot samples collected for routine WIDAL test were subjected to multiplex Real-time PCR assay targeting novel sequences of Salmonella ser. Typhi and Salmonella ser. Paratyphi A. The results of the new assay were compared to simultaneously requested blood culture, WIDAL tube agglutination tests and or final clinical impression.*
Results*: The multiplex Real-time PCR assay using DNA extracted from clot sample gave an accuracy of 70.7% with an overall sensitivity of 50.9% and specificity of 82.1% against all 116 enteric fever cases with positive predictive value of 92.2% and negative predictive value of 28.8%. The performance of this PCR was statistically significant (p = 0.001) in 116 patients with diagnosis of enteric fever either by laboratory tests or clinically confirmed cases.*
Conclusion: *To diagnose enteric fever using this test, further optimization is needed to improve the sensitivity by enrichment of clot samples and screening of additional sequences with a better detection limit could help to improve the overall performance of this diagnostic test.*
Key Word: *multiplex PCR; Real-time PCR; Salmonella ser. Typhi; Salmonella ser. Paratyphi A; enteric fever*

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I. Introduction

Salmonella ser. Typhi is usually associated with enteric fever, a major health problem in developing countries. However, in recent years Salmonella ser. Paratyphi A has emerged. Diagnosis of enteric fever is ever challenging, differentiating it from long list of febrile diseases with no specific & sensitive test to rely upon. (1) The lack of facilities for culture, primary care physicians are reliant serology and treat enteric fever empirically. They often prescribe a broad-spectrum antimicrobial often covering Salmonellae and targeting several microorganisms that may be causing the observed infection (3). Despite the increased sensitivity of bone marrow culture, obtaining bone marrow by standard methods is technically challenging, invasive and generally not performed.(1) The serological tests are hampered by lack of specificity and have no further scope of testing for antimicrobial resistance. Enteric fever diagnosis by PCR using different samples, such as whole blood, clot, stool, and urine has been studied with useful results. (3,4) Real-time PCR shows promise as an effective and accurate technology and has a high degree of agreement with conventional culture methods for Salmonella from food or clinical samples namely, whole blood, bone marrow and stool.(2,3) In a study by Nga et al., multiplex Real-time PCR assay was validated and demonstrated a high level of specificity and reproducibility under experimental conditions but concluded that PCR performed directly on blood samples may be an unsuitable methodology and a potentially unachievable target for the routine diagnosis of enteric fever.(3) Blood Clot cultures are excellent for isolation of enteric fever salmonellae.(4,5) Real-time assay using blood clot sample is a desirable alternative, as the microbiology laboratory receives the clotted blood sample for WIDAL test which eliminates the collection of an extra sample for PCR assay. The clot leftover after serum collection for WIDAL test is discarded after the collection of serum. Chaudhry et al. (6) have studied PCR diagnosis using clot sample, by amplifying the dH flagellin gene of Salmonella ser. Typhi but with prior enrichment by bile broth, with sensitivity and specificity of 100% and 93.7% respectively. Enrichment improves the sensitivity of the test, but the appeal of PCR assay which is speed, is missed along with an added-step. Recently, studies have validated new techniques to extract DNA

from clot. Clements et al.(7) have compared 3 methods for DNA extraction - namely, a phenol chloroform method, a silica gel membrane column method (Qiaamp Midi Kit) and a modified salt precipitation method - and revealed that the silica column method retrieves DNA from both clotted blood and ethylenediaminetetraacetic acid (EDTA)-preserved blood with equally good results.(8) Here in this study, a multiplex PCR targeting for differential diagnosis of enteric fever pathogens on clot samples is evaluated. The testing method, is multiplex-Real-time PCR (3) on suspected enteric fever patients' blood clot samples, using commercial column method to extract DNA.

II. Material And Methods

This retrospective non-randomized study was carried out jointly at Department of Microbiology, St. Johns Medical College, Bangalore and St. John's Research Institute, Bangalore. The study period was between September 2011 and September 2013. The new test was carried out on blood clot samples from patients from both outpatient and in patients at St John's Hospital, Bangalore, a tertiary care centre. A total of 160 non-consecutive patients of both genders and all age groups with clinically suspected enteric fever were included in the study. The demographic and clinical details from the hospital outpatient or inpatient records were collected. Ethics committee of St. John's Medical College approved the study protocol.

Study Design: Retrospective non-consecutive study for diagnostic test evaluation

Study Location: St John's medical college in collaboration with St John's Research Institute

Study Duration: September 2011 to September 2013.

Sample size: This is pilot study

Sample size calculation: Pilot study with minimum of 30 cases culture confirmed cases.

Subjects & selection method: The study population was drawn from patients who presented with acute febrile illness whose samples were sent for Tube WIDAL. A total of 160 non-consecutive patients of both genders and all age groups with clinically suspected enteric fever were included in the study. The demographic and clinical details from the hospital outpatient or inpatient records were collected. Ethics committee of St. John's Medical College approved the study protocol.

Inclusion criteria:

Clinically suspected of enteric fever patients or patients with any febrile illness with WIDAL and blood culture requested were included in the study.

1. GROUP 1: Automated or manual blood culture positive for Salmonella Typhi and Salmonella. Paratyphi A, irrespective of WIDAL titer.
2. GROUP 2: Blood culture negative and WIDAL positive defined as titer more than or equal to 1:160 for all 'STO' (Salmonella Typhi 'O'), 'STH' (Salmonella Typhi 'H') and 'SPAH' (Salmonella Paratyphi 'AH') antigens
3. GROUP 3: Blood culture negative and WIDAL Negative, defined as less than titer 1:80 for three antigens.
4. GROUP 4: Blood culture negative and WIDAL indeterminate, is defined as titer of 1:80 for any of the three antigens.

Exclusion criteria:

1. Any sample with no simultaneously collected blood sample for culture
2. Hemolysed sample

Note: Prior antimicrobial treatment was not exclusion criteria as, getting treatment naïve patient is challenging in tertiary care center. Data on antimicrobials was collected and analyzed in results.

Procedure methodology

This study was done to evaluate performance of RTPCR on blood clot DNA samples to compare with that of WIDAL and blood culture tests. The study consisted of two-part optimization i.e. first to get good quality DNA and in 2nd part to run RTPCR using the primers described by Nga et al. (6) After this process was set, patient samples were drawn as per the protocol, clot DNA was extracted and new test was performed. After the serum was used for WIDAL test, the clot along with vacutainer was stored at 4°C, until further sample processing for DNA extraction. The 30 µL of extracted DNA samples were stored at - 20°C till further use. This DNA sample

was used for the multiplex Real-time PCR assay for Salmonella ser. Typhi and Salmonella ser. Paratyphi A specific target sequences.

Real-time-Polymerase chain reaction

Bacterial strains used for optimization of Real-time PCR: Pure cultures of clinical isolate namely Salmonella ser. Typhi and Salmonella ser. Paratyphi A were used for the optimization of PCR. Same strains were used as the positive control in each run throughout the study. The identification of isolates was confirmed by serotyping (Central Research Institute, Kasouli, Himachal Pradesh).

PCR Optimization and Laboratory assay for detection limits

Crude lysate as template for colony PCR: For colony PCR, the template was prepared by picking up isolated colonies of bacteria using a sterile micropipette tip and suspending them in 10 µl of nuclease-free water and boiling them at 95°C for 10 min in a PCR tube. The supernatant was used as a template for optimization of PCR. Similar 36 lysates were made for both (Salmonella ser Typhi and Salmonella ser. Paratyphi A) isolates and stored at -20°C until further use.

Optimization experiments: The detection limit of assay was calculated using saline dilutions and subsequent results of repeated experiments were compared to assess variability. Initially, cultures of Salmonella ser Typhi and Salmonella ser. Paratyphi A were grown overnight at 37°C on MacConkey agar. Serial dilutions of the 1 MacFarland ($3x10^8$ CFU per ml) turbidity, up to 10-12 CFU per ml were made as described below. 200 µl of each dilution in saline was used for crude lysate preparation (as described above). Bacterial counts for each bacterial dilution in saline were performed in triplicate by pour plate method using nutrient agar medium (HiMedia Laboratories, Mumbai, India). The numbers of colony forming units were correlated with detection by Real-time PCR using melting curve analysis. The number of copies detected by PCR was correlated by calculating with the colony count in each dilution.

Optimization experiments:

1. Determining the viable count from 1 McFarland bacterial suspension and each dilution – by pour plate method
2. Real-time PCR was performed for Salmonella ser. Typhi and Salmonella ser. Paratyphi A for all dilutions, both in uniplex and multiplex reactions to find detection limit by assay.

The products were confirmed by running in 2.5% agarose gel with 0.5µg/ml ethidium bromide.

Extraction of DNA from clot samples: Samples were processed in biosafety cabinet. For DNA extraction, the blood clot is broken using sterile tip. Approximately 500µl of broken blood clot is transferred to 2 ml micro centrifuge tube. DNA was extracted using QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's protocol.(8) An additional step was included in the procedure, i.e. after clot lysis and proteinase K digestion the crude lysate was passed through PAXgene Shredder spin columns (Qiagen, Germany) to remove debris before proceeding for the extraction. The DNA was eluted in 30 µl of Elution Buffer provided in the kit. The eluted DNA was stored at -20°C until further use. The integrity and purity of DNA samples were checked by 1% agarose gel electrophoresis.

Primers and PCR conditions

Primers: Primers designed by Nga et al.,(3) were used for study. The primers were procured from Integrated DNA Technologies (IDT), USA. Primer sequences were as follows;

Salmonella ser. Typhi

ST-Frt 5'CGCGAAGTCAGAGTCGACATAG 3'
ST-Rrt 5'AAGACCTCAACGCCGATCAC 3'

Salmonella ser. Paratyphi A

S.Pa-Frt 5'ACGATGATGACTGATTTATCGAAC3'
S.Pa-Rrt 5' TGAAAAGATATCTCTCAGAGCTGG3'

Real-time-PCR conditions: Real-time PCR assay was done using SYBR Green (Sigma - Aldrich®) Chemistry. PCR reaction included, 5 mM MgCl2, 0.2 mM each deoxynucleotide triphosphate, 20 picomoles of each primer (For S. Typhi ST-Frt, ST-Rrt and for Paratyphi A S.Pa-Frt, S.Pa-Rrt), 10 U of Taq DNA polymerase (Amnion Biosciences Pvt Ltd, Bangalore, India) and 0.2x SYBR Green working strength in a 50 µl reaction. 10 µl of eluted DNA from blood clot sample was used as the template for the PCR. PCR was performed on Rotor Gene 6000

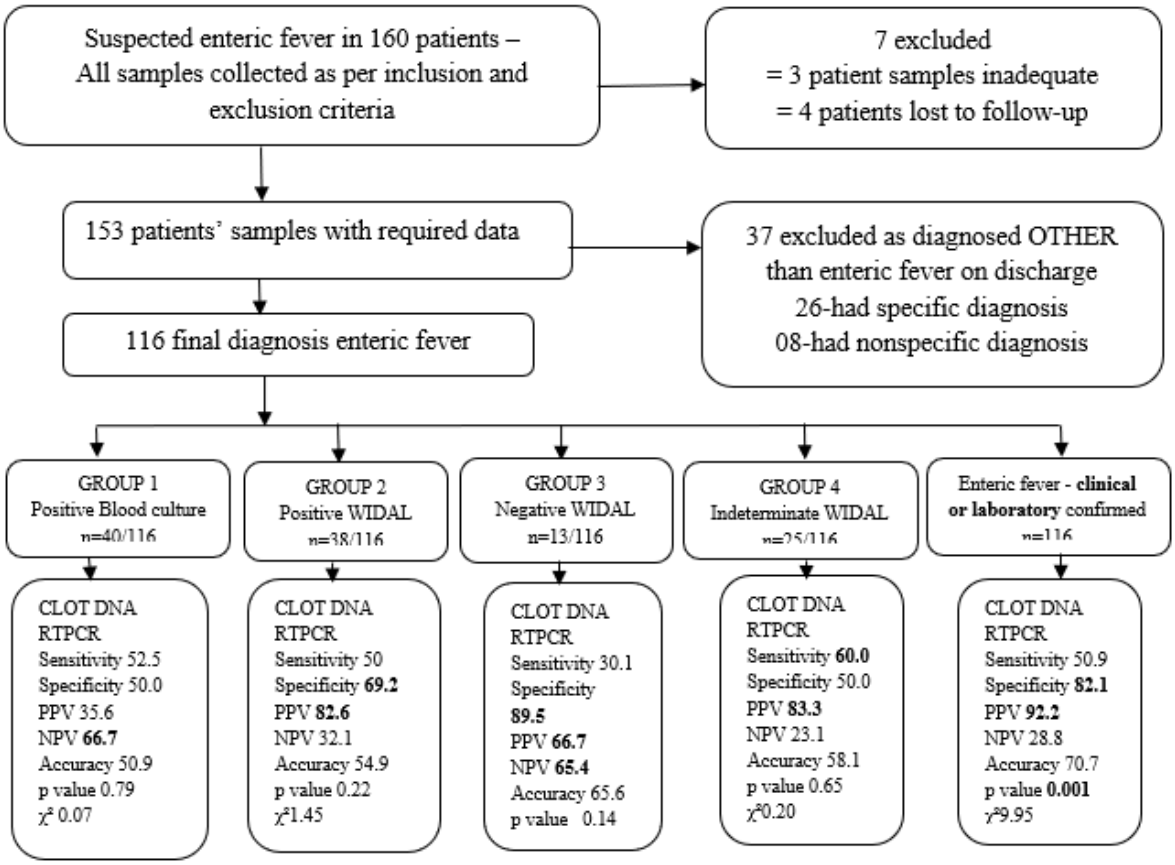
(Qiagen) Real-Time PCR Machine. All PCRs were cycled under the following conditions; Hold @ 95°C, 2 min 0 seconds and 40 repeats cycles of Step 1 @ 95°C, hold 30 seconds, Step 2 @ 55°C, hold 30 seconds and Step 3 @ 72°C, hold 30 seconds.

Real-time PCR Interpretation using Melting Temperature of Positive control: The detection is performed with dsDNA-specific dyes like SYBR Green, using melting curve that was recorded after the amplification of the target sequences by PCR. The primers allowed the amplification of PCR products having distinct melting temperature values, resulting in the formation of two distinct peaks representing the two targets. It was confirmed by the melting temperature (Tm), which was consistently specific for the amplicons obtained. In melting curves, the signal decreases gradually because of a temperature-dependent quench and more abruptly at a certain temperature because of the melting separation of the products/two strands. The melting temperature (Tm) of a product is defined as the temperature at which the steepest decrease of signal occurs. This can be identified conveniently as the peak value of the melting curve. The mean peak Tm obtained with curves specific for ser. Typhi ranged from 85±0.5°C and for ser. Paratyphi A 79.5 ± 0.5°C.(3) A negative PCR result was concluded if negative controls showed melting temperature as the positive controls, presence of contamination is considered and assay was treated as "INVALID". A positive result for Salmonella Typhi was interpreted, if the melting temperature of the sample coincided with melting temperature of positive control which was in the range of 85±0.5°C. For Salmonella Paratyphi A melting temperature ranged from 79.5 ± 0.5°C. The products were run on 2.5 percent agarose gel with ethidium bromide 0.5µg/ml and confirmed the findings from all the assays, including the controls. The Salmonella ser. Typhi specific primers produced as described by Nga et al(3) - a 131 bp amplicon and the S. Paratyphi A primers amplified a 104 bp fragment.

Isolation of Salmonella ser. Typhi and Salmonella ser. Paratyphi A from blood culture: Either method used was accepted i.e. manual culture or automated provided the blood culture was requested. The automated culture was either by BD BACTEC™ 9050 Blood Culture System or BacT/ALERT 3D System. For manual method, diphasic medium consisting of brain heart infusion (BHI) agar and BHI broth was used for blood cultures (HiMedia Laboratories, Mumbai, India). The bottles were screened daily for growth unto 7 days for manual and 5 days for automated culture bottles. Any isolate with Non-Lactose Fermenting colonies obtained on subculture on to MacConkey agar was identified by biochemical screening tests for gram negative bacilli. The identification was further confirmed by using Salmonella Polyvalent antisera against O9 and Hd for Salmonella ser. Typhi and against factor 2 and AH for Salmonella ser. Paratyphi A (Central Research Institute, Kasouli, Himachal Pradesh).

WIDAL tube agglutination test: The WIDAL test was performed within 24 h of collection of serum samples, using a colored antigen kit (Span Diagnostics, Surat, India.). WIDAL positive defined as titer more than or equal to 1:160 for STO Antigen (Salmonella ser. Typhi 'O'), either of STH Antigen (Salmonella ser. Typhi 'H') and SPAH Antigen (Salmonella ser. Paratyphi 'AH') antigens. A titer of 1 in 80 for any of STO, STH or SPAH antigens was considered as indeterminate result. WIDAL Negative, defined as titer up to 1:40 for three antigens. WIDAL borderline is defined as titer of 1:80 for any of the three antigens.

Real-time-PCR results: The Real-time PCR assay detection limit for uniplex and multiplex reactions was 3 x 10^3 CFU per ml. The products were also run in agarose gel, which were detected as clear bands upto 3 x 10^4 CFU per ml. Total of 160 patient clot samples were drawn as per the requirements. Out of these, seven were disqualified based on exclusion criteria. Out of 153, 116 were confirmed enteric fever either culture, WIDAL or clinically. Total of 40 were blood culture positive; 26 grew Salmonella ser. Typhi, and 14 grew Salmonella ser. Paratyphi A.

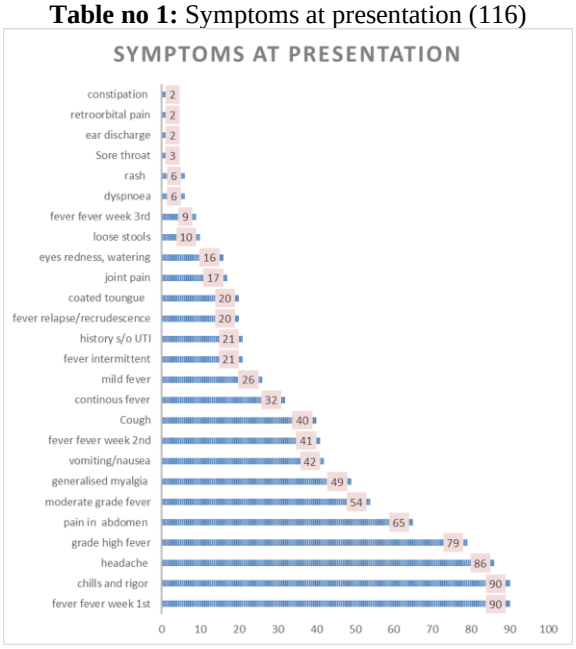


Statistical analysis - Data analysis (Pearson's chi-squared test) was done using the software Epi Info version 3.5. Chi-square test was performed to test for differences in proportions of categorical variables between two or more groups. The level $P < 0.05$ was considered as the cutoff value or significance. However, the results of the comparison should be interpreted with caution as the lack of an adequate gold standard is a major limitation for developing new enteric fever diagnostic tests. (10)

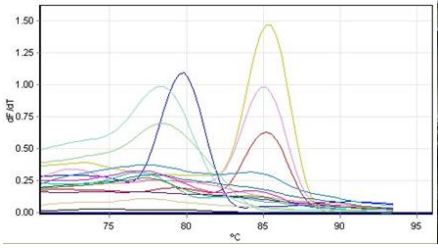
III. Results

The final diagnosis of enteric fever was established either by laboratory reports namely blood culture, Widal test or clinical basis in 116 patients. The results of the three tests in 116 established diagnoses of enteric fever are evaluated. The most common clinical presentation included, high grade fever with chills and rigor, headache, pain in abdomen, generalized myalgia, nausea, vomiting and cough in decreasing order of frequency. Mean age of the study population was 23.5 years and the highest number of patients were between 11 to 20 years of age i.e. 44 (28.8%). The total pediatric age group i.e. up to 18 consisted of 57(48.7%) patients and 96 (62.7%) patients were of more than 18 years. The study population (116) consisted of 59 (50.9%) males and 57 (49.1%) females. Out of 116 enteric fever patients, 57 patients had received prior treatment before visiting St. John's Hospital. However, the specific antimicrobial name was available only in 3 patients.

Table no 1: Symptoms at presentation (116)



Real-time PCR- Melting curve analysis Rotor Gene 6000 Real-Time PCR Machine With positive & negative controls



Colour	Name	Peak 1	Peak 2	Peak 3	Result
	multiplex Positive Control	72.0	79.2	85.2	Satisfactory
	Salmonella Typhi Positive control	73.3	81.0	85.3	Satisfactory
	Salmonella Paratyphi Positive Control	73.0	79.7	90.5	Satisfactory
	w 3367	74.0	77.0		Negative for enteric fever
	w 4441	72.5	77.8	85.0	Positive for Typhoid
	Negative Control - PCR hood	78.5	86.7	88.5	Satisfactory
	Negative Control –outside	78.3	89.5		Satisfactory

Blood culture status in 116 enteric fever patients with WIDAL and Real-time-PCR results

Blood cultures	**Reported**	**Widal result**			**Real-time PCR Positive in**
		Titers Suggestive of enteric fever	Negative	Indeterminate	
26(22.4)	*Salmonella* ser. Typhi	11*(9.5)	7(6.0)	8(6.9)	13(11.2)
14(12.1)	*Salmonella* ser. Paratyphi A	4(3.5)	9(7.8)	0(0)	8(6.9)
04(3.4)	CONS	1(0.1)	1(0.1)	2(0.2)	1(0.1)
72(62.1)	No growth	37(31.9)	12(10.3)	23(19.8)	37(31.9)
116	**Total**	**53(45.7%)**	**29(25%)**	**33(28.5)**	**59(50.9)**

IV. Discussion

Enteric fever is among the most common causes of acute febrile illness in India. The blood culture and WIDAL test are the commonly used tests for diagnosis in a tertiary care centre. Despite low sensitivity (40 to 60%)(9), blood culture is still considered as the gold standard for diagnosis of enteric fever. This is a major limitation for such studies as described by Parry et al.

In this study, the clot sample is evaluated for diagnosis of enteric fever. In a study by Nga et al(3), have shown that PCR performed directly on blood samples is an unsuitable methodology and a potentially unachievable target for the routine diagnosis of enteric fever. As discussed earlier, clot culture study has shown no added advantage in sensitivity over procedures which use whole blood; though they are excellent for isolation of enteric fever salmonellae.(4,5) In this study, the DNA from blood clot is extracted using a new methodology to extract DNA to be used as template for multiplex Real-time PCR assay. In this study, the new targets (3) are evaluated with respect to the blood clot sample without enrichment, for detection of the two important pathogens causing enteric fever in India.

Out of 153, only 116 patients were considered to have enteric fever based on culture, WIDAL and clinical impression or response. In 28/153 patients, the final diagnosis was other than enteric fever, and rest 9 out of 153 patients had no specific clinical or laboratory diagnosis. Of the 153-blood culture and WIDAL simultaneous requests, in 72(47%) patients, the tests were requested to rule in and in 26 (17%) patients to rule out enteric fever. In 55 (36%) patients no specific working diagnosis was considered. After sending the culture and WIDAL requests; irrespective of what patient had, empirical treatment was advised in enteric fever cases. All 153 patients received at least 1-2 days of standard medication (Namely, Tab Cefotaxime or Injection Ceftriaxone or Tab Ciprofloxacin).

Blood culture sensitivity was 35.5% against all 116 enteric fever patients. This is lower than most other studies where approximately positivity is 40–60% of presumptive cases.(9) The lower sensitivity also may be due to the treatment received in 57 patients before coming to St. John's Hospital. No growth among treated was 68.4% whereas without treatment 62.7% (CONS considered as no growth). This emphasizes the importance of culturing before administering antimicrobials at all care facilities. Availability of cost effective and technically feasible blood culture facility at all care levels, would also help bring down the empirical or improper treatment with precious antimicrobials.

With blood culture as gold standard, sensitivity of single tube WIDAL test in this study was 29.6% and specificity was 38.7%. Positive predictive value was 40.0% and negative predictive value of 50.0%. The accuracy was 46.6%. This is lower in comparison with similar study by Nagarajan et al. where WIDAL (slide agglutination) test showed sensitivity of 63%. The reason may be due to the method used, as slide agglutination method is known to give false positives. Also, WIDAL test continues to be plagued with controversies involving the quality of the antigens used and interpretation of the result, particularly in endemic areas.(10) But it is still used by many laboratories and clinicians, due to lack of facility to culture blood, or longer turnaround time or cost. Low sensitivity also may be due to the low titer in 1st week or lack of paired sera (64 /116 patients presented with fever 1st week). In this study, three patients had WIDAL significant titers suggestive of enteric fever however with the final diagnosis of culture positive UTI with E coli growing $> 10^5$ CFU per ml, Lower Respiratory Tract Infection with culture positive for Klebsiella and the third patient with Hepatitis E respectively. Though, the WIDAL result may be due to anamnestic reactions, dual infections cannot be ruled out due to lack of test result with convalescent sample. Realtime PCR and blood cultures were negative in these 3 patients.

The multiplex Real-time PCR assay using clot sample gave overall sensitivity of 50.9% & specificity of 82.1% against all 116 clinical and or laboratory confirmed enteric fever cases with PPV 92.2%. The new test performance is statistically significant (p value 0.001) in 116 patients with either laboratory or confirmation of enteric fever diagnosis. Among the blood culture positive the sensitivity of PCR was 52.5 % & specificity was 50.0%. The low sensitivity may be due to the low blood culture sensitivity itself, which may be due to the treatment prior to culture. Though, Real-time PCR assay performance appears to be above the blood culture or WIDAL performance with respect to the final clinical picture; all the three tests are complementary, since they are individually lacking in either sensitivity or specificity. This assay could be a useful adjunct as it needs further optimization to be used as single test. The sensitivity may improve with enrichment, but which is not appealing as speed takes the priority when choosing the tests like PCR for early diagnosis. However, the test could help in ruling out enteric fever in patients with negative WIDAL and blood culture tests with negative predictive value of 82.1% and accuracy of 65.9%. The test also helps in patients with WIDAL indeterminate but culture negative with positive predictive value of 63.6% and accuracy of 61.5%.

Limitations of the study

1. Storage time of clot samples before processing, eluted DNA after processing varied. For detection of target sequence products in this study by SYBR Green, but not with specific probes as used in the original study.

Though the melt temperature chosen may be specific, final confirmation is by sequencing of PCR products which could not be done.

2. Not having 100% sensitive test for confirming enteric fever; also, not having the specific tests for Rickettsial fever(typhus), viral fever, to assess the false positive tests.
3. Not having an arm with standard PCR validated test for enteric fever diagnosis or an enrichment group to compare.
4. Not having an arm with EDTA whole blood sample for comparison

V. Conclusion

Here, with blood culture as gold standard using specific target sequences (3) in blood clot samples the sensitivity, specificity, PPV and NPV were 52.5%, 50% 35.6% and 66.7% (p value of 0.79) respectively. However, among all 116 enteric fever cases overall sensitivity of 50.9% & specificity of 82.1%, PPV 92.2% was observed; which was statistically significant (p value of 0.001) in 116 patients with either laboratory or clinical diagnosis of enteric fever. We need further studies to explore clot sample to improve the sensitivity either by having an enrichment arm or using different targets with better detection limit or with specific probes, as an alternative to whole blood or bone marrow. The blood clot sample is not suitable exclusively for confirmatory diagnosis with this RTPCR test; however, the test could be a useful adjunct in particularly difficult cases where WIDAL and blood culture tests are not supporting the clinical diagnosis.

References

[1] Baker S, Favorov M, Dougan G. Searching For The Elusive Typhoid Diagnostic. BMC Infect Dis. 2010 Mar 5;10:45.
[2] Evaluation Of Real-Time PCR Vs Automated ELISA And A Conventional Culture Method Using A Semi-Solid Medium For Detection Of Salmonella | Request PDF. Researchgate [Internet]. 2024 Oct 22 [Cited 2024 Nov 11]; Available From: Https://Www.Researchgate.Net/Publication/8995234_Evaluation_Of_Real-Time_PCR_Vs_Automated_ELISA_And_A_Conventional_Culture_Method_Using_A_Semi-Solid_Medium_For_Detection_Of_Salmonella
[3] Nga TVT, Karkey A, Dongol S, Thuy HN, Dunstan S, Holt K, Et Al. The Sensitivity Of Real-Time PCR Amplification Targeting Invasive Salmonella Serovars In Biological Specimens. BMC Infect Dis. 2010 May 21;10:125.
[4] Watson KC. Isolation Of Salmonella Typhi From The Blood Stream. J Lab Clin Med. 1955 Jul;46(1):128–34.
[5] Escamilla J, Florez-Ugarte H, Kilpatrick ME. Evaluation Of Blood Clot Cultures For Isolation Of Salmonella Typhi, Salmonella Paratyphi-A, And Brucella Melitensis. J Clin Microbiol. 1986 Sep;24(3):388.
[6] Chaudhry R, Laxmi BV, Nisar N, Ray K, Kumar D. Standardisation Of Polymerase Chain Reaction For The Detection Of Salmonella Typhi In Typhoid Fever. J Clin Pathol. 1997 May;50(5):437–9.
[7] Clements DN, Wood S, Carter SD, Ollier WER. Assessment Of The Quality And Quantity Of Genomic DNA Recovered From Canine Blood Samples By Three Different Extraction Methods. Res Vet Sci. 2008 Aug;85(1):74–9.
[8] Qiaamp DNA Micro Handbook - QIAGEN [Internet]. [Cited 2024 Nov 12]. Available From: Https://Www.Qiagen.Com/Br/Resources/Resourcedetail?Id=085e6418-1ec0-45f2-89eb-62705f86f963&Lang=En
[9] Parry CM, Wijedoru L, Arjyal A, Baker S. The Utility Of Diagnostic Tests For Enteric Fever In Endemic Locations. Expert Rev Anti Infect Ther. 2011 Jun;9(6):711–25.
[10] Olopoenia LA, King AL. Widal Agglutination Test - 100 Years Later: Still Plagued By Controversy. Postgrad Med J. 2000 Feb;76(892):80–4.