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Evaluation of Rapid test in Comparison With Widal Test and Polymerase Chain Reaction for Diagnosis of Typhoid Fever

KEYWORDS: Salmonella, Widal test, Rapid test, PCR

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ABSTRACT *Typhoid fever, is a potentially fatal illness caused primarily by Salmonella typhi. The genus Salmonella contains over 2,000 sero-species. The diagnosis of typhoid fever always requires laboratory confirmation, either by isolation of the pathogen or by demonstration of specific antibodies. The PCR assays provide a rapid and highly sensitive method of differentiating the major Salmonella groups to detect all of the cases of acute disease. The current study was conducted on 30 patients that were suspected to have typhoid fever, based on history taking, clinical manifestations. All serum samples screened for Salmonella typhi antibodies by Widal test, then Rapid test and PCR. Widal test was positive in 100% of cases, while the Rapid test was positive in 83% of cases and the PCR was positive in 70% of cases. Rapid test was fast and cheap test showing higher specificity and diagnostic accuracy, while the Widal test was the cheapest test, and faster than PCR. As for the PCR method it is more sensitive and specific than culture and serology for diagnosis of typhoid fever.*

Introduction:
Typhoid fever is a disease caused by a bacteria known as Salmonella typhi, which is a major public health problem in many developing countries. It is endemic in South-East and Far- East Asia, the middle East, Africa, central and South America *(1)*. The infection is transmitted by feco-oral route through ingestion of food or water contaminated with faeces, it is also transmitted by either directly contaminated hands with faeces or urine of cases or carriers or indirectly by ingestion of contaminated water, milk, food or through flies. Contaminated ice, ice-cream and milk products are rich sources of infection *(2)*. Chronic typhoid carriers might be responsible for the endemicity and outbreaks of the disease. High prevalence of typhoid carriers occurs in patients with biliary, gastrointestinal and other related disease *(3)*.

It was estimated that typhoid fever caused about 21 million illnesses and more than 216,000 deaths in the world during 2000 *(4)*. Usually most of the patients (60% - 90%) are treated in the outpatient clinics, therefore hospital based studies usually underestimate the true incidence. *(5)*. Disease production depends on many factors: i) number of organisms swallowed, ii) state of gastric acidity and iii) possession of vi antigen by the organisms *(6)*. Typhoid fever is characterized by sudden onset of sustained fever, headache, malaise, anorexia, relative bradycardia, constipation or diarrhoea, and nonproductive cough. Epidemics are more common in hot weather as in spring and summer while it is sporadic in other seasons *(7)*. The disease may occur in all ages, but it's highest incidence is in children *(8)*.

Signs and symptoms of typhoid fever are nonspecific, a definitive diagnosis of the disease depending on the clinical presentation alone is no reliable. Therefore, the importance of the laboratory-based investigations in supporting the diagnosis of typhoid fever is highly focused on. *S. typhi* is isolated from appropriate samples including blood, bone marrow aspirates, stool, urine and rose spots *(9)*.

In Egypt, the diagnosis of typhoid fever is usually based on clinical presentation as well as Widal test, which are associated with numerous limitations *(10)*. The detection of IgM reveals acute phase of typhoid fever in the early stage of infection, the IgG detection reveals late phase as well as the carriage of infection, while the detection of both IgG and IgM suggests acute typhoid in the middle phase of the infection **(***11)*. It is well established that the development of molecular methods for diagnosis of infectious diseases, has improved the sensitivity and specificity of diagnosis. Polymerase chain reaction (PCR) is the most sensitive and rapid method to detect microbial pathogens in clinical specimens *(12)*.

The nested PCR had the best diagnostic value for detection of S. typhi among all the diagnostic tests used and also had higher efficacy in detecting the disease compared with the other methods like the Widal test, blood and urine cultures *(13)*.

The aim of our study was to analyze the diagnostic yield of rapid test in comparison with widal test and polymerase chain reaction for diagnosis of typhoid fever and to evaluate the sensitivity, specificity, accuracy, the cost and the time consuming of rapid test in comparison with widal test and polymerase chain reaction for diagnosis of typhoid fever.

Patients and Methods:
This study was conducted in co-operation between Tropical Medicine and Medical Microbiology & Immunology departments, Faculty of Medicine, Ain Shams University and Military Fever Hospital in the period from April

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2014 to April 2015. The study was performed on 30 patients suspected to be typhoid fever, based on history taking, clinical manifestations and positive Widal test (at titer ≥ 1\320), with positive inclusion criteria which were fever, often rising to (40 °C) or more in the afternoon - a rising and falling (undulating) fever, Chills, sweats, weakness, fatigue, joint, muscle, back pain, headache, vomiting, abdominal pain, constipation or diarrhea and lethargy. An informed consent was obtained from each of the participants before recruitment in the study. A full history and complete clinical examination was done for all the cases.

All the patients were subjected to the following laboratory investigations: Complete blood picture (with differential), Liver function tests (ALT, AST, Total and direct bilirubin), Widal test, Rapid test and PCR. Imaging with Ultrasonography; A real time scanning device, just vision 200 (SSA, 320A) with convex probe, 3.5-5 MHz, to measure the liver size, portal vein diameter and splenic size**.**

Blood samples were withdrawn from the patients by median cubital venipuncture method under complete aseptic condition. Blood samples were centrifuged 6000 rpm for 5min. Sera was withdrawn with and kept at -20°C and thawed at room temperature before being analyzed to perform Rapid test and PCR.

Widal test was done by A) slide agglutination technique and B) Tube agglutination technique. A) Using commercially available antigens of *S typhi*, a drop of the suspended antigen was added to an equal amount of the previously prepared serum. An initial positive screening test requires the determination of the strength of the antibody. This is done by adding together equal amounts of antigen suspension and serially diluted sera from suspected patients. Agglutinations are visualized as clumps. Weakly reactive agglutinations may require an adequate light source for proper visualization, while strongly reactive agglutinations are easily seen. The results of the tests are scored from 0 to 4+, ie, 0 (no agglutination), 1+ (25% agglutination), 2+ (50% agglutination), 3+ (75% agglutination) or 4+ (100% agglutination). The smallest quantity of serum that exhibits a 2+ or 50% agglutination is considered the end-point of serum activity or titer. B) Tube Agglutination Technique by making serial dilution of the patient serum with saline (0.85%) in 7 Wassermann tubes and the eighth tube contained only saline as a control. One drop of the antigen suspension was added into each tube and mixed well then incubated for48 hours at 37°C in the incubator. The tubes were examined after incubation time and checked for agglutination, the titer to be taken was the last tube to show agglutination. Titers equal or in excess of 1: 320 considered significant *(14).*

Rapid test (TYPHIDOT Rapid IgM) supplied by Reszon Diagnostic International is an immunochromatographic assay designed for the qualitative detection \of specific IgM antibodies against specific S.typhi OPM antigen in human serum or plasma. TYPHIDOT Rapid IgG/IgM (Combo) cassette (25 pieces packed in individually sealed aluminium pouch). 35 µl serum/plasma was added to each sample well serum which wicked up the membrane wait until the wet sample front of the serum reaches area marked (C) (Figure 1). After this three drops of chase buffer were added to each sample well and results were available with in15-20 minutes. (*15)*

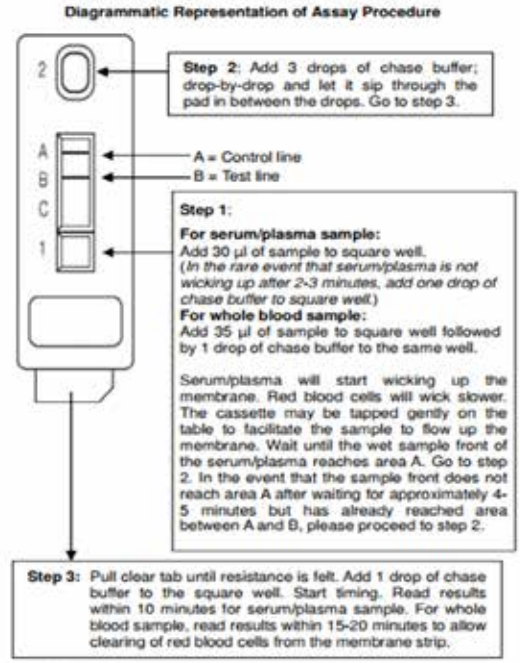

Figure (1): Rapid test (TYPHIDOT Rapid IgM) *(13)*

PCR was according to (ref) , briefly200 µl of the sample were added to 20 µl QIAGEN Protease into the bottom of a 1.5 ml microcentrifuge tube, 200 µl buffer AL added to the sample then mixing by pulse-vortexing for 15 s. Samples incubated at 56°C for 10 min.

Then 200 µl ethanol (96–100%) added to the sample, and mixed again by pulse-vortexing for 15 s. After mixing, briefly the 1.5 ml microcentrifuge tube centrifuged to remove drops from the inside of the lid. Carefully the mixture was applied to the QIAamp spin column and centrifuged at 6000 x g (8000 rpm) for 1 min the QIAamp spin column placed in a clean 2 ml collection tube and the tube containing the filtrate discarded. The QIAamp spin column opened and 500 µl buffer AW2 added without wetting the rim .The cap closed and centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min. After gently mixing and brief centrifugation of each PCR tube, all tubes were placed in the thermal cycler, and amplification with primer set (f& r) for detection of genus salmonella species was done *(16)* **(Table 1)**.

	Sequence(5` to 3`)	Product Size	Species Specificity
F	ACTCAGGCTTCCCG-TAACGC	162 bp	All Salmonella SPP
R	GGCTAGTATTGTCCT-TATCGG.		

Table (1): Primer sets used in this study

Statistical Methodology

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013. Descriptive statistics were done for quantitative data as minimum & maximum of the range as well as mean±SD (standard deviation) for quantitative parametric data, while it was done for qualitative data as number and percentage. The level of significance was taken at P value < 0.050 is significant, otherwise is non-significant.

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Results:
All the 30 cases in our study were males with age mean±SD 21.6±1.1. All the patients (100%) suffered from fever, headache and abdominal pain while 73.3% suffered from diarrhea. For their lab evaluation the Widal test was positive in 100%. Rapid test showed 83.3% positive and 16.7% negative. On the other hand, the PCR showed positivity in 70% and negativity in 30% as shown in *figure 2*.

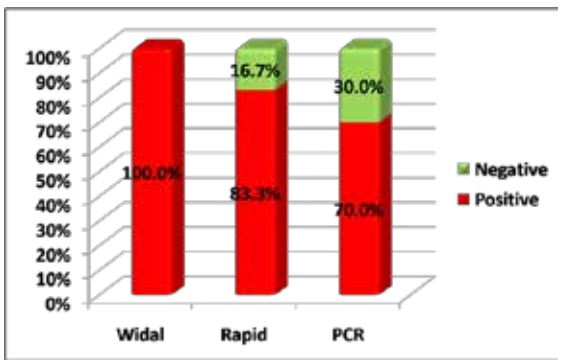

Figure (2): Typhoid tests among the studied cases

PCR is our reference point for positivity of typhoid infection. All Widal findings were positive even in all negative PCR findings. There was some agreement between Rapid and PCR results shown in table (2)

Test	Findings	PCR		^P
		Positive (N=21)	Negative (N=9)	
Widal	Positive (30 cases)	21 (70.0%)	9 (30.0%)	--
Rapid	Positive (25 cases)	21 (70.0%)	4 (13.3%)	0.125
	Negative (5 cases)	0 (0.0%)	5 (16.7%)	

Table (2): Agreement between PCR and Widal & Rapid tests.

Rapid test showed better characteristics than Widal test; both had perfect sensitivity but Rapid test had higher specificity and diagnostic accuracy as shown in Figure 3.

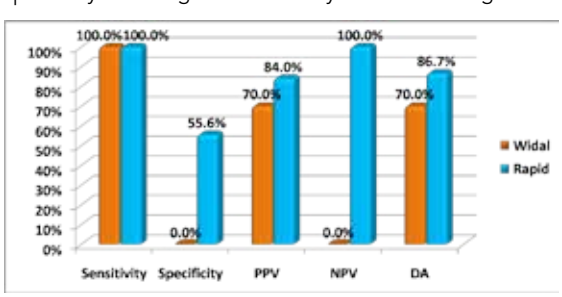

Figure (3): Diagnostic characteristics of Widal and Rapid tests in diagnosis of Typhoid

Table (3): Liver functions and CBC results among the studied cases :

Variables	Mean±SD	Range
ALT (up to 40 IU/L)	37.3±9.0	22.0–55.0
AST (up to 37 IU/L)	40.5±9.9	25.0–59.0
T. bilirubin (up to 1.5 mg/dl)	1.0±0.1	0.9–1.1
WBC (11-14 x10^3/mL)	5.8±1.6	3.0–9.0
HB (12-16g/dl)	11.9±1.0	10.0–13.0
Platelets (150 – 400 x10^3/mL)	176.8±37.8	123.0–255.0
Abnormal Values	N	%
ALT> 37.0	13	43.3
AST> 32.0	22	73.3
T. bilirubin> 1.2	0	0.0
WBC< 4.0	3	10.0
HB< 13.0	22	73.3
Platelets < 150.0	6	20.0

WBC was significantly lower among positive PCR cases. Platelets were significantly higher among positive PCR cases. ALT > 37.0(IU/L) was significantly more frequent among positive PCR cases as shown in table 4.

Table (4): Comparison between positive and negative Typhoid PCR regarding Liver functions and CBC

Variable	Positive (N=21)	Negative (N=9)	P
ALT *(up to 40 IU/L)*	39.0±10.1	33.3±3.5	^0.032
AST *(up to 37 IU/L)*	41.8±10.7	37.7±7.5	^0.245
T. bilirubin *(up to 1.5 mg/dl)*	1.0±0.1	1.0±0.1	^0.736
WBC *(11-14 x10^3/mL)*	5.0±1.2	7.6±0.8	^<0.001*
HB *(12-16g/dl)*	11.9±1.1	11.7±0.8	^0.640
Platelets *(150 – 400 x10^3/mL)*	187.3±39.0	152.4±20.8	^0.004*
Abnormal Values	N (%)	N (%)	
ALT> 37.0	12 (57.1%)	1 (11.1%)	#0.042*
AST> 32.0	15 (71.4%)	7 (77.8%)	#1.000
WBC< 4.0	3 (14.3%)	0 (0.0%)	#0.534
HB< 13.0	14 (66.7%)	8 (88.9%)	#0.374
Platelets < 150.0	3 (14.3%)	3 (33.3%)	#0.329

^Independent t-test, #Fisher's Exact test, *Significant

Hepatomegaly& splenomegaly was significantly more frequent among positive PCR cases as shown in figure 4.

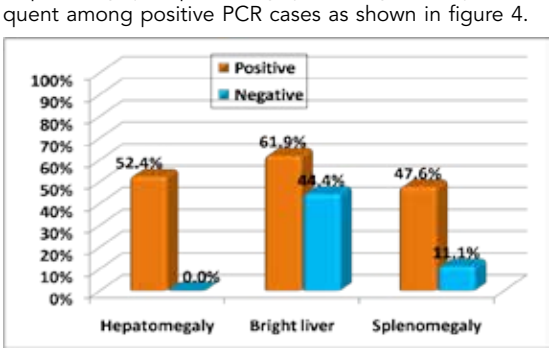

Figure (4): Comparison between positive and negative Typhoid PCR regarding Abdominal US findings

The laboratory tests done in our study showed that Rapid test was fast and cheap test. Widal test was the cheapest test, and faster than PCR as shown in table 4.

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Table (5): Comparison between Time and Cost of different typhoid diagnostic tests:

Variables	Widal test	Rapid test	PCR
Time	5 hours	15 minutes	24 hours
Cost	50 pounds	70 pounds	250 pounds

Discussion:

Diagnosis of enteric fever in endemic areas is still reliant on clinical presentation *(17)*. However recent studies tend to prefer rapid, accurate and affordable tests, that will not rely on expensive equipment, nor highly skilled and trained clinical and laboratory personnel *(18)*.

Regarding the clinical presentation of the studied cases fever, headache and abdominal pain were the main presenting symptoms in all cases (100%). Otherwise sweating was detecting in 26 cases (86.7%), vomiting in 16 cases (53.3%) and diarrhea in 22 cases (73.3%). *Islam et al. (19)* did a study on 88 cases, all cases were presented with fever (100%), vomiting was detected in (68.6%), diarrhea in (50%), headache in (17%), constipation in (26.1%).

Regarding the laboratory findings in the present study, the most common haematological manifestation in our study was anaemia 22/30 (73.3%), 3patients (10%) had leucopenia, 6 patients (20%) had thrombocytopenia. This is similar to with *Abro et al. (20)* study that was done on 75 patients diagnosed as typhoid fever, at the Infectious Diseases unit and medical wards at Rashid Hospital Dubai, United Arab Emirates, from March 2005 to February 2008. The most common hematological changes observed were; anemia (61.3%), thrombocytopenia (40%), leucocytosis (10.6%) & leucopenia (4%).

In the current study, Widal test≥ 320 was positive in 100% (30 cases), 25 samples had positive rapid test and 21 samples had positive PCR for typhoid .We are comparing to the sensitivity of PCR and the sensitivity of Widal≥ 320 which was100% but it lacks the specificity, while the sensitivity and specificity of Rapid test was 100% and 55.6%, respectively. The accuracy value of Widal test≥ 320 and Rapid test was70% and 86.7%, respectively. So, rapid test had better characteristics than widal test in diagnosis of typhoid. Regarding the results of PCR test that was done (30 cases give 21 positive cases (70.0%) and give 9 negative cases (30.0%). All of them had positive Widal test more than1\320 (100%). In another study, in Pakistan, 55 cases of suspected typhoid fever were diagnosed by the PCR and blood culture, gave 58.2% and 14.5% positivity, respectively, showing significantly better results by PCR *(21)*. A large, well-designed study in Indonesia by *Hatta and Smith (22)* reported a sensitivity of 61.8% by blood culture and 84.5% by PCR from blood samples. A study from Nepal reported sensitivity of PCR 82.7% which is much higher than blood culture (26.9%) *(23)*.

Khan et al. (16) conducted a study on 80 patients with clinical diagnosis of typhoid fever and 40 controls were included in the study. The sensitivity of PCR on blood was found to be 100 % whereas the specificity was 76.9%. The positive predictive value (PPV) of PCR was calculated to be 76.9% with an accuracy of 86%.Reporting that nested PCR can be used as a useful tool to diagnose clinically suspected, culture negative cases of typhoid fever.

In the current study the rapid test gave positive results in 25cases (75 %) and negative results in 5 cases (25%) comparatively to the PCR which gave positive results in 21cases (70.0%) and negative results in 4 cases (13.3%). In another study, 38 patients (68%) of the Group-I (clinically suspected to be cases of enteric fever) were positive for blood culture, 32 (57%) were Widal positive, and 44(79%) were positive for typhidot test (rapid test).While Group-II(had non-typhoidal febrile illness) had all patients sterile on blood culture, 4 (17%) were Widal positive, while 3 (12.5%) tested positive for typhidot test (Rapid test). Among the38 culture positive cases in Group-I, typhidot was positive in 35 patients, while Widal was positive in 28 patients, giving sensitivity of 92% and specificity of 87.5% as compared to Widal which had sensitivity of 74% and specificity of 83% (24).

So Typhidot test (rapid test) is an equally reliable, simple test that gives rapid diagnosis and can be helpful in early institution of therapy. In the current study, rapid test offer good results for the diagnosis of typhoid in areas of endemicity (as the specificity and sensitivity are 55.6% and 100%, respectively) and Widal test Specificity and sensitivity are (0%) and (100%), respectively.

In a similar vessel, a study conducted in Abassia fever hospital-Cairo-Egypt between September 2012 and March 2014, a total of 154 patients that had been clinically suspected as typhoid fever were investigated by the blood culture, Widal tube agglutination test and rapid test in addition to 46 healthy controls were investigated by Widal tube agglutination test and rapid test. Rapid test showed higher sensitivity and specificity, 86% and 89% than widal tube agglutination test, 81% and 71%, respectively. Rapid test had higher sensitivity when fever duration<5 days (100%). Rapid test is a useful, rapid easy serological test for early diagnosis of typhoid fever especially for primary healthcare centers and outpatient clinics as well as hospitals (25).

So the current series revealed that rapid test was fast and cheap test. Widal test was the cheapest test, and faster than PCR. This may make Rapid test to be suitable for national serological surveys, and laboratories with large diagnostic workloads.

Conclusion:

The most frequent symptoms among patients were fever, headache, abdominal pain, sweating, vomiting and diarrhea or constipation. Typhoid disease still represents a significant public health problem in Egypt to be diagnosed only by clinical presentations. The Rapid test offer good results than Widal test for the diagnosis of typhoid in areas of endemicity (as the specificity, sensitivity and accuracy are 55.6%, 100% and 86.7%, respectively). The Rapid test is less costly, easier to perform (i.e. simple and rapid), that making it suitable for national serological surveys, and laboratories with large diagnostic workloads.

Recommendations

Larger scale multi-center studies are recommended to study the sensitivity and specificity of the Rapid test and Widal test to confirm the values calculated in the present study. Also, research and further studies should also continue to focus on the diagnostic value of Rapid test and Widal test in comparison with PCR.

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