Regional Review

Typhoid fever: misuse of Widal test in Libya

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Abstract

The worldwide gold standard of diagnosing of enteric fever depends on the isolation of *Salmonella enterica* serovar Typhi from a patient's bone marrow and/or blood culture. In Libya clinicians are heavily dependent on the Widal test for diagnosis of enteric fever which has been used without determining the locally appropriate threshold titer, because the laboratories lack the skilled, experienced personnel and appropriate facilities to detect and serotype *Salmonella* isolates. To improve the diagnosis process, clinical management and reliability of public health measures, there is an urgent need for the effective training of laboratory technicians and to provide resources to culture *Salmonella* species according to published guidelines. Clinicians should understand the limitations of Widal test and recognize that it cannot be expected to give a reliable diagnosis.

Key words: Widal test; Typhoid *Salmonella*; diagnosis; Libya

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Introduction

The World Health Organization (WHO) estimates that the annual global incidence of typhoid fever is about 21 million cases with 200,000 deaths caused by typhoid fever each year [1-3]. This data is probably underestimated because of poor disease diagnosis. Furthermore, data on which this estimate is based is limited, and comes from isolated studies conducted in countries with healthcare infrastructures not capable of assessing the burden of enteric fever. In most African countries the incidence of typhoid is 10-100 cases/100,000 person years with highest incidence in children [1]. The WHO Global Salm-Surv program reported that *Salmonella enterica* serovar Typhi ranked sixth (5%) after *Salmonella enterica* serovar *Typhimurium*, *Salmonella enterica* serovar *Enteritidis*, *Salmonella enterica* serovar *Isangi*, *Salmonella enterica* serovar *Livingstone*, and *Salmonella enterica* serovar *Corvalis*. Typhoid is considered an endemic disease in Mediterranean North African countries [4]. In Libya, low prevalence of *S.* Typhi and *S. Paratyphi* A, B and C were detected in stool samples from 30,163 patients hospitalized with acute diarrhea during 1975-1980 [5], this might be attributed to the type of sample obtained from these patients. The Libyan Centre for Information and Documentation (CID) published that the incidence for years 2004, 2005 and 2006 was 7, 21 and 16/100,000 persons/year respectively [6-8]. Worldwide, the disease is mainly associated with low socio-economic status and poor hygiene. It is considered as one of the most serious infectious disease threats to public health globally with particular concern over the rapid and widespread emergence of multiple antibiotic resistance among the species [9-11]. According to our knowledge, the validity of Widal test has not been assessed in Libya. Therefore, the objectives of this article are to provide a comprehensive review to assess the value of the diagnostic methods of typhoid fever with emphasis on Widal test as a diagnostic tool commonly used in Libya. The information presented in this review was obtained from Highwire Press (including PubMed) search for the period 1950-2013 in titles and abstracts, using the terms 'widal test in Libya', 'enteric fever in Mediterranean', 'widal test in Africa and Asia', 'single, tube widal test', 'widal agglutination titre', 'serology of typhoid fever', 'specificity and sensitivity of widal test' and 'diagnosis of typhoid fever'. Additional data were also obtained from a Google search using the aforementioned terms. Furthermore, papers published in local biomedical journals, and when available, abstracts presented in local and international meetings on the subject were included.

Diagnosis methods of typhoid fever

Diagnosis of typhoid fever can be made on the following factors: clinical symptoms, signs, serological markers, bacterial culture, antigen detection, and DNA amplification. However, none is entirely satisfactory. Detection yield of the organism from bone marrow (85-95%), blood (70%) and/or stool (45-65%) is currently considered the most reliable diagnostic method [12]. In a large study conducted in Saudi Arabia using 1,114 samples to monitor the validity of Widal test compared with blood culture, 74.8% were found to be Widal agglutination test positive, but the sensitivity of Widal test increased to 77.6% when the cut-off was taken as 1/60 for O antigen and 1/320 for H antigen of *S.* Typhi. [13]. In countries with limited resources were bone marrow and blood culture are expensive and require equipment, supplies and trained laboratory personnel seldom found in primary health-care facilities, the Widal test remain the predominant diagnostic tool [14-16]. For practical purposes, a treatment decision must be based on results obtained with a single acute phase sample. The cut-off for a positive Widal, chosen in a particular community depends on the background level of typhoid fever (i.e., the prior probability) and the level of typhoid vaccination, which may vary with time. The result may lack sensitivity and specificity particularly in a community with endemic typhoid fever.

Two studies indicated that the passive haemagglutination test (70% sensitive and 92% specific) is comparable with the Widal test [17,18]. Recently, a novel microplate agglutination assay using the absorbed sheep red blood cells to enhance the Widal test reactivity appeared to be a useful alternative technique (19). The clinical application of a dot blot test (Typhidott and Typhidot-Mt) to detect IgG and IgM antibodies to the bacteria has been evaluated and gave superior results to the Widal test [20,21]. A dipstick assay developed for use in developing countries gave unacceptable results for sensitivity or specificity [22]. The TUBEX commercial kit which detected IgM antibodies appeared to provide the most accurate results but has some limitations [23]. These new commercially available typhoid rapid antibody tests have shown variable performance [24-26] and have not fully been evaluated in Africa [27-29] (Table 1).

Polymerase chain reaction (PCR) has not become an established method for diagnosis typhoid fever [30]. In a recent study performed in India, sensitivity of PCR-based diagnosis was 95% compared to the Widal test which has a sensitivity of only 63%. In certain cases, the PCR assay was more sensitive than the blood culture [31]. In Turkey, the Widal test was not useful for differential diagnosis compared with blood culture and PCR [32]. The diagnostic sensitivity of PCR may be increased by simultaneously testing blood, stool and urine samples [33,34], or using Real-time PCR and multiplex PCR that may have even higher sensitivities [34,35].

Somily *et al* suggested that the availability of such facilities would still remain limited to specialized centers, and reliance on Widal test for diagnosis of enteric fever will probably continue until the introduction of a relatively simple, cost effective, and reliable test for detection of *Salmonella* infection [36].

Diagnosis of typhoid fever by Widal test

The Widal test has been used for more than 100 years for diagnosis of typhoid fever [37-39]. It is a tube dilution test to measure agglutinating antibodies against the lipopolysaccharide O and the protein flagellar antigens (Hd) of *S.* Typhi. The value of the test for diagnosis of typhoid fever has long been debated [40-42]. For some patients, the Widal test does not detect antibodies even in blood culture-confirmed cases [43,44]. There is significant cross-reactivity with other infectious agents, which can produce false-positive results, leading to an over-diagnosis of typhoid fever. The Philippine Society for Microbiology and Infectious Diseases proposed some recommendations published in a position paper in 1991 on the use of Widal test in the diagnosis of enteric/typhoid fever. It stated that: 1. blood and/or bone marrow culture are the diagnostic tests for confirmation of typhoid fever; 2. a single test in an endemic area is of no value and it should not be used as a screening test for asymptomatic individuals; 3. a negative test does not rule out typhoid fever in patients with signs and symptoms of the disease and it should not be used as a basis for deciding duration of treatment [45]. Furthermore, Reynolds *et al* [43] concluded that diagnosis of typhoid fever based on serology alone is frequently inaccurate. There are reports of a large number of false-positive cases especially in areas where typhoid fever is endemic and in patients who previously had typhoid fever [46]. Finally, WHO has issued no recommendations on the use of typhoid rapid antibody tests [47].

Table 1. The sensitivity and specificity of Widal test with counterpart other methods

Brand	Country	Sensitivity %	Specificity %	Technique	Compared method	Ref.
Wellcome Diagnostics, England)	South Africa	O 71 H 81.6	O 98.8 H 93.4	Tube	Passive haemaglutination test	17
SPAN Diagnostics Pvt, Ltd	India	>70	>92	Tube	Reverse Passive Haemagglutination test	18
Murex Biotech Limited, UK	Saudi Arabia	Not determined	Not determined	Tube	SRBC Microplate agglut. assay	19
Wellcome Diagnostic, Dartford, UK	Pakistan	55	81	Tube	Typhidott and Typhidot-M	20
Murex Biotech limited ,UK	Bangladesh	42.8	85	Slide	DOT EIA IgM (Typhidot)	21
Murex Biotech,Ltd. UK	Indonesia	60.7	88.4	Tube	Dipstick IgM	22
Difco	Turkey	52	88	Slide and tube	Blood culture	24
Bio-Rad	Vietnam	64	76	Tube	Multi-Test Dip-S-Ticks, TyphiDot, and TUBEX	26
Cromotest	South Africa + Tanzania	S- O 95.2 S- H 80.3 T- O 87.3 T- H 95.2	S- O 3.6 S- H 50.0 T- O 6.9 T- H 13.8	Slide and Single Tube	TUBEX and Typhidot	29
Arsitha Diatech	India	63	Not determined	Slide	PCR Blood culture	31
Murex Biotech limited ,UK	Saudi Arabia	Not determined	Not determined	Tube	No comparison	36
Sanofi Diagnostics Pasteur, France	Vietnam	74	95	Tube	Blood culture	38
Not stated	Central Africa	Not determined	Not determined	Not stated	Blood culture	39
(Wellcome Diagnostics, England	Malaysia	98	67	Tube	Dot enzyme immunoassay	40
(Wellcome Reagents Ltd, England	Malaysia	Not determined	Not determined	Tube	Blood culture and feces	41
Nirmal Laboratories, India	India	Not determined	Not determined	Slide and Tube	Blood culture	49
Murex Diagnostic, UK	Bangladesh	88	98	Slide	Blood culture	50
Institute of preventative medicine, Taiwan	China	91	77.8	Tube	Blood culture	58
Difco Antigens	Ethiopia	Not determined	Not determined	Slide	Blood culture	61
(Sanofi Diagnostics Pasteur, France	Vietnam	O 0.92 H 0.60	O 0.57 H 90	Tube	ELISA IgM dipstick IDeaL TUBEX	62
Bacto widal antigen set, Difco	Jakarta	O 53	O 98	Slide	Blood culture	64
Gamma Biological Co.	Thailand	86	98	Tube	Blood culture	65
Biosystem Febrile Antigen Kit (UK)	Nigeria	Not determined	Not determined	Tube	No comparison	66

Table 1. (continued) The sensitivity and specificity of Widal test with counterpart other methods

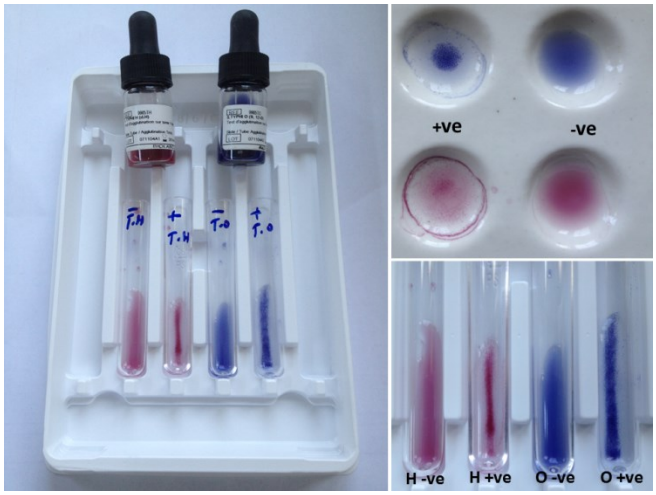
Brand	Country	Sensitivity %	Specificity %	Technique	Compared method	Ref.
Becton Dickinson, NJ, USA	Tanzania	75	98	Slide and Tube	Blood culture	67
Remel Europe Ltd, UK	Kenya	Not determined	Not determined	Not stated	Blood, Bone marrow and stool culture	68
Remel Biosystems Dialab Biotec	Egypt	O 100 H 94.02 O 100 H 91.04 O 97 H 86.56 O 88.1	O 54.16 H 8.3 O 58.3 H 16.6 O 58.3 H 20.83 O 91.6 H 45.83	Slide and Tube	ELISA	69
SA Scientific, San Antonio, TX	Egypt	56	79	Slide	Blood culture	70
Local Jordan Vaccine Institute & Wecllome Co, USA	Jordan	Not determined	Not determined	Tube	Blood culture	72
SPAN Diagnostics Ltd, India	DR Congo	Not determined	Not determined	Tube	Blood culture	74
BIO-RAD	Togo	60	91.08	Tube	plate agglutination	76
BIO-RAD	Togo	Not determined	Not determined	Tube	No comparison	77

Limitations of Widal test

The test is easy to perform which makes it practical for use in the field for presumptive diagnosis of typhoid fever. In endemic areas a substantial proportion of healthy population exhibit sero-positivity against O and H antigens for *S.* Typhi or Paratyphi (Figure 1). In a seroprevalence study conducted by Turkish Ministry of Health on O and H antibodies against *S.* Typhi or Paratyphi (by using slide agglutination test), it was demonstrated that 25% of healthy adults were seropositive for O and H antibodies [48]. In a study from Malaysia, 61% of healthy adults were seropositive against the H antigen and 6% were seropositive against O antigen [49]. One of the reasons for these high rates of sero-positivity against serotype Typhi is the widespread presence of *Salmonella* infections in the community, and O and H antigens shared with other *Salmonella* serotypes and other bacteria [37, 50-53]. Agglutination antibodies to Vi antigen can be detected by the Widal assay, but even with the appropriate control antigens the results are unreliable [54]; an ELISA was also developed which successfully detect serum antibodies to Vi antigen, but because of the kinetic of Vi antigen serum antibody production, the authors suggest that these antibodies may be of limited value in the serodiagnosis of acute typhoid infection [55]. However, the detection of Vi antibodies can be used for detection of carriers during specific investigations [14,56,57]. Furthermore, the Widal test can be falsely positive in patients with previous vaccination or infection with *S. Typhi* [37]. Vaccination is not a factor that influences results in the Libyan population because there is no national program of typhoid vaccination. Raised Widal titers have also been reported in association with other diseases [58-61].

Numerous studies indicate that the sensitivity, specificity, and predictive values of the test vary dramatically among laboratories; this makes the value of the test questionable to both epidemiologists and clinicians [36,40,50,52,62–64]. The Widal reaction is

Figure 1. Slide and tube agglutination of Widal test



indicative of typhoid fever in only 40–60% of patients at the time of admission. Interpretation of the test in endemic areas is difficult since the majority of normal healthy individuals have detectable antibodies [65]. High seroprevalence rates may also be found in normal population [66] indicating that testing a single serum sample is inadequate for the confirmation of typhoid fever.

The Widal tube agglutination test was assessed among febrile hospitalized Tanzanian children with culture-confirmed typhoid fever cases. A Widal titer of ≥ 1:80 was found the be the optimal indicator of typhoid fever among the population studied, and was performed relatively well in terms of sensitivity (75%) and specificity (98%). For economic considerations, the authors expect the Widal test to remain the major option in many developing countries [67]. Widal testing performed on acute phase sera of Kenyan patients with a clinical evidence of typhoid fever had limited diagnostic efficacy, revealing that 26% of patients had the diagnostic titers of infection [68]. A cross sectional comparative study performed in Egypt detected marked discrepancies among antigens from four different locally available sources at three different cut-off values, when sensitivity and specificity were compared [69]. These discrepancies among different brands were supported by different studies [70,71]. In addition, a semi-quantitative slide agglutination and single-tube Widal test was performed poorly in two sub-Saharan African cites [29]. Data obtained from various studies indicate a major limitation of the test with variable ranges of sensitivity and specificity in different populations, precluding its acceptance as the definitive diagnostic assay [72,73]. Most recently, Widal test was assessed on-site in the Democratic Republic of the Congo. It concluded that clinicians highly rely on Widal test for the diagnosis of typhoid fever despite the poor performance and inaccurate interpretation [74]. However, it would be possible to upgrade the performance of laboratories in rural and remote areas by adopting a centripetal program of external quality assessment as an introduction to internal quality control [75]. Misdiagnosis based only on Widal test resulted in several hundred of over-treatment cases and might also perpetuate the perception that typhoid is common. This led to the belief that more than 30% of patients were assumed having typhoid fever in some hospitals of Togo [76]. Moreover, inadequate interpretation and incorrect labeling contributed to wrong interpretations and raised the need of a simpler and reliable immunologic test for the diagnosis of typhoid fever in Togo [77]. To improve the specificity of the Widal test, a recommended standardization of interpretation criteria and use of tube agglutination must be applied [78].

Application and limitation of Widal test in Libya

Typhoid and paratyphoid fevers are endemic in the Mediterranean North Africa countries and multidrug resistance is common among *S. Typhi* and *S. Paratyphi* isolated in this region [73]. The variable range of sensitivity and specificity of the Widal test in different populations casts doubt on the systematic use for definitive diagnosis in patients presenting fever and on initiation of antibiotic treatment based on agglutination of a single antigen. Currently, there is no established standard procedure in any laboratory in Libya to detect and report *S. Typhi*. These laboratories have limited resources and lack the skilled personnel; therefore, clinicians are more dependent on the Widal test. Poor laboratory skills and erroneous interpretation of the test might lead to misdiagnosis and mismanagement of the patients. To ensure consistent results by different sources of the antigens used in Libya, we suggest the following: 1. Widal test should be interpreted in relation to baseline antibody titers (cut-off value) obtained by paired tube dilution using sera from a healthy local population, and to determine the specificity, sensitivity and predictive values, rather than depending on the titers stated by the manufacturers (there are six different diagnostic kits available in Libya); 2. a single Widal test is not reliable for the diagnosis of typhoid fever and it will remain an issue of contention (this practice is widely applied in Libya); 3. a negative test does not rule out typhoid fever in patients with signs and symptoms of the disease; and it should not be used as a basis to make decisions on treatment duration and 4. Detecting the *Salmonella* from bone marrow, blood and/or stool culture before initiating antimicrobial therapy remains the diagnostic method of choice that can be achieved by introduction of full scale upgrading of the laboratory resources and updating the personnel with continual training. It is important to remember that antimicrobial susceptibility testing and molecular epidemiological linkage cannot be elicited on serological diagnosis.

Conclusions

Since the clinical laboratory plays a significant role in typhoid fever diagnosis, there is an urgent need for effective training of laboratory staff and the

provision of appropriate resources for bone marrow and blood culture according to published guidelines. Introduction of newer serological methods for early diagnosis of typhoid fever remains critical. Both laboratory technicians and clinicians should understand the limitations of the Widal test interpretation. It is important to remember that antimicrobial susceptibility testing and molecular epidemiological linkage cannot be elicited on serological diagnosis. Blood culture before initiating antimicrobial therapy remains the diagnostic method of choice.

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