

Comparison between the Widal Test and Modifiedal using 2-mercaptoethanol in the Diagnosis of Typhoid Fevers in Basrah City

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ABSTRACT: A total of one hundred and twenty blood samples were collected from males and females who visited different hospitals in Basrah city. They suffered from Typhoid fever syndroms. The study aimed to isolate and identify *Salmonella typhi* from blood samples using Widal test and compared with modified Widal test using 2-mercaptoethanol(2ME) test in the diagnosis of Typhoid fever. Result showed using 2ME is a favorite from that conventional Widal test to assess diagnosis and negative result of 2ME is strong evidence against diagnosis of chronic infection.

KEYWORDS: typhoid fever, Widal test, 2-mercaptoethanol

I. INTRODUCTION

Typhoid fever continue to be a global health problem, especially in tropics and subtropics with an annual global burden of about 21,600,000 illness and 220,000 death during 2000 and that paratyphoid cause 5,410,000 illness [1]. The diagnosis of typhoid fever in developing countries is based mainly on clinical ground and is difficult, as the presenting symptoms are diverse and similar to those observed in other common febrile illness [2,3]. The traditional salmonella laboratory confirmation of clinical suspicion of typhoid fever is based on culture on selective media and identification of suspected colonies by biochemical and serological tests [4]. These methods are generally time consuming, laborious and may give false negative results [5]. An alternative is typhoid serology using the Widal test that is widely used in many developing countries including Iraq, because of its feasible, fast and easy. Over 100 years since its introduction as a serological means of detecting the presence of typhoid fever, the Widal test continues to be plagued with controversies involving the quality of the antigens used and interpretation of the result, particularly in endemic areas [6,7]. However, several reports affirmed that when performed reliably and interpreted with care it can be of value in the diagnosis of typhoid fever [8,9].

Although Widal test has been in use for more than a century, the value of the test to diagnose typhoid fever has been debated for as many years as it has been available [10]. It relies classically on the demonstration of a rising titer of antibodies in paired samples 10 to 14 days apart. In typhoid fever, however, such a rise is not always demonstrable, even in blood culture-confirmed cases [10]. In addition, Interpreting the test has been such a problem that different cut-offs have been reported from different places [11,12]. Furthermore, patient management cannot wait for results obtained with a convalescent-phase sample. For practical purposes, a treatment decision must be made on the basis of the results obtained with a single acute-phase sample [13,14]. So evaluating the result of a single Widal test is necessary for correct interpretation.

This study was carried out to evaluate the value of Widal test with using 2-mercaptoethanol test for the diagnosis of typhoid fever patients in Basrah hospitals.

II. MATERIALS AND METHODS

Study design :

Two hundred and twenty individuals suspected to have typhoid fever were studied. Patients were either admitted to the AL-Basrah general hospital, AL-Sader teaching hospital, AL-Fayhaa general hospital, AL-Shafaa general hospital, AL-Zubair general hospital, Basrah Children specialized hospital and Basrah Maternity and Children hospital. The period of sampling collection from February to the end of October 2014, and the age of patients ranged from 1-60 years.

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Samples :

Ten milliliters of venous blood were taken from all patients at the time of temperature spikers, before antibiotic administration. Six milliliters of blood were used for blood culture; the remaining 4 milliliters were collected in plain tubes and centrifuged for 10 minutes at 2500 rpm. Sera were separated and used for serological tests either immediately or kept frozen at -20°C until used [15]. Then 6ml of blood was dispensed into the culture medium bottle containing 45ml of Tryptic Soy Broth (OXOID, England) mixed with the broth and then incubated at 37°C. [16].

Subculturing and biochemical identification:

After 24 hours incubation, sub-culturing was performed from the Tryptic Soya Broth on XLD agar (OXOID, England). After overnight incubation positive culture were proceed further while negative broth cultures were incubated for seven days and sub cultured before reported negative, Suspected colonies obtained on the above media were screened by biochemical tests using Triple Sugar Iron agar (TSI)(BBL), citrate utilization test, motility (Difco). Ureas test (Himedia Ltd, India) and lysine decarboxylation (LDC, Difco) test [15].

API 20 E system:

This system is devised for the biochemical identification of Enterobacteriaceae and other gram negative bacilli. It consists of 20 microtubes containing dehydrated media.

Widal test and 2-ME-Modified Widal test:

Rapid slide agglutination test for the conventional and the 2-ME modified tests were done using stained febrile antigens purchased from Plasmatic Laboratory products (LTL)(UK). The tests were conducted by placing equal volumes of 20µl of serum and stained *Salmonella* antigens, mixed and examined visually after 1 minute. If agglutination was observed, the antibody titer against *Salmonella* was recorded as 1/80 according to the manufactures. Also, by conducting the same procedure on serum dilution of 1/2 , 1/4 and 1/8 antibody titer of 1/160, 1/320, and 1/640 were recorded respectively if agglutination was observed [17]

The 2-ME modified Widal test (2-ME MWT) was done after incubation of the patient serum with an equal amount of 2-mercaptothanol (0.05 M; Fluka, U.K), prepared in phosphate buffered saline (pH7.2, 0.15M) for 1 hour at 37°C. Consequently in calculating the titers, the 1/2 pre-dilution of sera with 2-ME was taken into account.

The positive Widal test was based on the detection of significant ($\geq 1/160$) anti-O agglutinin titer with or without significant ($\geq 1/160$) anti-agglutinin titer [18]

Statistical analysis:

The Chi-square test was used to determine the statistical significance of the data by using SPSS program (Statistical Package for Social Science) version 11, and significance was assumed at $p \leq 0.05$.

III. RESULTS

Out of 120 blood samples only 50 isolates of *Salmonella typhi* were obtained from blood samples as shown in (Table 1). The statistically significant ($p \leq 0.05$)(Table 1).

Table 1: Distribution of *Salmonella typhi* isolates in different hospitals.

Hospital	No. of samples	Isolaes	
		No.	%
AL-Zubbair General Hospital	18	10	20
AL-Basrah General Hospital	21	13	26
AL-Mawani General Hospital	27	10	20
AL-Fayhaa General Hospital	20	8	16
AL-Shafaa General Hospital	13	0	0
AL-Sader Teaching Hospital	10	3	6
Basrah Maternity and Children Hospital	6	4	8
Basrah Children Specialty Hospital	5	2	4
Total	120	50	100

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Age and gender distribution of *S. typhi*:

The age and gender distribution of the patients can be seen in (Table 2). The lowest incidence was among the 40-60 age group whereas the highest incidence was among the 15-20 age group. The table also shows that the incidence was higher among males (56%) than that of females (44%). There was not statistically significant ($p \leq 0.05$).

Table 2: Age and sex of patients infected with *S. typhi*.

Age group	Male		Female	
	No.	%	No.	%
15-20 years	14	50	12	54.5
20-40 years	8	28.5	6	27.2
40-60 years	6	21.4	4	18.1
Total %	28	56%	22	44%

Relationship between blood culture and widal test:

Single Widal Slide Agglutination Tests and bacteriological culture. Out of 120 included samples, 50(41.6%) were *S. typhi* and 10(8.3%) were *S. paratyphi* A (Figure1). The Widal test was found to be significant with blood culture ($p=0.58$).

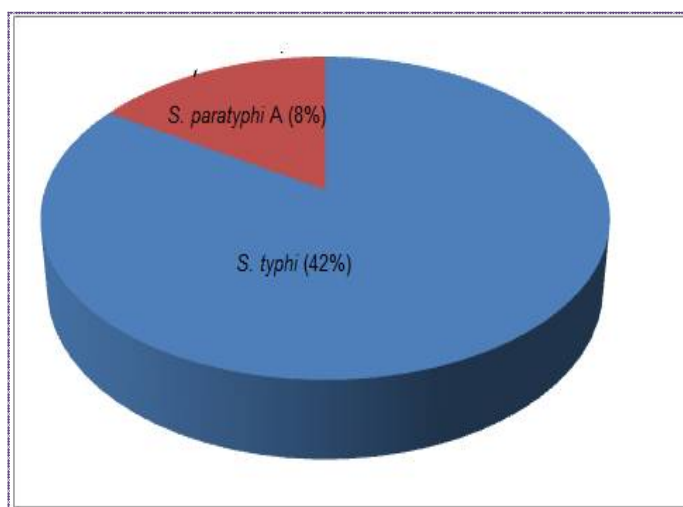


Figure (1): Percentage distribution of *Salmonella* spp. isolated from blood culture.

On the other hand, when the significant anti-O titer was considered to be 1/160, the widal test had a sensitivity of 50.1% and specificity of 50%. Also, the sensitivity decreased to 37%, but the specificity increased to 77% when the significant anti-O titer was elevated to 1/320 (Table 3).

Table 3: Assesment of the conventional widal test with cultural results.

Positive titers (O)	Sensitivity %	Specificity %	PPV %	NPV %
1/160	50.1	50	4.2	90
1/320	37	77	13.5	92.5
1/640	14.6	95.5	65.6	91.3

PPV: Positive Predictive Value

NPV: Negative Predictive Value

As comparison, the sensitivity and specificity of the 2-ME were considered in the light of the results of the conventional Widal test. When the significant their of the conventional test was $\geq 1/160$ the 2-ME had sensitivity of 10% and specificity of 99% (Table 4). On the other hand, according to the culture results of the three clinical samples studied collectively, 2-ME had sensitivity of 68.2% and specificity of 96.5% (Table 4).

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Table 4 : Comparison results of 2- Mercaptoethanol (2-ME) with culture and widal test.

Results	Percentage of 2- ME			
	Sensitivity %	Specificity %	PPV %	NPV %
Culture	68.2	96.5	76	95.4
Widal test	10	99	100	43.3

PPV: Positive Predictive Value

NPV: Negative Predictive Value

IV. DISCUSSION

Typhoid fever has an important socio-economic impact, so accurate diagnosis of the disease at an early stage is important not for etiological diagnosis but also for identifying individuals that may serve as potential carrier who may be responsible for acute typhoid fever outbreak [19].

This study was carried out in Basrah hospitals. The patients were randomly selected from the out-patients clinic of Basrah hospitals. One hundred and twenty patients suspected of having enteric fever with widal test application result were used. The widal test was done to those patients and results which had been obtained show that there were 50 patients significant for enteric fever. It is clear from table (1) that the majority of patients are AL-Zubair General Hospital, AL-Mawani General Hospital and AL-Basrah General Hospital respectively.

In Iraq, few studies on typhoid fever were carried out [20]. These studies emphasized on the epidemiological of *S.typhi*, has studied the outbreaks of *S. typhi* in larger area within a short time favored the possibility of water borne disease [21,22]

Regarding the age-group, which were affected by this disease, a higher incidence was in the age group 15-20 years (Table 2). This results similar with the previous studies [23,24]. This may be due to their mobility, consumption of unhygienic food and water in school and colleges [25].

A total of 150 samples were included in the analysis. The statistical analysis of result revealed no significant differences between the Widal agglutination reaction and culture diagnosis of clinical samples. Throng blood culture is taken as gold standard but the results shows that even Widal test may be reliable alone for diagnosis of enteric fever infections. Similar findings revealed that Widal test can be diagnostic value when blood cultures are not available or practical [26,27].

In the present study, it was found to have sensitivity of 50.1% and specificity 50%, when the cut-off titer for anti-O was considered to be the commonly used 1/160. In order to improve the utility of Widal test the cut-off titer for anti-O was increased from 1/160 up to 1/320 (Table 3). In applying this titer, the sensitivity was decreased from 50.1% to 37% and the specificity increased from 50% to 77%. This modification in the interpretative criteria did not solve the problem because the specificity was still low. Also, some cases might be lost in the early stages of the disease. Therefor, an attempt was made to over come some of these drawbacks by using the 2-ME.

The immune response against both H and O antigen of *Salmonella typhi* and *paratyphi* starts by the formation of IgM which may subsequently which on to IgG [28,29]. This was reflected on the finding that high frequencies of the culture positive cases were also 2-ME positive and these results could be attributed to the prescence of 2-ME resistant anti-O of IgG class. In addition, in support to the suggestion of the prescence of anti-O of two classes (IgG and IgM) is the finding of reduction of one to two folds in titers recorded by 2-ME in comparison with the conventional widal test. Moreover, it was reported that the formation of anti-H antibody precedes the formation of anti-O agglutinin [30].

In conclusion, 2-ME has a higher specificity and accuracy in the laboratory diagnosis of enteric fever than the conventional Widal test.

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