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IDENTIFICATION OF ENTERIC FEVER BY DIFFERENT METHODS

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Abstract

This study was conducted in Babylon governorate in the period from January 2019 to April 2019 .Patients were randomly selected from Al-Musayyib General Hospital and Marjan Medical city, clinics and laboratories in the Musayyib of Babylon governorate for patients suspected of typhoid fever, according to the diagnosis of the specialist physician. 150 samples of blood, urine and stool were collected from people with typhoid fever. These samples were divided into 150 blood sample, 96 urine sample and 34 stool sample for the same patients .Initial results showed that 102 of total patients had a positive result in widal test while 48 others had a negative result and the highest antibody titer in the examination widal test was 1/320 compared with negative control 1/20. On the basis of the Whole killed Antigen(WKA) test , which was prepared from isolate obtaining from Al – kifl hospital diagnosis by the public health laboratories in Bagdad as bacteria *Salmonella typhi*, the result showed 65 cases of the total number of patients who were positive for the widal test to be tested for WkA. The results showed that the rate of infection for females was 46%, while the rate of infection for males was 53.8 % ,and the rate 12.3 % for patient under the age of 20 years and 56.9 % for patients between the age of (21- 40) years, whereas 23 % the rate of age is between (41- 60) years, while the proportion of age group between (61 – 80) years has reached the lowest rate of infection 7%. The result of the study showed most of the samples that have been culture were negative result, as the percentage samples, does not appear was (83.3%) of total number samples that have been cultured. While the sample which gave a positive result for *Salmonella typhi* and *Salmonella paratyphi* was the ratio of (16.6 %). Either in the examination widal were more isolates indicate of two types of bacteria in the serum of an infected person with typhoid fever (Enteric fever).

***Keywords*:** *Salmonella*, widal test, Whole killed Antigen, Enteric fever

Introduction

Typhoid fever is an acute, generalized infection of the reticulo-endothelial system, intestinal lymphoid tissues and gallbladder caused by the species of bacteria *Salmonella typhi*, so it's mostly an intracellular infection (AFM *et al*., 2013). The bacterium enters the body orally after the intake of contaminated food or water, and then reach to the small intestine, adheres to and invades the specialized M–cells and enterocytes. The pathogen is trans located to the intestinal submucosa and subsequently disseminates throughout reticuloendothelial system (Amal *et al.,* 2012).

The infection form of the disease is intestinal and/or extra intestinal following ingestion *S. typhi* that penetrate the gastrointestinal tract and are taken up by reticuloendothelial system (RES) when the bacterium multiplication, there is bimodal incubation period with peaks at about 8-18 days, the duration reflecting the balance between the infecting dose and the host immune defense mechanisms (Al-Ammar, 2006). *Salmonella typhi*, the causative agent, is most frequently isolated from blood during the first week of illness but can also be isolated during the second or third week of illness, through the first week of antimicrobial therapy and during clinical relapse (Baker *et al*., 2010). Isolation of *Salmonella typhi* from bone marrow is the current gold standard method for confirming a case of typhoid fever. However, these are requires equipment, supplies and trained laboratory personnel seldom found in primary health-care facilities in the developing world (Wain and Hosoglu, 2008). Cultivation (blood culture) is a more practical albeit less sensitive alternative to bone marrow culture. However, it is not always available and, when it is, it takes 2 to 3 days. For this reason, diagnosis may be delayed or overlooked and patients without typhoid fever may receive unnecessary and inappropriate antimicrobial treatment. As a result, in developing countries, typhoid rapid antibody tests can facilitate diagnosis and disease management (Karen *et al*., 2011).

Materials and Methods

Serological Test

1. **Widal test :** The test was based on demonstrating the presence of agglutinin (antibody) in the serum of an infected individual, against the H (flagellar) and O (somatic) antigens of *Salmonella typhi* (Safia *et al*., 2016).

2 **Preparation of heat killed bacterial antigen of *Salmonella typhi***

- The isolate were cultured at 37 ºC overnight on nutrient agar by spreading method.
- The bacterial growth were harvested by adding sterile normal saline in test tube, and then heat–killed (30 min at 70 ºC), centrifuged at 2500 rpm for 10 min.
- The supernatant was avoided; the precipitate was washed three times with normal saline and suspended in normal at concentration of 1×10^9 and then testing by culturing on blood agar
- Killed suspension stored in refrigerator - 20 ºC for use.

After culturing test, it there was growth of the bacterium, so the step 2 (heat–killed for 30 min at 70 ºC) must be repeated (De Beaufort *et al*., 1997).

Cultivation

According to (Barrow and Feltham, 1993) the culture media that used in this study (for isolation and identification enteric fever (all media were prepared depended on the manufactures 's) include : Bismuth Sulfite Agar (BSA), *Salmonella, Shigella* Agar (SS agar) Xylose-Lysine Deoxycholate (XLD) (Oxoid-England) and Hektoen Enteric (HE) Agar, Blood agar, Macconkey agar, Brain Heart

Infusion broth, Tetrathionate broth with iodine (Himedia-India) and 5% fresh human blood was add to blood agar base after sterilization to prepare blood agar.

Results

Results for the test were obtained, tabulated, analysed and compared based on widal test, WKA test and positive predictive value of blood, urine and stool culture. Of the 150 patients among whom were 87 (58%) male and 63 (42 %) female. The total number of widal test positive were 102 (68%) and negative result were 48 (32%), the total number of WKA test were positive 65 (63.7 %) and negative result were 37 (36.2%), while the total number of culture positive were 25 (16.6 %) among which were 18 (12%) for blood culture, 1(0.6 %) for urine culture and 6 (4%) for stool culture, Table 1.

Table 1: Profile of the enteric fever diagnosis methods

Total Numbers of Patients	150
Number of males	87 (58 %)
Number of females	63 (42 %)
Total number of patients with widal test positive result	102 (68 %)
Total number of patients with widal test negative result	48 (32 %)
Total number of patients with killed antigen positive result	65 (63.7 %)
Total number of patients with killed antigen negative result	37 (36.2 %)
Total number of patients with culture positive	25 (16.6 %)
Total number of patients with culture positive from blood specimen	18 (12 %)
Total number of patients with culture positive from urine specimen	1 (0.6 %)
Total number of patients with culture positive from stool specimen.	6 (4%)

The results of widal test showed 102 (68%) of 150 sample were positive and 48 (32%) were negative, for the same manner, among which were 52 (50.9 %) males and 50 (49%) females that distributed depending on age, Table 2.

Table 2 : Gender and age groups distribution of enteric fever patients depend on widal test positive result

Total N (%)	Female N (%)	Male N (%)	Age
16 (15.6 %)	7 (6.8 %)	9 (8.8 %)	≥ 20
43 (42.1 %)	18 (17.6 %)	25 (24.5 %)	21 - 40
25 (24.5 %)	17 (16.6 %)	8 (7.8 %)	41 – 60
18 (17.6 %)	8 (7.8 %)	10 (9.8 %)	61 - 80
102 (99.9 %)	50 (49 %)	52 (50.9 %)	Total

The result of WKA test showed that 65 (63.7 %) from 102 samples were positive. The male percentage was different than female in enteric fever patient were distributed as 35 male percentage (53. 8 %) and 30 female percentage (46.1 %) as illustrated in Table 3.

Table 3 : Gender and age groups distribution of enteric fever patient depend on killed antigen positive result.

Total N (%)	Female N(%)	Male N(%)	Age
8 (12.0 %)	1 (1.5 %)	7 (10.7 %)	≥ 20
37 (56.9 %)	15 (23 %)	22 (33.8 %)	21- 40
15 (23 %)	11 (16.9 %)	4(6.1 %)	41 - 60
5 (7.6 %)	3 (4.6 %)	2 (3 %)	61- 80
65	30 (46.1)	35 (53.8 %)	Total

Discussion

Currently, the diagnosis of enteric fever depends on the isolation of *Salmonella* from a patient, most commonly by blood culture. Antigen detection has not been investigated much and detecting an immune response specific for typhoid fever has been done only with antibody detection. Serodiagnosis depends on the age-old and widal test the detection of raised titre of agglutinating serum antibodies against the lipopolysaccharide (Lps) [O] or flagella antigens of serotype typhi and other serological diagnostic tools (Wain and Hosoglu, 2008)

The results of culture (16.6 %) among whom, the rate of blood culture (12 %) was elevated compared with the rate of urine culture (0.6 %) and stool culture (4 %). Blood cultures are the standard diagnostic method, the rate of isolation bacteria from blood approaches 90% in untreated patients in the first week; however, the figure falls to less than 50% by the third week. The sensitivity of blood culture is higher in the first week of the illness which is reduced by prior use of antibiotics and increases with the volume of blood cultured and the ratio of blood to broth (Keusch, 1993). Stool culture alone is less than 50% sensitive and urine even less so (AFM *et al*., 2013).

In the present study; in Table 1, out of 150 patients whose blood samples were tested ; 87 were males and 63 were females. Of the 87 males, 52 (50.9 %) responded positive to widal test. Out of the 63 females were 50 (49 %) give positive result to widal test. Also depended on WKA test, from 102 patients who were positive to widal test. The number of males were 35 (53.8 %) and 30 (46.1 %) were females (as illustrated in Table 2). The number of males positive to widal test and WKA test were more than those of females . In a research conducted by Chalya in 2012, the study representing 8.7% typhoid fever cases, he observed that males were more affected two times than the females (Charles *et al*., 2012), also these result Compatible with (Ramyil *et al*., 2013).

In Table 2; out of 102 patient were 65 cases positive to WKA test were 35 (53.8 %) males and 30 (46.1) females. Human sera from patient that contain antibacterial antibody were incubated with heat – killed bacteria (antigen) at 37 ºC for 30 min, the agglutination appearance were consider the positive result after mixing the serum with heat killed antigen (De Beaufort *et al*., 1997).

Rapid slide agglutination of Widal test is non-specific for diagnosis of typhoid fever because the cross reactivity that observed when gave positive results in healthy control persons (Mohammed, 2017)using Widal test as the only laboratory test for the diagnosis of typhoid fever will result in misleading diagnosis. Therefore, it is very essential to use culture technique to diagnose enteric fever (Yagoub and Elhaj, 2015).

Reference

AFM, A.S.; Abdullah, M.Y.; Bodrul, M.I. and Waseka, A.J. (2013). Different diagnostic procedure of typhoid fever: A Review Update Journal of Current and Advance Medical Research, 1(2).

Amal, H.A.; Eman, S.S.; Ann, A.K. and Kawther, K.J. (2012). The Effect of typhoidfever on cytokines (Interleukin-6 And 8) a nd C–reactive protein

concentration. Journal of Advanced Medical Research 1(1): 114-122.

Al–Ammar, M.H. (2006). Some aspects of specific immunity for *Salmonella typhi* in typhoid patients and rabbits. Alkufa university.

Baker, S.; Favorov, M.O. and Dougan, G. (2010). Searching for the elusive typhoid diagnostic. BMC Infect Dis., 10: 45.

Wain, J. and Hosoglu, S. (2008). The laboratory diagnosis of enteric fever. J Infect Dev Ctries, 2: 421–5.

Karen, H.K.; Arvinda, S.; Maupi, E.; Greta, H. and Claire, L.C.; Anne, B.M. and John, A.C. (2011). Sensitivity and specificity of typhoid fever rapid antibody tests for laboratory diagnosis at two sub-saharan african sites . Bull World Health Organ, 89: 640–647.

Safia, S.; Md.AbdoullahAl, M.; Rabeka, S. and Shahanaz, J. (2016). Laboratory diagnosis of enteric fever. Bangladesh Journal of Infectious Diseases. 3(2).

De Beaufort, A.J.; Langermans, J.A.M.; Matze–vanderlans, A.M.; Hiemstra, P.S.; Vosswn, J.M. and Van Furth, R. (1997). Difference in binding of killed and live Streptococcus pneumoniae serotype by c- reactive protein . Scandinavian Journal of Immunology, 46(6): 597 – 600.

Barrow, G.I. and Feltham, R.K. (1993). Cowan and steel's manual for the identification of medical bacteria, 3rd Ed, Cambridge University Press.

Charles, A.M. and Adam, M.H.; Gad, El Rab, M.O.; Morshed, M.G. and Shakoor, Z. (2012). Detection of *Salmonella typhi* agglutinatinins in sera of patients with other febrile illnesses and healthy individuals. Journal of African Medicine, 10(1): 41-44.

Ramyil, M.S.; Ihuoma, O.J.; Ogundeko, T.O.; Ameh, J.M.; Olorundare, F.; Adeniyi, O.G.; Amapu, T.Y.; Izam, M.M. (2013). Comparative study on the use of widal test and stool culture in the laboratory diagnosis of *Salmonella* infection in adult and children in Jos Metropolis, Plateau State, Nigeria . International Journal of Science and Research, 2(12): 02013633.

Keusch, G.T. (1994). Salmonellosis. In: Harrison's Principles of Internal Medicine (13rd ed). Ed: Isselbacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci AS, Kasper DL. McGraw-Hill Book Company, New York, 671-676.

Mohammed, A.H. (2017). Use of C-reactive protein in the evaluation of widal test and typhoid stripe test in the diagnosis of typhoid fever. Journal Immunol. Clin. Microbiol., 2(1): 16-20.

Yagoub, H.A. and Elhaj, A.E. (2015). Comparison between the Widal test and culturing technique in the diagnosis of enteric fever in Khartoum State, Sudan. African Journal of Bacteriology Research, 7(5): 56–59.