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Study of *Salmonella typhi* isolated from patient suffering from typhoid fever in AL-Samawah city, Iraq

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Abstract

Bachground: Salmonellae are food-born pathogens transfer through oral ingestion of contaminated food or water and causing disease in both healthy and immune-compromised people. Objective: current study was designed for isolating and identifying *Salmonella typhi* from blood and stool samples, and detecting IgM antibody to *S. typhi* specific antigen using Immunochromatographic (ICT) method. Methods: This study was conducted on 180 patients from both genders suffering from typhoid fever. Their age range was 1 to 50 years. The samples were collected from different hospitals in AL-Samawah city. The effect of age, gender and residence on the frequency distribution of typhoid fever was also determined. The collected samples were tested by culture, ICT and then results were analyzed using appropriate statistical methods. Results: The results showed that Immunochromatographic (ICT) method was highly sensitive when applied to blood samples as compared to blood culture and serology.

Key words: typhoid fever, ICT, salmonella typhi**,** blood culture, stool culture.

INTRODUCTION

Typhoid fever is a systemic human infectious disease caused by Salmonella typhi. Symptoms during the acute phase of infection comprise nausea, abdominal pain, headache and fever [Ref]. According to the World Health Organization (2014), the annual rate of typhoid fever reaches 21 million cases and 222,000 fatalities (1).This gram-negative enteric bacillus belongs to the family Enterobacteriaceae. It is a motile, facultative anaerobe and there is no animal reservoir that is susceptible to various antibiotics (2). Typhoid can be diagnosed with certainty only by isolation of *Salmonella typhi* from the blood, urine, feces or other body fluids [Ref]. This is often not possible in developing countries where the disease is common and endemic, because bacteriological facilities are not available in many of the smaller hospitals. Under these circumstances the diagnosis has to be made by the association of a clinical picture compatible with typhoid and a significant titer of agglutination antibodies in the blood against the H and/or O antigen of Salmonella typhi (3).Complications of typhoid fever include intestinal hemorrhage or perforation, pneumonia, myocarditis, hepatitis, acute cholecystitis and meningitis. Following an initial recovery, relapses may occur in about 10–20% of untreated patients (4).

MATERIALS AND METHODS

1- Collection of Specimens

Blood and stool samples were collected aseptically from 180 Patients suffering from typhoid fever who visited Al-Samawah general hospitals for the period from (March 2017-Februray 2018). The age range of patients was 1 to 50 years. A total of 7 mls blood sample was drawn aseptically from each patient; a 2-ml blood sample was tested for widal test using O Somatic Antigens and H Flagellar Antigens (5) and a 5-ml blood sample was inoculated in 50 ml of Brain heart infusion (BHI) for culture of *S. typhi* (6). These samples waere collected aseptically following universal safety precaution.

2- Samples Culture

Blood culture: A volume of 5ml of blood was aseptically injected into sterile bottle contained 50 ml of sterilized Brain heart infusion (BHI) broth and then incubated at 37ºC. Blood culture was regularly examined for checking the turbidity and color change which referred to microbial growth. Culture should be incubated for at least 7 days before result is reported as negative. Nevertheless, bottle was discarded after 14 days (7). Subcultures were performed as follows: from each positive blood bottle, a loopfull was transferred to MacConkey agar and Salmonella-Shigella agar (S.S agar) and Chrom??? agar , streaked, incubated for 24 hours at 37 ºC. The isolates were stained with Gram stain and examined under light microscope (8). All culture media were prepared according to the information of the manufactures.

Stool culture: In typhoid fever, stool cultures are usually positive from the second week of the infection. Stool is usually plated on desoxycholate-citrate agar and also inoculated into fluid enrichment media such as tetrathionate or selenite broth. Suspicious colonies from culture plates are tested directly for the presence of salmonella O antigens by slide agglutination and sub cultured to peptone water for determination of H antigen structure and for further biochemical analysis (9).

3. Biochemical test : Important relevant biochemical tests such as Oxidase test, Indol test, Urease test, Methyl Red/ Voges-Proskauer test, Citrate utilization, Kligler test, Cytochrome oxidase tests and Catalase test were conducted according to (10,11).

4. Api20E system: This system was devised for biochemical identification of Enterobacteriaceae and other gram negative bacilli. It consists of 20 micro-tubes containing dehydrated media (each micro-tube consists of a tube and cupul section). The Api20E system was performed according to manufacture's instructions.

5- *Salmonella typhi* antibody detection: Detection of *S. typhi* antibody by ICT is a qualitative test. The ICT utilizes a unique combination of monoclonal antibody/colloidal gold dye conjugate and a polyclonal antibody immobilized on the solid phase. This is selectively identifying *S. typhi* antibody associated with *S. typhi* infection with high degree of sensitivity and specificity.

6- Serological identification: Serological identification of *Salmonella* isolates was done according to (12). All isolates were doing with polyvalent O and H antisera by using slide agglutination test as follows:

- One drop from physiological normal saline was placed on each of the glass slides at each side and then a loopfull from bacterial culture was mixed with each drop.
- One drop from each O/ H polyvalent antisera was added to one of the previous drop and then mixed by plastic rod and rocked. The other drop was used as control.
- The clear agglutination occurred within 1-2 minute indicated a positive result.

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

RESULTS AND DISCUSSION

Identification of salmonella on the culture media: The results of blood cultures of patients' samples showed that 45 isolates out of 190 cases were found positive for *Salmonella typhi* as shown in Fig. (1).

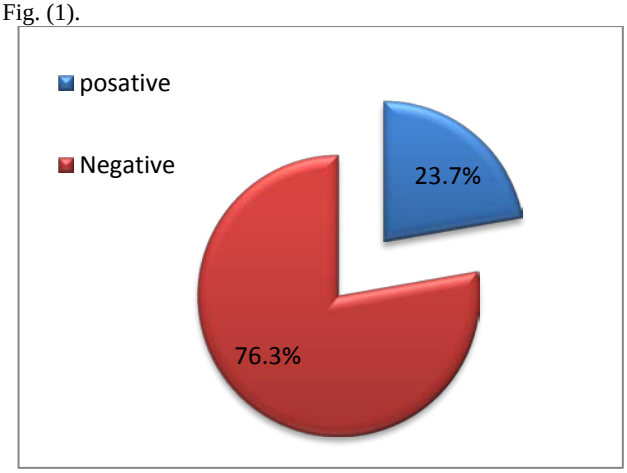


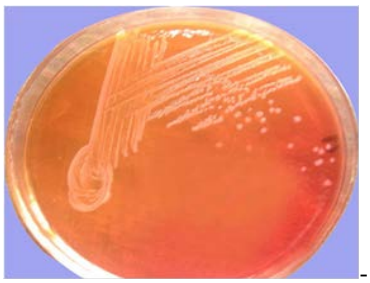
Figure 1 Percentage of blood cultures positive for *Salmonella typhi*

Blood culture is the gold standard diagnostic method for typhoid fever (13). Its sensitivity is poor, because a very small number of bacteria is needed to cause severe infection. The sensitivity of blood culture is maximum during first week of the illness and decreases with progression of the illnesses (14).

Bacteria may be found in bloodstream at any stage of the illness, but most commonly found in the first 7-10 days and during relapses (9). If the patient is untreated, blood culture is usually positive in about 75% during first week and decreasing to 15%-26% during later stages of the disease (15). In current study, it was found that 50 ml of medium was sufficient for 5 ml of blood apparently because of very low degrees of bacteraemia in some patients (16).

Morphological properties: After 24 hrs of incubation at 37 Cº, the bacterium showed convex, (2–4 mm) in diameter, and smooth colonies. On MacConkey agar, they looked pale due to their inability to ferment lactose, but on Salmonella shigella (S–S) agar, they were colorless. On Blood agar *S. typhi* produce grey white 2-3 mm in diameter colonies as (Figure 2).

A-



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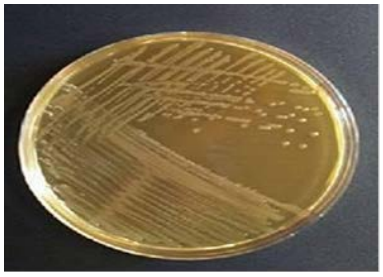

C-

Figure 2 Growth of *Salmonella typhi* on: Blood agar (A), MacConkey agar (B) and Salmonella shigella agar (C)

On Xylose–Lysine–desoxycholate (XLD) agar, the colonies were red in color with black colony center while on nutrient agar; the colonies of most strains were moderately large, 2-3 mm, in diameter after 24 hours at 370 ºC. SS agar is highly selective medium formulated to inhibit the growth of most coliform organisms and permit the growth of species of Salmonella and shigella from environmental and clinical specimens. The high concentrations of bile salts and sodium citrate inhibit all Gram-positive and many Gram-negative organisms including coliforms. Some strain produce mucoid colonies (17).

Salmonellae require enrichment of the minimal medium with one or more amino acids or vitamins e.g., cysteine or nicotinamide. Also, most *S. typhi* strains require tryptophan (18).

Microscopic Examination: Gram staining was done for morphological identification of *S.* typhi, where *S.* typhi was found (Figure 3) to be Gram-negative short bacilli (19).

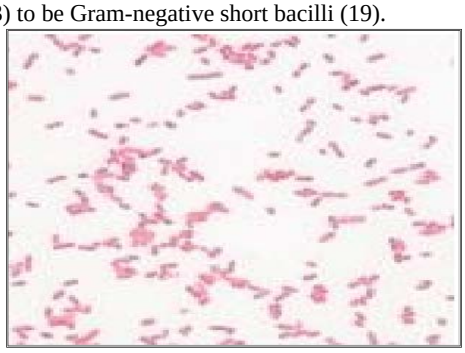

Figure 3 Microscopical appearance of *Salmonella typhi* as Gram-negative short bacilli

Biochemical Reactions: Table 1 presents biochemical reactions of *salmonella typhi*

Table 1 Biochemical reactions of *salmonella typhi* isolate

Biochemical test	Result	Biochemical test	Result
Indole	–	Methyl Red	-
Catalase	+	citrate utilization	-
Voges–Proskaure	–	oxidase, and ureas	+
Production of H2S	+		

(+) positive reaction, (–) negative reaction.

Identification using api20E: The results of api20 system had supported biochemical identification of *S. typhi* (Table 2 and Figure 4).

Figure 4 API20E results for *Salmonella typhi*

Table 2 Results of biochemical test of API20E system

Test	result	Test	Result
Tryptophan Deaminase	-	Urease	-
Indol Production	-	H_2 S Production	+
Acetone production	-	Citrate Utilization	+
Gelatiase Liquefaction	-	B–Galactosides	-
Glucose fermentation	+	Arginine Dihydrolase	+
Mannitol fermentation	+	Lysine Decarboxylase	+
Inositol fermentation	+	Decarboxylase	+
Sorbitol fermentation	+	Melibiose fermentation	+
Rhamnose fermentation	+	Amygdalin fermentation	-
Sucrose fermentation	-	Arabinose fermentation	+

Serological tests

Widal test (Figure5): This introduced as a serologic technique to aid in diagnosis of typhoid fever. A total of the 190??? clinically suspected typhoid cases examined in the period between March 2017 to the end of February 2018 for widal test. 110 (57.9%) were positive for anti O antigen and anti H antigen while 80 (42.1%) of blood samples were negative.

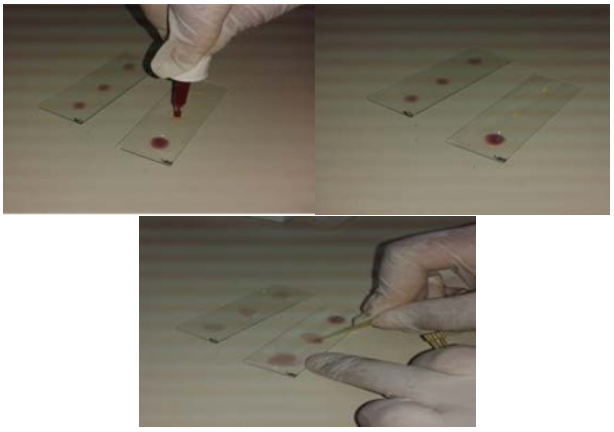

Figure 5 Widal agglutination test

The test was based on demonstrating the presence of agglutinin (antibody) in the serum of an infected individual against the H (flagella) and O (somatic) antigens of *Salmonella typhi* (15).The O Antigen is the somatic antigen of *S. typhi* and is shared by *S. paratyphi* A, *S. paratyphi* B, other Salmonella species and other members of the Enterobacteriaceae family. Antibodies against the O antigen are predominantly IgM rise early (appear on day 6-8) in the illness and disappear early. The H antigens are flagella antigens of *S. typhi*, *S. paratyphi* A and *S. paratyphi* B. Antibodies to H antigens are both IgM and IgG rise late (on days 10-12) in the illness and persist for a longer time (20). Serological diagnosis relies classically on the demonstration of a rising titer of antibodies in paired samples at an interval of 10-14 days (13).

In typhoid fever, however, a four-fold rise after 2 weeks in not always demonstrable even in blood culture confirmed cases. This situation may occur when the acute phase sample is obtained late in the natural history of the disease, because of high levels of probable background antibodies in an endemic region or because in some individuals the antibody response is blunted by the early administration of an antibiotic (21).

False negativity is one of the drawbacks of the Widal test. Hosoglu et al. [22] conducted a study to evaluate the associated factors with Widal test negativity in an endemic area. Widal test negativity was retrospectively analyzed by the authors among culture-proven typhoid fever cases. Factors like age, gender, previous antibiotic usage, duration of symptoms, leucopoenia, haematocrit value and erythrocyte sedimentation rate (ESR) were evaluated for potential association with Widal test negativity (22).

Detection of Salmonella typhi antibody by ICT is a qualitative test. By ICT, among 190???????? blood samples from the suspected cases, 119 (62.6%) were positive for IgM of *S. typhi*. Among the ICT positive cases, it was found that 93 (78.2%) cases had IgM antibody, 17 (14.3%) cases had both IgM and IgG antibody and only 9 (7.5%) cases positive for IgG antibody.

Among the three different tests for the diagnosis of typhoid patients, Immunochromatography (ICT) test showed maximum 119 (62.6%) positive result followed by blood culture 45 (23.7%) and stool culture 26 (13.7%) (Figure 6).

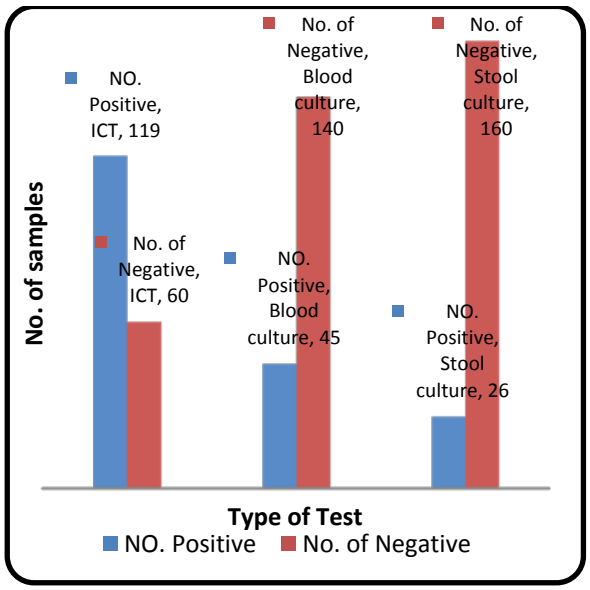

Figure 6 Comparison among ICT , blood culture and stool culture for diagnosis of typhoid fever

Age and gender of typhoid cases: The age and gender distributions of patients can be seen in table (3). The highest rate of typhoid cases was among the age group 16-20 (26%). The results also showed that the incidence was higher among males (55%) than among females (45%).

Table 3 Age and gender distributions of typhoid cases

Age group	No. of Isolates			
	Male		Female	
	No.	(%)	No.	(%)
1-5	2	7.4	2	8.7
6-10	3	11.1	3	13.0
11-15	4	14.8	4	17.4
16-20	7	26	6	26.1
21-25	3	11.1	2	8.7
26-30	2	7.4	0	0.0
31-35	2	7.4	2	8.7
36-40	2	7.4	2	8.7
41-45	0	0	2	8.7
46-50	2	7.4	0	0.0
Total	27	55	23	45

Moreover, results showed that the prevalence of typhoid fever, within the studied population, was higher among patients living in rural areas than among those living in cities ($P < 0.01$; Table 4).

These findings could be attributed to life style of people living in rural areas where people drink water without any sterilization so that bacterial pathogens are easily and directly transmitted via water.

Table 4 Prevalence of salmonella isolation according to residence

Residence	No. of cases examined	No. of positive cases	% of positive cases	*P* value
Urban	90	17	34	<0.01 **
Rural	100	33	66	
Total	190	50	100	

** Highly significant.

Further more, results of current revealed that the incidence of typhoid fever cases caused by *Salmonella typhi* was highest during May, 2017 and lowest during November, 2017 (Table 7).

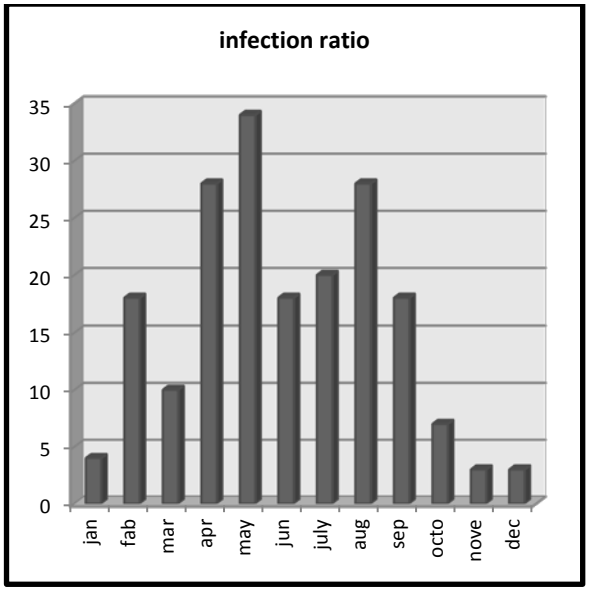

Figure (7): Distribution of typhoid fever infection rate according to month of the year.

CONCLUSION

In concluded, it was found that 45 out of 190 (23.7%) had positive blood cultures and the isolates belong to *salmonella typhi* whereas 26 isolate (13.7%) had positive stool cultures. Also, ICT test showed higher rate of typhoid cases 119 (62.6%).

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