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Isolation and Identification of *Salmonella typhi* Caused Typhoid Fever from Hospitals in Basrah City

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Abstract

A total of two hundred and sixty blood samples were collected from males and females who visited different hospitals in Basrah city for the period from February to the end of October 2013. The stady aimed to isolate and identify *Salmonella typhi* from blood samples collected from clinically suspected cases to predict the diagnostic value of the clinical suspicion versus the bacteriologic investigation including isolation and characterization of the main causative pathogen, *Salmonella typhi*. The study aimed to determine the effect of age and gender on the frequency distribution of typhoid fever during the study period.

Key word: *Salmonella typhi* , Typhoid fever

1.Introduction:

Typhoid fever, also known as enteric fever, is a potentially fatal multisystemic illness caused primarily by *Salmonella typhi*.It affects roughly 21.6 million people (incidence of 3.6 per 1,000 populations) and kills an estimated 200,000 people every year [1] .It has been documented that food sources such as eggs, poultry and meat products comprise major risk of transmission of enteric fever to humans. However, typhoid fever can largely be passed via fecal-oral route through contact with active, convalescent cases or chronic carriers [2,3].

Herin, try to predict the diagnostic value of the clinical suspicion versus the bacteriologic investigation including isolation and characterization of the main causative pathogen, *Salmonella typhi*. In addition, it is an attempt to determine the effect of some epidemiologic factors including age, gender variations on the occurrence of typhoid fever.

The lack of specificity of the clinical spectrum together with the difficulty of achieving a definitive bacteriologic or serologic diagnosis, impede making early and accurate diagnosis of typhoid fever[4,5]. . However the bacteriologic identification, namely blood culture, is still the mainstay of the diagnosis [6].

Aims of study:

Isolate and identify *Salmonella typhi* from blood samples collected from clinically suspected cases to predict the diagnostic value of the clinical suspicion versus the bacteriologic investigation including isolation and characterization of the main causative pathogen, *Salmonella typhi*. The study aims to determine the effect of age and gender on the frequency distribution of typhoid fever during the study period.

2.Materials and Methods

Blood sample were collected aseptically from 260 Patients taken from patients suffering from typhoid visited of (Al-Basrah general hospital, Al-Sadr teaching hospital, Al–Fayhaa general hospital, AL-Shafaa General Hospital AL-Zubair General Hospital, Basrah childrens specialized hospital, Basrah Maternity and Children Hospital and Al-Mawani general hospital in Basrah city) for the period from February to the end of October 2013, the age of patients ranged from 1-60 years. A total 7 ml blood sample was drawn aseptically from each patient ;2ml was tested for widal test and 5ml of blood was inoculated in 50ml of Brain heart infusion (BHI) for culture of *S. typhi*. [7]..

Widal test

Widal testing was done using O Somatic Antigens and H Flagellar Antigens, by using Widal slide agglutination method [8].

The result was considered to be suspected when both flagellar(H) and somatic(O) antibody titers were 1/ 160 and 1/320 and over for patients [9,10]..

Culturing

The volume of 5ml of blood was aseptically injected into sterile bottle contained 50 ml of sterilized Brain heart infusion (BHI) broth with 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 was reported as negative. Neverthless bottle was discarded after 14 days[1]. .

Subcultures were as follows: from each positive blood bottle, First: a loopfull was transferred to MacConkey agar and Salmonella-Shigella agar(S.S agar), streaked, incubated for 24 hours at 37 ºC. The isolates were stained by Gram staind and examined by light microscope[11]. .

Biochemical test

Important biochemical tests (Oxidase test, Indol test, Urease test, Methyl Red/ Voges-Proskauer test, Citrate utilization, Kligler test, Urease test and Catalase test) were conducted according to[11,12,13]. .

Serological Identification

Serological identification of *Salmonella* isolates was done according to[14]. . All isolates were doing with polyvalent O and H antisera by using slide agglutination test as follows:

1-One drop from physiological normal saline was placed on each of the glass slides at each side, and then a loopful from bacterial culture was mixed with each drop.

2-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.

3-The clear agglutination occurred within 1-2 minute indicated a positive result.

Api20E system

This system is devised for the biochemical identification of Enterobacteriaceae and other gram negative bacilli. It consists of 20 microtubes containing dehydrated media (each microtube consist of a tube and cupul section). The Api 20E system was performed according to the manufacture instructions.

Also Cytochrome oxidase tests were carried out according to [13]..

Statisties 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 $p \leq 0.05$.

3.Results

Widal test

A total of the 260 clinically suspected typhoid patients examined in the period between Februray to the end of October 2013. Atotal of the 260 clinically suspected typhoid patients examined for widal test , 142(54.61%)were positive for anti O antigen and anti H antigen. While 118(45.38%) samples were negative.

Isolation and identification of samples and distribution of *S. typhi*

Out of 260 cases, 70 isolates were identified as belong to *S.typhi.*

Morphology and Microscopy of *Salmonella*

The isolates were identified as related to the *Salmonella* by their pale colonies (non lactose fermenters) on MacConkey agar. In addition, on SS agar appear as colorless colonies ,production of H_2S turn the center of colony to black as shown in figure (1,2).Microscopic examination of the isolates appeared as gram negative bacilli figure(3)

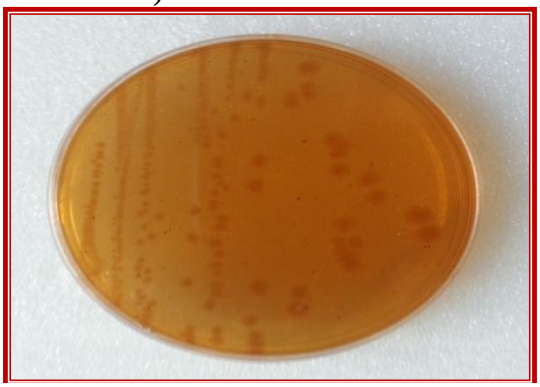


Figure (1): Growth of *Salmonella* on MacConkey agar

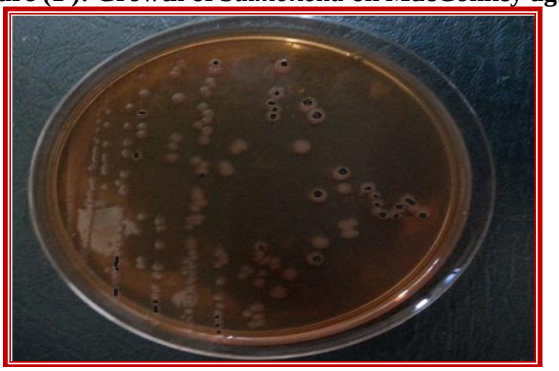
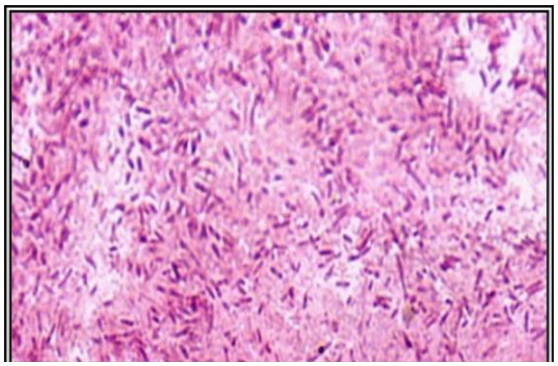


Figure (2): Growth of *Salmonella* on SS agar



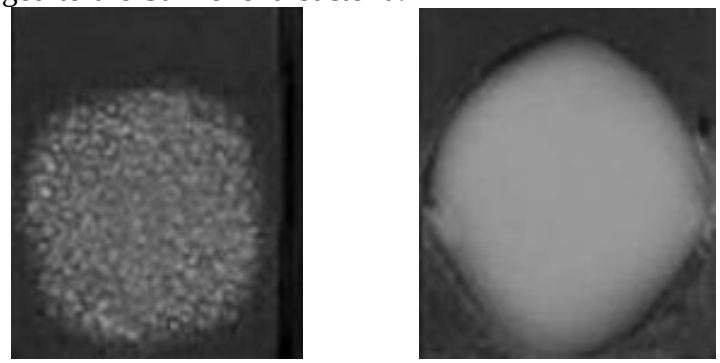
Figure(3): Gram staining of *Salmonella*

Culturing

All of *Salmonella* (70 isolates) gave alkaline (red) slant and acid (yellow) butt,with production of H2S (blackening of agar) in Kilgler Iron agar, and all of them were positive for methyle red test, this is indicated by diffuse red color in medium and indicated positive for fermentation of glucose. They also gave negative reaction for Voges- Proskauer test indicated by absence of development of pink-to red. Also all isolates gave negative reaction for citrate utilization, oxidase, indole and ureas tests.While they gave positive for motility and catalase test.

Serological identification

Seventy isolates were serologically diagnosis using salmonella antisera to make sure it belonged to the Salmonella bacteria. The agglutination with specific antisera showed in figure (4).



A B

Figure(4): Agglutination with specific antisera. A: positive for *salmonella*, B: negative.

The API 20E test

To confirm and complete the biochemical and serological results, the API 20E system was used for identification of *S. typhi*. The system which contains 20 different biochemical reactions including 10 enzymatic reactions,10 fermentation oxidation reactions an oxidase test was used. The results were interpreted after 24 h. at 37°C, which revealed that tested isolates (20) belong to *S. typhi* . Biochemical reactions on API 20E strip and calculation chart are shown in (Figure 5,a and b) respectively.

Figure (5,a): API 20E results for isolated bacteria *Salmonella* serotype Typhi.

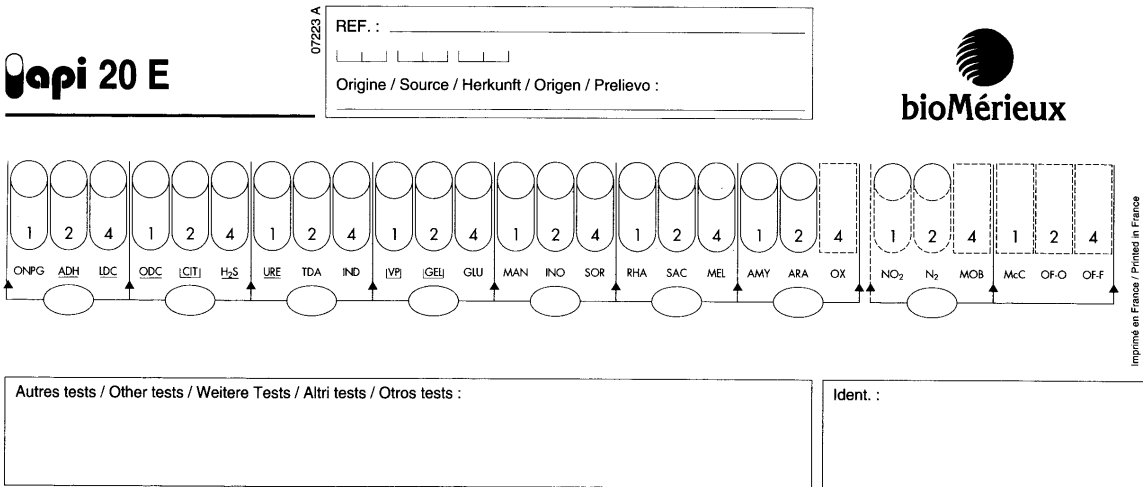


Figure (5,b): A representative photograph of proforma used to tabulate the results obtained from API 20E strip, which are later used for the identification of *Salmonella typhi*

Geographical distribution of *Salmonella typhi*

Out of 260 blood samples only 70 isolates were obtained from blood samples as shown in table (1). The statistically significant (P≤ 0.05

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

No.	Hospital	No. of samples	Isolates	
			No.	%
1	AL-Zubair General Hospital	45	17	37.77
2	AL-Basrah General Hospital	56	20	35.71
3	AL-Mawani General Hospital	51	17	33.33
4	AL-Fayhaa General Hospital	47	11	23.4
5	AL-Shafaa General Hospital	22	3	13.63
6	AL-Sader teaching Hospital	17	1	5.88
7	Basrah Maternity and Children Hospital	20	1	5
8	Basrah childrens specialty hospital	2	0	0
Total		260	70	26.92

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 51-60 age group (2.85%) whereas the highest incidence was among the 11-20 age group (35.71%). The table also shows that the incidence was higher among males (52.85%) than that of females (47.14%). There was not statistically significant (p≤0.05).

Table(2) Distribution of *Salmonella typhi* in different age and gender groups.

Age group	Isolates		Male		Femal	
	No.	%	No.	%	No.	%
1-10	7	10	3	8.1	4	12.12
11-20	25	35.71	14	37.83	11	33.33
21-30	20	28.57	11	29.72	9	27.27
31-40	13	18.57	7	18.91	6	18.18
41-50	3	4.28	2	5.4	1	3.03
51-60	2	2.85	0	0	2	6.06
Total	70	100	37	52.85	33	47.14

4. Discussion

In spite of recent advances in public health and sanitation, Typhoid fever continues to be a major cause of morbidity and mortality[15] . In Iraq, few studies on typhoid fever were carried out. [16] conduced 2-year surveillance while[17] conduced 5 years surveillance (1985-1989) in Iraq. These studies emphasized on the epidemiological of *S. typhi***.** [18] , has studied the outbreaks of typhoid fever and reported that the distribution of cases over a large area within a short time favored the possibility of water borne disease. It is clear from table (1) that the majority of patients are AL-Zubair General Hospital, AL-Basrah General Hospital and AL-Mawani General Hospital respectively.

Regarding the age-group, which were affected by this disease, a higher incidence was in age group 11-20 years (Table 2). This results similar with the previous studies of [6] and [19], [20] , also[21] . This may be due to their mobility, consumption of unhygienic food and water in school and colleges[22,23] . In addition, the little cases were in age groups (41-50) and (51-60) years old. The reason may be related to frequent boosting of immunity [24].

On the other hand, males were found to be more infected (52.85%) than females (47.14%) as indicated in (Table 2); this results is similar to other results of studies in AL-Musaib district[25] and Diyala governorate[21] . This might be due to that most males were out-doored and from this point of view they could be regarded as food eating and handing or contact with other patients[26].

In the present study, out of the 260 clinically suspected typhoid fever, 70(26.92%) were blood culture positive for *S. typhi* and 142(54.61%) widal positive patients. Similar findings were also reported from another study in Basrah and Mosul provinces where the isolation rate in blood culture is 28.89%, 25% and 100%,57.1% were positive for widal test respectively[20,27] . In the other hand, study that made in Najaf province recorded 22.6% in blood culture and 51.4% for widal test.

Blood culture has the promise diagnosis in the first week and is very specific, but its sensitivity is poor due to various factors. The most important factor is the very few numbers of bacteria needed to cause severe infection, which can be as low as 10/ml. Hence, positive culture yield are very low and elude definitive diagnosis. Other limiting factors, beside the bacteriostatic effect of antibiotics (already administered before the culture sample is taken), may be the nature of culture medium employed, the time of blood collection, the hosts immune response system, and the intracellular characteristics of *Salmonella typhi* [27]. In spite of, isolation of the causative agent by culture has remained the gold standard for diagnosis of enteric fever [28,29]

Although the Widal test has been used for more than a century in many developing countries but it is non-specific, poorly standardized, often confusing and difficult to interpret [30] . Moreover, sharing of O and H antigens by other *Salmonella* serotypes and other members of Enterobacteriaceae makes the role of Widal test even more controversial in diagnosing typhoid fever [6].

In the other hand, (142) samples were taken for culturing. After held out the biochemical tests and serological

identification Figure (4). Then complete and confirm the diagnosis, depending on API20E system as shown in the figure (5 a,b). The tests led to isolate (70)(26.92%) *S. typhi.* This percent was higher than that obtained by[31] in Baghdad (8.23%) and [32] in Sulaimani(11.6%) who used blood culture for primary isolation and lower than the percent obtained by [28] in Basrah (28.89%) and [33] in Karbala (52.8%) who used API20E system for identification. Mentioned [34] that API20E at the 99.9% like hood level was demonstrated to be accurate method for *Salmonella enteric* identification.

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عزل وتشخيص *Salmonella typhi* المسببة للحمى التايفوئيدية من مستشفيات مدينة البصرة

منى عبد الامام المازني و محمد عبد المحسن الحجاج
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الخلاصة

جمعت 260 عينة دم من الذكور والاناث الذين راجعواعدد من المستشفيات في مدينة البصرة للفترة من شباط الى نهاية تشرين الاول 2013 . هدفت الدراسة الى عزل وتشخيص البكتريا المسببة الرئيسية للمرض (*Salmonella typhi*) من نماذج الدم المأخوذة من المشتبه سريريا باصابتهم بالمرض وذلك لغرض اختبار القيمة التشخيصية للاعراض السريرية مقابل الطرق البكتريولوجية متمثلة بعزل وتشخيص المسبب المرضي الرئيسي الا وهو *Salmonella typhi* . وكذلك تهدف الى تحديد تاثير الجنس والعمر على معدل الاصابة بالحمى التيفوئيدية خلال فترة الدراسة.

الكلمات المفتاحية: السالمونيلا تيفي , حمى التيفوئيد