Bacteriological and serological study of typhoid fever caused bysalmonella isolation from patient in Samawah city.

Hedaa M. Nahab&Nuha Mohammed Mousa
Biological Dept. ,College of Science, Al-Muthanna University, Al-Muthanna, Iraq
Key words:typhoid fever, ICT, salmonella typhi.

Abstract: Salmonellae are food borne pathogens, acquired by the oral ingestion of contaminated food or water, causing disease in both healthy and immune compromised individuals. This study was done on 180 patient at many age group range from one to fifty five years old and for both sexes were collected from patient suffering from typhoid fever who attend the different hospitals in samawah city.this study aimed to isolate and identify Salmonella typhifrom blood and stool samples,and to detect IgM antibodyto S. typhispecific antigen by Immunochromatographic (ICT) method, and which highly sensitive in blood samples of the present study and was compared to blood culture and serology.and aimed to determine the effect of age, gender, residence and months of years on the frequency distribution of typhoid fever during the study period(march 2015-februray 2016).The collected samples were tested by culture, ICT and then results were analyzed using appropriate statistical methods.

The results revealed that 40 out of 180 (22.2%) were positive of blood culture and the isolates belong to salmonella typhi.while the stool culture gave 20 isolate at a percentage (11.1%). ICT test give higher rate of typhoid cases 155 (86.1%).

Introduction

Typhoid fever is an acute systemic disease resulting from infection with Salmonella typhi, which is gram negative, nonsporting rode, motile bacteria [1]. Typhoid can be diagnosed with certainty only by isolation of Salmonella typhi from the blood, urine, feces, or other body fluids, 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[2]. Complications include intestinal hemorrhage or perforation, pneumonia, myocarditis, hepatitis, acute cholecystitis and meningitis. Following an initial recovery, relapses occur in about 10–20% of untreated patients[3].

Materials and Methods:-

1-Specimen and collection:

Blood and stool samples were collected aseptically from 180 Patients suffering from typhoid fever who visited of Al-samawah general hospitals, for the period from (march 2015-februray 2016). the age of patients ranged from 1-55 years. A total 7 ml blood sample was drawn aseptically from each patient ;2ml was tested for widal test using O Somatic Antigens and H Flagellar Antigens, [4].and 5ml of blood was inoculated in 50ml of Brain heart infusion (BHI) for culture of *S. typhi*. [5].These sampleswas collected aseptically following universal safety precaution.

2-Serological Identification

Serological identification of *Salmonella* isolates was done according to[6]. 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 loop full 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.

3- Detection of *Salmonella typhi* antibody

Principle of the test: 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 was selectively identifying *S. typhi* antibody associated *S. typhi* infection with a high degree of sensitivity and specificity.

4- Culturing of samples:-

Blood culture :-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. Nevertheless bottle was discarded after 14 days[7]. .Subcultures

were as follows: from each positive blood bottle, First: a loopfull was transferred to MacConkey agar and Salmonella-Shigella agar(S.S agar) and Chromagar , streaked, incubated for 24 hours at 37 ºC. The isolates were stained by Gram staind and examined by light microscope [8]. All culture media were prepared according to the information of the manufactures.

StoolCulture :-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].

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[10,11].

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 [11].

StatistiesAnalysis :-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 $p \leqslant 0.05$.

Resultsand discussion

Identification of salmonella on the Culture media :-in this study , Out of 180 cases, 40 isolates were identified as belong to *Salmonella typhi.* According to laboratory tests which are used in this study as shown in fig (1).

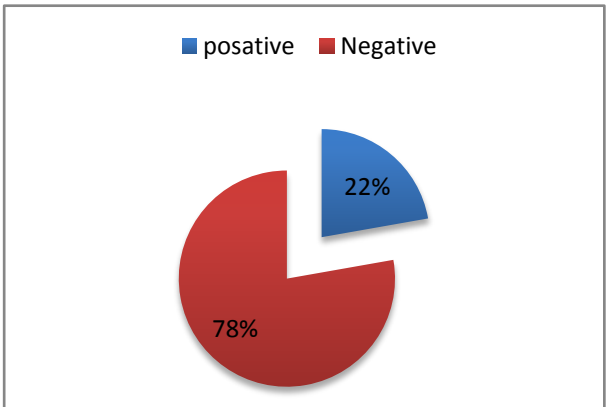


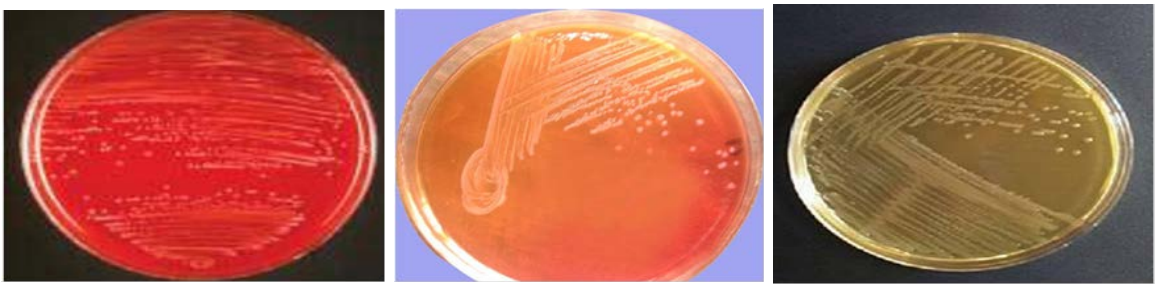
Figure (1): distribution of*Salmonella typhi*which are isolated

from blood culture of typhoid cases.

Blood culture is the gold standard diagnostic method for diagnosis of typhoid fever [12].But its sensitivity is poor due to various factors. The most important factor is the very few numbers of bacteria needed to cause severe infectionthe sensitivity of blood culture is highest in the first week of the illness and reduces with advancing illnesses [13].

Microorganisms may be found in bloodstream at any stage of the illness, but are most commonly found duringthe first 7-10 days and during relapses [9].Thepatient ifuntreated, blood culture is usually positive in about 75% during first week and decreasing15% - 26% later in the course of the disease [14].Inthis study we found that 50 ml of medium was adequate for 5ml of blood, presumably because of very low degrees of bacteremia in some patients [15].

morphological properties:- after 24 hrs of incubation at 37 C^{o}, the bacterium showed convex (2–4) mm in diameter, and smooth colonies.On Macconkey agar they looked pale due to their inability for fermenting lactose, but on Salmonella shigella (S–S) agar, they were colorless.On Blood agarS. typhi produce grey white 2-3 mm in diameter colonies as figure (2) bellow.



-A--B- -C-

Figure (2): Growth of Salmonella typhi: -A-on blood agar.B- on Macconkey

agar.C-on Salmonella shigella agar media.

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 are moderately large 2-3 mm in diameter after 24 hours at 370c.SS agar is highly selective medium formulated to inhibit the growth of most coli form organisms and permit the growth of species of Salmonella and shigella from environmental and clinical specimens. The high bile salts concentration and sodium citrate inhibit all Gram positive bacteria and many Gram negative organisms, including coliforms,Some strain produce mucoid colonies [16, 17].

Salmonellae require enrichment of the minimal medium with one or more amino acids or vitamins e.g, cystin or Nicotinamide; most S. typhi strains require tryptophan [18].

Microscopic Examination: -Grams staining was done for morphological identification of S. typhi,whereS. typhi was found to be Gram negative short bacilli see figure (3)[19].

Figure (3)showingGram negative short bacilli of Salmonellatyphi under the microscope

Biochemical Reactions:- The results of biochemical reaction of *salmonella typhi* showed in table (1).

Table (1): biochemical reaction of *salmonella typhi* isolate.

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

Key: (+) positive reaction; (–) negative reaction .

Identification using api20 E:- The results of api 20 system came to ensure the biochemical identification of *S. typhis* table(2) and figure (4)

Figure (4): API 20E results for *Salmonella* serotype Typhi.

Table (2): results of biochemical test of api 20 E system.

Test	result	Test	Result
TryptophaneDeaminase	-	Urease	-
Indol Production	-	H_2 S Production	+
Acetone production	-	Citrate Utilization	+
Gelatiase Liquefaction	-	B – Galactosides	-
Glucose fermentation	+	A rginineDihydrolase	+
Manitol fermentation	+	Lysin Decarboxylase	+
Lnositol fermentation	+	Decarboxylase	+
Sorbitol fermentation	+	Melibiose fermentation	+
Rhamnose fermentation	+	Amygdalin fermentation	-
Sucrose fermentation	-	Arabinose fermentation	+

Serological test

Widaltest :-The Widal agglutination test was introduced as a serologic technique to aid in diagnosis of typhoid fever.A total of the 180 clinically suspected typhoid cases examined in the period between march to the end of Februray 2016 for widal test , 105(58.3%) were positive for anti O antigen and anti H antigen. While 75(41. 6%) of samples were negativeas shown infigure(5).

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[14].The 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, paratyphi A and 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 [12].

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

Detection of Salmonella typhi antibody:-Principle of the test: Detection of S. typhi antibodyby

ICT is aqualitative test.By ICT, among 180 blood samples from the suspected cases, 120(66.6%) were positive forIgM of *S. typhi*..Among the ICT positive cases, it was found that 93(77.5%) cases had IgM antibody, 18(15%) cases had both IgM and IgGantibodyand only 9 (7.5%) cases positive for IgGantibody.

Among the three different test for the diagnosis of typhoid patients, Immunochromatography (ICT) test showed maximum 120(66.6%) positive result followed by blood culture 40 (22.2%)and stool culture 20(11.1%) (table 3).

Table (3) :- comparation between ICT , blood culture and stool culture of typhoid cases.

Study cases	N. (%) positive	N. (%) negative
ICT	120(66.6%)	60(33.3%)
Blood culture	40 (22.2%)	140(77.7%)
Stool culture	20(11.1%)	160(88.8%)
Total	180	

Age and gender of typhoid cases.

The age and gender distribution of the patients can be seen in table (4). The lower rate of typhoid cases incidence was among the age group 16-20 (25%) whereas the highest incidence was among the < 41 age group (2.5 %). Thetable also shows that the incidence washigher among males (55%) than that of females (45 %).

Table (4):- Age and gender distribution of typhoid cases.

Age group	Isolates		Male		Female	
	No.	(%)	No.	(%)	No.	(%)
1-5	2	5	1	4.5	1	5.5
6-10	3	7.5	2	9	2	11.1
11-15	9	22.5	4	18	4	22.2
16-20	10	25	6	27.2	5	27.7
21-25	4	10	3	13.6	2	11.1
26-30	3	7.5	2	9	0	0
31-35	3	7.5	1	4.5	1	5.5
36-40	3	7.5	1	4.5	2	
41-45	1	2.5	0	0	1	5.5
46-50	1	2.5	1	4.5	0	0
51-55	1	2.5	1	4.5	0	0
Total	40	100	22	55	18	45

The difference in distribution could be explained as a result of the nature of life style in our rural area and the peoples drinks water without any sterilization ,so the bacterial pathogens transmitted by water . The resultsRepresented by the table (5) follow.

Table (5) :- prevalence of salmonella isolation according to the residence.

Residential	No. of examination	No. of positive cases.	% of positive cases.	P value
Arban	85	12	30	<0.01 **

Rural	95	28	70	
Total	180	40	100	

** highly significant.

Figure (6) shows that the higher rate of typhoid fever which cause by salmonella typhi was during may followed by august and April while the lowest incidence in November.

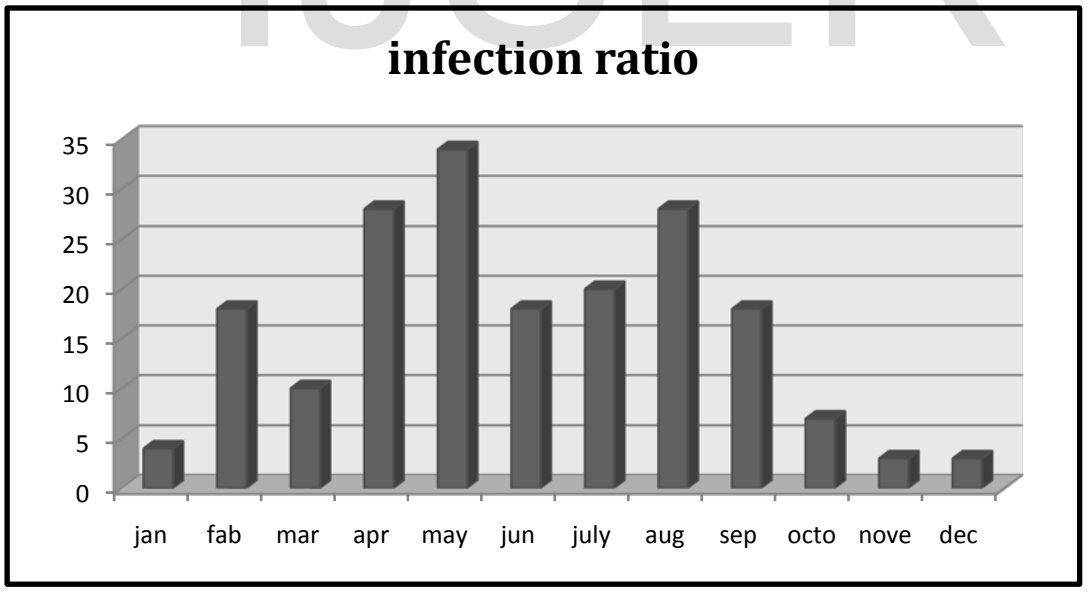


Figure (6): Distribution of infection rate according to the month ofyear.

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