Identification of salmonella typhi by serological and molecular tests isolated from blood

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Abstract

Background: Salmonella Typhoid Diagnosis by Widal Test and Polymerase Chain Reaction (PCR).

Objective: To study the Isolation and diagnosis of Salmonella typhi,diagnosis of the use of the widal test , diagnosis of Salmonella typhi) using PCR technique and Detection of Salmonella typhi.

Patients and Methods: The study included the collection of 120 blood samples, with 59.16% (71) and female patients (40.83%), 49 years of age (60-1 years), 24.16% of patients in hospital and 75.83% Patients who are not in hospital.

Results:The samples were initially identified using the Widal test as a traditional method of diagnosis and the rate was 75 positive and 45 negative, 20 blood samples were isolated from the suspected disease with typhoid fever, and the diagnosis of bacterial isolates was 17 Widal test (85% (P> 0.01) The isolates studied under the Polymerase Chain Reaction were detected as carriers of the Flic gene and 16.7%. Based on the emergence of a 599 bp package, a base pair of Nasted PCR was the size of 360bp base pair in the gel A significant difference was found (P> 0.01).

Conclusion:Serological methods have been shown to be less effective in laboratory diagnosis to diagnose typhoid fever. The molecular diagnosis of PCR can be relied upon as a more accurate diagnosis of typhoid fever infection than the bacterial isolates that own a Flic gene.

Key words: *Salmonella typh*i ,*Salmonella enteric serover* Typhi, Widal test ,Flic gene.

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Introduction

Typhoid fever Salmonella enteric serovar Typhi, one of the most common human pathogens and cause typhoid (Salmonellosis) which includes gastroenteritis and enteric fever and septicemia. These bacteria are important human nurses in all age groups but is more dangerous in children [1]. Salmonella enteric serovar Typhi bacteria belonging to the family Enterobacteriaceae it named relative to the world (salmon) which isolate Bacillus, (salmonella cholerae) from pigs in (1885) [2] .The typhoid fever the most important problems faced by health organizations and institutions in the world for being systemic infectious diseases (Systemic infections) [3]. The typhoid fever

(Salmonella enteric serovar Typhi) was negative-valued bacilli Does not produce capsule and consume citrate does not fermentation lactose and Sucrose does not produce indol and liquefaction gelatin and don't decompose urea produces H2S gas. Salmonella typhi bacteria has several most important physical antigens (O-Ag) with a sugary physical antigens nature (H-Ag) antigens intensity (Vi-Ag) with a protein nature [4]. Typhoid fever spread from the intestines to the blood stream and the rest of the body and may cause death if not redeeming fluid therapy and antibiotics and have babies more vulnerable to infection and those with arthritis and chronic diseases [5]. And highways to detect these bacteria is (Widal test) which is cheap and easy ways to use [6].

And other technologies that have underlying molecular biology, including PCR (Polymerase Chain Reaction) has proved to be a quick and quality and sensitive to detect live microorganisms (Microbiology) in various clinical models. [7] Typhoid fever was examined in our country, dramatically and most of the provinces of Iraq as well as triggering had been addressed in many ways patients. current study relied on a comparative study of causative diagnosis of patients (Salmonella enteric serover Typhi) by serologic methods In addition to molecular methods [8]. Flic gene is the gene that encodes the flagin protein whips in bacteria (Salmonella typhi) and diagnosed these bacteria infection [9].

PCR Nasted this type of specialized prefixes, and is composed of more than one part can link with more than one site on DNA objective. the initiator is designed to double the large-size piece of DNA cannot duplicate the other standard prefixes [10].

Therefore, the study dealt with the following axes:

1- Isolation and diagnosis of Salmonella typhi.
2- Diagnosis of the use of the widal test.
3- Diagnosis of Salmonella typhi) using PCR technique.
4- Detection of Salmonella typhi.

Patients and Methods

120 blood sample were collected from patients suspected infection by typhoid fever. (collected blood samples) from Baghdad hospital internal consultation/city of medicine, child protection, medical city hospital and hospital educational dignity. recorded information concerning patients or who are lying in hospitals such as age gender, sample source. Clinical samples included a blood planted immediately after taking for diagnosis. Followed [11] to isolate salmonella bacteria from blood, was traditional and modern ways as follows:

With drawal amount (2.5ml) for adults and children (2.5 ml) of blood, Separating serum from the blood sample drawn from patients where put in special tubes free of any anti-clotting substance and left for laboratory temperature (10-15) minutes, then abandoned by a central exclusion Centrifuge speed 3000 rpm for serum separation from the rest of the

ingredients to make : Check Widal test as a traditional way to diagnosis .

Laboratory diagnosis by Serological methoed:The test was used in the test, consisting of tubes containing Salmonella enteric Typhi antigens.

1- *Salmonella typhi* H
2- *Salmonella typhi* O

This selection depends on Agglutination obtained after mixing drops of the examination with a series of fear of the patient's serum, and to receive the vaccine for typhoid fever followed two methods.

1- Serum obtained after separation from blood cells by the use of the central evaporation device and speed (3000) cycle / minute for 10 minutes.
2- The serum is separated by vacuum tube steril (Gel & clot activater), which does not need centrifugation and does not require electrical energy. With the help of the silicon gel contained in the tube, the patient's sutures are separated after a few minutes of blood withdrawal.

(20µl) of the test material was added to a glass slide, then 20µl of the patient's serum was added and mixed with a stick. The slide was then moved for two minutes and the balance was observed. The correlation was positive, And do not get.

Means that the test is negative, and then a series of positive patient serotonin antibodies are used to identify antibodies in the blood of the patient against Salmonella typhi, where the following strains are taken (1:80, 1: 160 , 1: 320, 1: 640). A drop of each tube was added in the test kit to a drop of diluted serum and the slide was moved for 2 minutes. The resultant balance was then investigated and the antibody score was recorded when the test was positive at the highest dilution. [24], and the positive results for the VIDAL test were adopted as a screening test.

Molecular diagnostics

Bacterial DNA extraction:DNA extracted bacteria by the method described by the manufacturer for several reclamation and as instructed by the company (Promega) and was in sterile conditions as follows:-

1-Transfer 1 ml of bacterial isolates implanted the developing central 24 hours old. Brian heart infusion broth to (1.5) abendrov mL tubes and failed germ centrifuge for a minute 16000-13000 RPM speeds and pulling trapped by pipette and leave Deposit.

2-Added 300 microliter by lysis solution Pipet Nuclei of and mix the contents thoroughly by the mixer (Vortex) for 5 seconds and then incubated tubes in a water bath rocking (water bath) with temperature of 70 m for 5 minutes then left the pipe to cool to room temperature, then put in your package. Supermarket 5 minutes 16000-13000 RPM speeds for surfacing the content.

3-Add 3ml Solution RNase and mix content by a carburetor (Vortex) and incubated at 37 c for 15-60 minutes and then left to cool at room temperature.

4-Added the contents of tube 250 microliter from Protein Precipitation Solution and mix the contents thoroughly by the mixer (Vortex) and then incubated tubes in the

freezer for 5 minutes and then placed in a centrifuge for 10 minutes and 16000-13000 rpm speed to precipitate proteins.

5-Then pull the fluid in pipes and transferred to new tubes.

6- Added to the new pipeline diluted DNA 500 microliter of Isopropenol and then put in the freezer for 5 minutes to increase precipitation DNA and then placed in a centrifuge for 10 minutes 16000-13000 RPM speeds.

Polymerase Chain Reaction (PCR): Molecular diagnosis was made for bacterial isolates in the study by revealing Flic gene using polymerase chain reaction PCR and follow the following steps. Mix Master series of enzyme reaction mixture PCR polymerization preparation according to manufacturer's instructions Promga In the following steps.Solution of max master dissolved (2x) Green and pre record company equipped by hydrofoil (5) which consists of:

Taq DNA polymerase 2 U/ml

MgCl2 3Mm

Reaction buffer PH=8.5

dNTPs 400Um

User (PH = 8.5) at room temperature and blend in the mixer for his like before useprefixes solutions prepared for gene at room temperature and mix with a carburetor for smooth before use, and lotions have been added as shown in table 1. Mix contents well pipes using the mixer then put in your PCR reaction was performed multiplier as hypocracy [13]. of the bacteria salmonella. Were transferred (10). output of microlitr Diplo for posting. Then save the remainder of Diplo in degrees (-20).

Table (1): Sequence of the quality parameters of the Flic gene.

Source	Output size bp	Number of rules bp	Sequence of initiator 5`——3`	The initiator	N
(Khan *et al.*, (2012	599	20	F/5-TCT CAC ACA CCA TTG CA -3	*Flic*	1
		19	R/ 5-AGC AGG TTT ACC ATC AGA A -3		
	360	21	F /5-TGA ATT TCT GCC CTT CCC ATT -3	Nasted *Flic*	2
		21	R/ 35-GGT TCA GGG GTG ACA CCA TTT -		

* F= Forward / R = Revers

Table (2): The main interaction Mix Master mix components.

N	compoents	Size reaction 1	concentration	Size reaction 2
1	PCR PreMix	10µl	2x	l µ10
2	forward	l µ1	l µ10	l µ1
3	Reverse	l µ1	l µ10	l µ1
4	DNA	l µ2	50-100mg	l µ1
5	ddH_2O	lµ6		l µ7
	Final size	lµ20		l µ20

Table (3): PCR conditions first interaction.

Gene	Programing	Temperature	Time	No of cycle
Flic	Initial denaturation	95^0c	5min	1
	Denaturation	95^0c	30s	35
	Annealing	58^0c	30s	35
	Extension	72^0c	30s	35
	Final Extension	72^0c	7min	1
	Hold	4^0c	&	&

Nasted PCR for gene Flic: It is magnified and multiplied process DNA Molecular help to reduce background from layman amplification of DNA molecules [14], which can describe by the following steps: Add all substances (mix master, F(Forward) and R (Revese) primer, primers, and H2O) with the same concentrations prior to 1ml of PCR product for Flic gene.

Table (4): Second Nasted PCR reaction conditions.

Gene	Programing	Temperature	Time	No of cycle
Nasted *Flic*	Initial denaturation	95^0c	5min	1
	Denaturation	95^0c	30s	35
	Annealing	56^0c	30s	35
	Extension	72^0c	30s	35
	Final Extension	72^0c	7min	1
	Hold	4^0c	&	&

Gel Electrophoresis: Electric relay method using agaros gel of rapid methods used to separate DNA molecules used in different size and shape (15) as follows: The agaros has been prepared (2%) Add (1) g agaros (50) ml PVR (1X TBE). Hot agaros to a boil and leave to cool down to (50-45) ° c, then add (6) microliter of Ethedium promied the focus (0.5) micogram/ml.

The agaros has been prepared (2%) Add (1) g agaros (50) ml PVR (1X TBE). Hot agaros to a boil and leave to cool down to (50-45) ° c, then add (6) microliter of Ethedium promied the focus (0.5) micogram/ml. Then pour the coolant agaros quietly to prevent the formation of air bubbles in the backup plate Tray after installing comb for excavation (Wells) in the plate to load the samples. Leaving agaros for solidifies at room

temperature and for a (30) minutes to raise coolly comb of agaros horizontal migration unit plate installation of the basin used for electric relay, fill the tub then(TBE) buffer to cover surface of gel. transfer (7) microliter of the sample to be tested, And added (3-5) microliter of(Loading buffer) loaded into drilling. as for the matrix-Diplo (PCR) were carried over directly, that contains the migration dye Master mix pass electric current to voltage of 100 volts and (400) ampere per cm (45) Minutes after posting, check the gel using a spectroscope optical wavelength (265) nanometers. The gel was conceived using a digital camera filters for U.R rays and installed above the UV source UV-Tran illuminator.

Statistical Analysis

To complete the current research, the statistical program SPSS version 21 and Microsoft Excel 2010 were used in the graph. The statistical system has two basic parts:

1-descriptive statistics. A create statistical table and containing duplicates and percentage of arithmetic mean and standard deviation and standard and mis-minimum and higher (range). The graph includes the vertical shape and the curve (ROC curve).

2- Indicative statistics: A-test square Kai. Chi-Square test (22), B - Binomial test (Z-test), C-test Kolmogorov-Smirnov (Z), D-Test assay variation Analysis of variation (ANOVA) test & multi-comparison less significant difference (LSD) test. E-Validity Test Validity test:Sensitivity Sensitivity (%).Privacy Specificity (%) . Positive Predictive Value (PPV%). Negative predictive value (NPV%) Accuracy (%). Area under the curve (AUC). The value of the Cutoff value.

Results

The results were showed that positive tests 75 out of 120 in all samples in the study. The percentage of positive samples was 62.5% as shown in Table (5). The percentage of positive balance of Salmonella isolates was17(85%) were to be Salmonella enteric typhi, while the other isolates were other strains40 (85.1%) and finally isolates with no bacterial growth but positive results were obtained 18 (34%). ($p<0.01$).

Table (5): Widal test results depending on bacterial isolates.

Widal test			Bacterial isolation			Pearson Chi-Square Test (P-value)
			Salmonella typhi	Others	NO growth	
	Positive	N	17	40	18	P=0.00 Highly sign. (P<0.01)
		%	85%	85.1%	34%	
	Negative	N	3	7	35	
		%	15%	14.9%	66%	
Total		N	20	47	53	
		%	100%	100%	100%	

Molecular Identification

Flic, which encodes the flagellin, has been detected in the Salmonella enteric server Typhi, a diagnostic gene for the infection of the bacteria. It was designed according

to the modalities and the interactive program [9], in 18 isolates, 90% of the isolates included in the study , The detection results for the first interaction 3 isolates were carrying the gene Flic by 16.7%, while 15 isolates negative for gene (83.3%)With significant statistical difference at P = 0.00, P <0.01. The results of the polymerization sequence showed that it had a size of 599 base pairs when comparing the multiplying beams with the DNA Ladder, known for the molecular size and supplied by promega, where it was observed that the beam sizes were similar to the expected size.

The second reaction was Nased PCR for samples that had a Flic gene. It was also observed that there were three samples with this gene but with a smaller size of 360bp basal pair when comparing the multiplication packets with the DNA Ladder. Known as molecular sizes and supplied by promega. It is also noted that the beam sizes are similar to the expected size.

Table (6): Flic Genetics genetic screening test (PCR) test for bacterial isolates.

Genetics		Positive	Negative	Binomial (Z) Test (P-value)
Flic testl (PCR)	N	3	15	P=0.008 Highly Sign. (P<0.01)
	%	16.7%	83.3%	

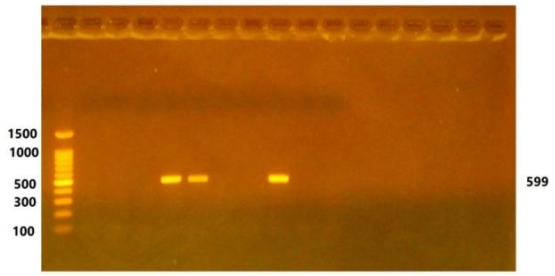

Figure (1): Electric relay to first interaction Flic enteric Salmonella bacteria gene.

Typhi by gel agaros 2% concentration containing 0.5 µg/ML of tincture of Ethidium promide using DNA ladder (100bp-1000bp), and voltage 100 v/cm 65 minutes, found tracks 4, 5, 8 a gene Flic either tracks 1, 2, 3, 6, 7, 9, 10 does not contain the gene.

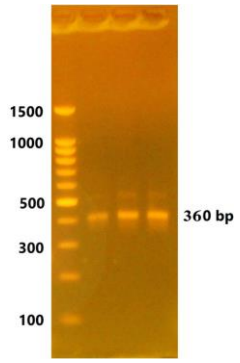

Figure (2): posting to the second interaction of Salmonella enteric bacteria Flic gene

Typhi by gel agaros 2% concentration containing 0.5 µg/ML of Ethidium bromide and dye DNA ladder (100bp-1000bp), and voltage 100 v/cm 65 minutes, found tracks 4, 5, 8 a gene Flic.

Comparison of the results of the Widal test on the basis of PCR

The study showed that there was no statistically significant difference (P = 0.502, P> 0.05) according to the results shown in Table (6). The Widal test gave a positive result for the three positive samples in the Flic-PCR test and 13 samples Negative for the PCR test but positive within the Widal test, while the negative result of the Widal test was also negative in the PCR test (2,13.3%). The PCR test is the most accurate test in the diagnosis of pathogenic bacteria, including typhoid fever. All stages of clinical tests used to diagnose typhoid fever such as the Widal test and increased incidence of typhoid fever led to efforts to find a way to diagnose these bacteria. Were Characterized by high speed, accuracy and sensitivity [22].

The reliance on traditional methods such as the Widal test, which is currently adopted in hospitals and government and private laboratories, depends on the antigen and antigen dependence, which depends on the appearance of antigen antibodies (H) within (8-6) days and the appearance of antigen antibodies (O) during 10-12 [23]. This results in delay in detection of infection and then the urgency of the disease. Therefore, the PCR technique takes only a few hours.

Table (7): Comparison of the results of the Widal test with PCR results.

Widal test		Fli-c (PCR) test		Pearson Chi-Square Test (P-value)
		Positive	Negative	
Positive	N	3	13	P=0.502 Non sign. (P>0.05)
	%	100%	88.7%	
Negative	N	0	2	
	%	0%	13.3%	
Total	N	18	15	
	%	100%	100%	

Discussion

The results of this study were different from the results of the study obtained by in [8].

The percentage of Salmonella isolates was 11.7% positive and was the Widal test, where it was not at the exact level of diagnosis of typhoid fever. Several studies have confirmed the sensitivity and specificity of the test (WID) test from one laboratory to another. This difference arises as a result of the different method of implementation and technique of this test and the interpretation of its results from one laboratory to another and from one town to another. This result is not consistent with the results of the researchers [17], [16] that the most serological tests are accredited in hospitals and the laboratory is a traditional test to determine the incidence of typhoid fever, which depends on the correlation between the antigens and antibodies found in the patient's serum. Determining the type of infection, whether acute or chronic, is the antigen antigen's antigen primacy and velocity. In the acute infection, the antigen (H) antibodies rapidly appear and then the antigen (O) antibodies appear and increase, disappearing after months, while antigen antibodies remain for a long time.

These results coincided with the results of [18], with 58.33% of the Salmonella bacteria containing 587bp. The present study coincided with the study of [9], in detecting a gene in negative bacteria. The percentage of Salmonella isolates containing gene was 5.26%. These results were consistent with the results of a study by[19], with a baseline size of 343bp. These results coincided with the results of a study by[20], with a baseline size of 364bp. These results coincided with the results of a study by[14], and a size of 366bp base pair. These results are consistent with the results demonstrated by[9], of the PCR test compared with the Widal test in the diagnosis of Salmonella enteric Typhi.

Conclusion

Serologic methods have been shown to be less effective in laboratory diagnosis to diagnose typhoid fever. PCR can be relied upon as a more accurate diagnosis in the identification of typhoid fever from bacterial isolates that own a Flic gene.The average age of persons (20-40 years) is the most vulnerable age group.It was observed that the highest incidence of infection in males is female.

Recommendation

1-Adopting modern and rapid methods for diagnosis of typhoid fever bacteria in periodic testing using more efficient Widal test techniques as a PCR technique for all food preparation workers in restaurants to ensure that they are free from the pathogenic bacteria.

2-The use of molecular methods (PCR) to diagnose serological patterns of Salmonella enteric server Typhi bacteria found in the country.

3-Conduct prospective studies on other diagnostic genes for Salmonella bacteria.

4-Studying the genetic sequence of genotyping and sequencing to accurately

identify the epidemiology of bacteria and their various sources.

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