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SEROPREVALENCE AND MOLECULAR METHODOLOGY FOR IDENTIFICATION OF *SALMONELLA TYPHI* AT UNIVERSITY

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Abstract

Salmonella typhi and *paratyphi* A serological diagnosis for 125 university students, including a Widal test and then a molecular method for 18 isolates of *Salmonella typhi*, is the result of serological diagnosis and finally the genetic method used to ensure the result.

These include various steps to first collect samples (125 cases) and then use the Widal test for diagnosis of both bacteria involving typhus and Para typhus A than the molecular method also used, including PCR, Gel electrophoresis among students for cases of *Salmonella typhi* infection.

Collect samples from students in the Department of Biology at Zakho University about 125 cases. First, the maximum percentage among age groups is typhoid cases (21-20 years), which is smaller cases in students, about 6 (4.8%), especially in male cases, 3 (2.4%) female cases, Furthermore, typhi cases more than paratyphi A cases by (7.2,5.6%) respectively so that the total number of infected cases already increases by 18 cases infected with typhi and 11 cases infected with paratyphi because of this the total number of infected cases already increases by 18 cases infected with typhi and 11 cases infected with paratyphi .Based on the emergence of a 599 bp package, a base pair of Nasted PCR was found to be 360bp base pair in gel A substantial difference (P>0.01).

There is a growing incidence of *Salmonella paratyphi* A and *Salmonella typhi*, might attributed to variety of factors. Serological methods have been shown to be less effective in laboratory diagnosis to diagnose typhoid fever. The culturing method, biochemical tests and 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 enterica serovar Typhi, Widal test, Flic gene, PCR, Gel electrophoresis.

Introduction

Typhoid fever is a chronic condition of infection representes with typical manifestations of headache, fever, abdominal pain, etc. (Shen *et al.*, 2007; Hanan, 2016). *S. Typhi* caused typhoid fever mostly by followed by *S. Paratyphi* A. Human-adapted Salmonella enteric, serovar *Paratyphi* A, B and C, cause one or two cases of paratyphoid fever for every 10 cases of *Salmonella typhi* infection. Since the clinical course of typhoid fever does not distinct from paratyphoid fever, collectively of *Salmonella enterica* serovars Typhi, Paratyphi A, B and C are denoted to being typhoidal *Salmonella* serovars (Kumar *et al.*, 2013). *S. Typhi* is the typhoid fever etiologic agent. It is a major cause of morbidity in many parts of the world and is also one of the most common human diseases, causing salmonellosis, including gastroenteritis, enteric fever and septicemia (Yoon *et al.*, 2009). It belongs to the Enterobacteriaceae family and is named in (1885) relevant to the (salmon) world, that isolates (salmonella cholera) from pigs (Quinn *et al.*, 2002). This bacteria is negatively regarded bacilli Does not create capsules and eat citrate does not cause lactose fermentation, and Sucrose does not generate indol and gelatin liquefaction and does not disintegrate urea generates H_2S steam. Several major physical antigens of *Salmonella typhi* such as O antigen with a naturally occurring sugar physical antigens H antigen, Vi-Antigen strength with protein nature (Farhan *et al.*, 2018). Typhoid fever epidemiology that spreads to the bloodstream from the intestine and the rest of the body and might be leading to death (Zhou

and Pollard, 2010). Widal test easy to detect *Salmonella typhi*, which is simple to diagnose and cheap (Dieffenbach and Dveksler, 2003). Molecular biology such as PCR technique is sensitive and quick methods for detecting microorganisms in various clinical forms (Higgins and Hames, 1994). In my country, many ways used to diagnosis of Typhoid fever, the patient treated dramatically and most regions of Iraq as well as triggering. The current study used the comparative causative diagnosis of infection (Forbes *et al.*, 2002). Flic gene is the gene that encodes and identifies flagin protein whips in bacteria (*Salmonella typhi*) (Kumar *et al.*, 2012). PCR This type of standardized prefixes has been clustered and consists of more than one component that can be connected to more than one DNA target site (Hamzah and Hasso, 2019). The initiator is programmed to double DNA piece, the other generic prefixes cannot be duplicated (Khan *et al.*, 2012), the research, therefore, dealt with the following axes, using different methodologies to classify *Salmonella typhi* and *paratyphi* A.

Material and Methods

Collect specimens (125 cases): This research was carried out at the University of Zakho and the University of Polytechnic Erbil. Work began with the drawing of blood approximately (3ml) for both genders, including two different samples, age plus gender, then age associated with *Salmonella typhi* and *paratyphi* A. Works continuously with the Centrifuge blood sample separating serum at speed 6000 rpm

Laboratory diagnosis by Serological method:

The test consisting of tubes Serum obtained by centrifuge, (20µl) of the test material was added to kits *Salmonella typhi* O and *Salmonella typhi* H used white plate to clearly show agglutination reaction this work for identify *Salmonella typhi* after that *salmonella paratyphi* A diagnosis by take 20µl of the patient's serum was added to kits special for this bacteria and mixed with a stick used same plate in previous step. The contents were moved for two minutes and the result was observed by agglutination reaction, the amount of serum plus kit calculates by micropipette.

PCR and Gel electrophoresis:

Molecular methods including DNA extraction: bacterial DNA extraction using technique explained by the manufacturer for multiple reclamations and as the company instructed (name of company QIAGEN), applied sterile conditions as follows:- A- 1 ml of isolates cultivated bacteria in the central growth for 24-hour in Brian heart infusion broth was transferred to (1.5) mL abandorof tubes, centrifuge of germ 16000-13000 RPM speeds about one minute, then by pipette, picking stuck and leaving precipitate. B- 300 µL of lysis solution was added and the contents mix by Vortex, then used the water bath to incubated of the tubes at 70 m temperature for five minutes then in the room temperature, let cool the pipe and placed in the box. Then 16000-13000 RPM velocities for 5 minutes to surface the content. C- Add 3ml RNase solution and combine the material by Vortex and incubate at 37 for 15-60 min. D-Added 250 µL tube contents from Protein sediment solution and contents mixed by Vortex, then the tubes incubated in for 5 minutes, then precipitate proteins centrifuge in 16000-13000 rpm for 10 minutes. E - Bring the water into the tubes and move to new pipes. F -Added DNA 500 microliter of Isopropanol to the new pipeline and placed 5 minutes in the freezer to increase DNA precipitate, then centrifuged 16000-13000 RPM for 10 minutes. PCR: In the study, bacterial isolates diagnosed by molecular methods was made by exhibition of Flic gene using PCR technique in the polymerase chain and following steps. Master Mix, polymerization enzyme mixture PCR preparation in compliance with Qiagen Company instructions from the manufacturer in the as steps. Master max solved solution (2x) Clean and pre-record hydrofoil ready business (5) Consisting of: Taq DNA polymerase, Reaction buffer PH=8.5 400Um User (PH=8.5), 2 U / ml $MgCl_2$ 3Mm and dNTPs at room temperature and mix before using prefixes of gene-prepared solutions at room temperature and mixing by carburetor, then added lotions as in Table 1. Mix well contents the mixer then placed the reaction

Table 1: show structures of both sequences.

Source	Output size bp	Number of rules bp	Sequence of initiator 5-3	The initiator	N
Internet Gen BANK	599	20	F/5-TCTCACACACCATTGCA-3	Flic	1
		19	R/5-AGCAGGTTTACCATCAGAA-3		
	360	21	F/5-TGAATTTCTGCCCTTCCCATT-3	Nasted d Flic	2
		21	R/35-GGTTCAGGGGTGACACCATTT		

F=Forward / R=Reverse

Nasted PCR for Flic gene: Add to Flic gene all substances (mix master, F (Forward) and R (Revese) primers, primers, and H2O) in a similar concentrations before 1ml of PCR.

Table 2: The core elements of the Mix Master Mix interface.

N	Compo-nents	Size reaction 1	concent-ration	Size reaction 2
1	PCR Premix	10µl	2x	1µ10
2	Forward	1µ1	1µ10	1µ1
3	Reverse	1µ1	1µ10	1µ1
4	DNA	1µ2	50 -100mg	1µ1
5	ddH20	1µ6		1µ7
	Final size	1µ20		1µ20

Table 3: First contact with PCR conditions and second Nasted PCR.

Gene	Programing	Temperature	Time	No of cycle
	Initial denaturation	950c	5 min	1
Flic and Nasted flic	Denaturation	950c	30 s	35
	Annealing	56 0c	30 s	35
	Extension	72 0c	30 s	35
	Final extension	72 0c	7 min	1
	Hold	4 0c	*	*

of PCR were multiplier performed as bacteria hypocrisy. Store it then in degrees (-20).

Gel Electrophoresis: Electrical relay technique with agaros gel consider quick technique used to DNA molecules separation of different sizes and shapes (15) as follows: Add (1)g agaros (50) ml PVR (1X TBE) to the agaros (2%). Warm agaros to cool down to (50-45)°c, then add (6) Ethedium microliter promised concentrate (0.5) micogram / ml. Add (1)g agaros (50) ml PVR (1X TBE) to the agaros (2 percent). Warm agaros to cool to (50-45)°C, then add (6) Ethedium microliter promised concentrate (0.5) microgram / ml. To prevent air bubbles, pour the agaros coolant quietly to forming in the Tray backup plate after placing the comb for (Wells) in the plate to fill the samples allowing agaros to solidify at room temperature, for (30) minutes to increase the agaros cool comb migration horizontally, installation of unit plate used for electrical relay, then fill buffer (TBE) to cover the gel surface. Pass (7) microliter of the specimen to be tested and insert (3-5) microliter packed into the drill. As for the matrix-Diplo (PCR) were done directly test, (265) nanometers optical wavelength of spectroscope are using, which includes the dye migration, Master mix pass electricity about 100 volts and 400 ampere per cm (45) minutes after publication. The gel was equipped with U.R. lens filters and mounted above the UV-Tran light source.

Statistical analysis

The data were executed using the Social Sciences Statistical Package (SPSS), version 18. As frequencies and percentages, all variables are expressed.

Results

Serological test (Widal test): this test was performed for 125 blood samples from students including first study age associates with the gender of the results shown in Table 3. Typhoid cases were the highest in the age group (21 years and below) 6 (4.8%) for male cases higher than females, about 3(2.4%) calculating by total percentages. Clearly highlights the Table below.

Table 4 shows the rate of *Salmonella typhi* with *paratyphi* A among same age: from the table 5 the higher number present among (21 year and below) groups also for both types of bacteria but what is the most important thing from this table 6 that are typhi cases more than paratyphi A cases about 9, 7 respectively so because of this reasons the total numbers of infected cases already increases by 18 cases infected by typhi and 11 cases infected by paratyphi A these percentages results from variety of factors.

Table 3: age distribution of *Salmonella typhi* among gender.

Age group	Numbers of isolates			
	Male		Female	
	No	%	No	%
20-21 years	6	4.8%	3	2.4%
21-22 years	4	3.2%	2	1.6%
23 year and above	2	1.6%	1	0.8%
Total	12	9.6%	6	4.8%

Table 4: Age distribution of *salmonella paratyphi* A and *Salmonella typhi.*

Age group	*Salmonella typhi*	*Salmonella paratyphi* A	Total
20-21 years	9(7.2%)	7(5.6%)	16
21-22 year	6(4.8%)	3(2.4%)	9
23 year and above	3(2.4%)	1(0.8%)	4
Total	18(14.4%)	11(8.8%)	29

Molecular Identification:

The positive result of *Salmonella typhi* among students send to the genetic lab for molecular methodology do it including PCR, Gel electrophoresis after that they make comparison between the results of serological test and molecular methodology. *Flic* gene Which encodes the flagellin (H antigen) detected in the Typhi enteric server of Salmonella, a bacterial infection diagnostic gene. Accordance to the modalities and interactive programmed it was designed (Shanahan *et al.*, 1998), in 18 isolates, The detection results for the first interaction 3 isolates contained 16.7 percent of the *Flic* gene, while 15 isolates were negative for the gene (83.3 percent). The sequence results showed that the sizes of beam were similar to the

expected size, when comparing the DNA Ladder to the multiplying beams, informed for the molecular size and supplied by QIAGEN Company, it had a size of 599 base pairs, where it was found that the beam sizes were similar to the expected size. In specimens that had a Flic mutation, the second reaction was Nasted PCR. It was also found that when multiplication packets comparing to the Ladder of DNA, with this gene, there are three 360bp samples smaller size. Registered and supplied by QIAGEN business as molecular sizes. It is also noted that the size of the beams is close to the size predicted.

Table 6: highlights these items: showing the results.

Gene	+VE	- VE	*p*-value
Flic test (PCR)	3	15	*P*=0.008 highly sign
	16.7%	83.3%	*P*<0.01

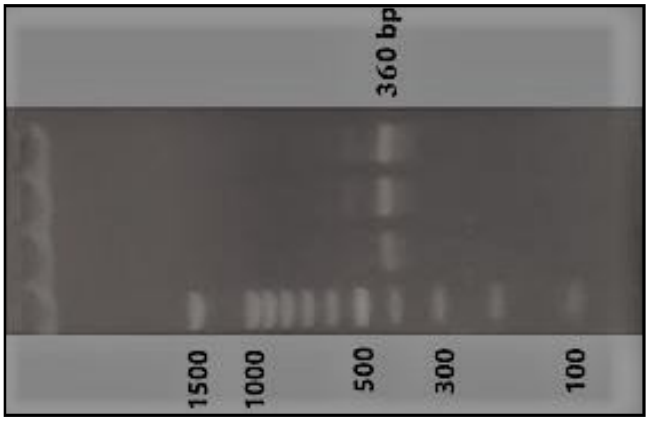

Fig. 1: Electric relay of enteric Salmonella first interaction Flic gene.

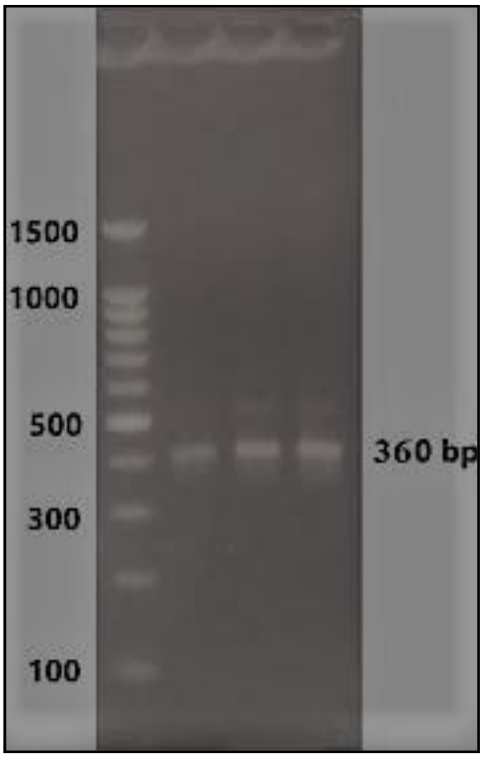

Fig. 2: Presentation of the second association of Flic gene Typhi of Salmonella enteric by 2% agaros, 0.5 µg / ML containing of Ethidium promised using, voltage 100 v/cm 65 minutes, DNA ladder (100bp-1000bp), finding tracks 4, 5, 8 of the gene Flic either tracks 1, 2, 3, 6, 7, 9, 10 does not contain the Typhi gene by 2% agaros containing 0.5 µg/ML of ethi.

Discussion

Enteric fever is a concern for public health throughout the state. Males with M: F ratios of 2.3:1 were more infected than females. This could be because of cultural background, where males are more hospital report, while being more susceptible to become infected because of outdoor activities (Parande *et al.*, 2011). This results agreed with (Sood and Taneja, 1961; Khosla *et al.*, 1995). Health Majority (4.8% percent) of cases infected by *Salmonella typhi* among males was only about (2.4%) in female cases especially in the age group 20-21 years,. Enteric fever possible being prevalent in this age group and in males include in general, mobility of them, take of unhygienic water and food in colleges, our culture in Iraq country which always includes man responsible for outdoor activity. Health Education is an critical role in this age, as low educational status and illiteracy is correlated to ignorance, especially in their house, poverty and poor personal hygiene also play an important role. These observations were consistent with various studies (Walia *et al.*, 2006; Kanungo *et al.*, 2008; Parande *et al.*, 2011). Occurred of Enteric fever cases in the our sample year, which suggests that there has been little change in drinking water protection and sanitation over the time or that a carriers are present in large number in society (Aggarwal *et al.*, 2007). *Salmonella typhi* cases ranged from (7.2%) in 21 years and below the other age, but in the case of paratyphi A, the rate of typhi is less than (5.6%) below that of paratyphi A. The rise in the proportion of cases of *Salmonella typhi* may probably be due to a high degree of medical concern with mild fever cases being treated for enteric fever, changes in host sensitivity, improvements in organism virulence and extensive use of *Salmonella typhi* vaccines and quinolones over the past decade (Gupta *et al.*, 2009). 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

1- There is a rise in the occurrence of Salmonella typhi and Salmonella paratyphi A due to improved testing methods or increased drug resistance.

2- The parents educational status, the mothers particularly, must be improved through classes in education of adult. In the rainy season, more reports were seen, which could be attributed to inadequate

sanitation and drainage.

3- The *Salmonella Para typhi* A and *Salmonella typhi* proportion of cases is nearly equal, which might due to a higher degree of medical concern with mild cases of fever being treated for enteric fever, improvements in host sensitivity, and changes in organism virulence, and extensive use of Salmonella typhi vaccines over the past decade.

4- 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

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