Molecular Investigation of *fliC* and bla_{TEM1} Gene among Clinical Isolates of *Salmonella Typhi*

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Abstract

This study aimed to isolate and diagnose *Salmonella typhi* by *flic* gene and investigated the presence of bla_{TEM1} gene by using PCR technique and electrophoresis systems in Najaf Province includes 40 clinical specimens blood for both sexes with an age ranged between)5-60) years old .The study result by using a *flic* gene was detected in 30 of the 40 isolates of *S. typhi* isolated from typhoid patients , which was conducted in the diagnosis of bacteria concluded that the technique PCR are the most sensitive in the detection of *S. typhi* compare with other way, bla_{TEM-1} was detected in 16 of the 40 isolates from *S.typhi* . The conseder the bla_{TEM} gene encoding TEM-1 b-lactamase is think to give resistance only to penicillins and early cephalosporins; thus , the spectrum resistance of TEM-1 descendants may extend to second, third and fourth-generation cephalosporins.

Keyword: *Clinical cases; PCR; health diagnoses; S.typhi, typhoid ,*bla_{tem1} .

Introduction

Typhoid fever is an acute, potentially fatal systemic disease caused via *Salmonella enterica serovar typhi* , *paretyphi,* pathogens only precise to people. *S.typhi* is a genus of rod formed gram-negative enterobactereaceae that cause fever typhoid [(1)] . This pathogen has develop marked mechanisms for persistence in its host that assisted to include its transmission and survival. *S.typhi* can be transmitted via ingestion of water or food contaminated via urinary carriers excreting *S. typhi* [(2)]. PCR have been arise as a salutary process for the diagnosis of several diseases infectious lately. value diagnostic is important precisely wherever the a long period cultivation or little load bacterial . PCR the test consistent in the diegnosis & the administration of the illness typhoid [(3)].

Methods and Materials

The study was done at Laboratories of Molecular and Bacteriology in Departmant of the Biology , Faculty of Sciences, University of Kufa , Iraq.

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Specimens collection and bacterial identification

A total of 40 samples were collected from blood with clinical suspicion of typhoid fever in Al- Najaf provenance, was involved the study fundamental, of contenuous rise-grade fever with temperature medium - 38°C . The duration median of disease at consultetion at 6 days (about 6 to18 days) [(4)]..

Blood sample was collected from patients , four ml of blood venous fresh samples was collacted of doubtful typhoid patients via syringes sterile. The blood was delivered in to screw cupped having 30 ml of brain heart infusion broth and placed in bact/alert 3D apparatus for a week .If positive sample , each sample was protected utilizing the process direct of inoculation on culture of discerning means namely MacConekey, Blood ,XLD and S.S agar thereafter, inoculated at 37°C for 18 to 24 hr [(5)].

DNA Extraction

Genomic DNA was extracted via utilizing the extraction commercial method(Genomic DNA promega Kit).

Molecular Identification

The PCR assay was performed to detect the *bla-tem1* gene and *flic* gene for identification of *Styphi* as appear in table2 This primers were designed via Alpha DNA company, Canad as in table (1) . the produces amplified ware curtained utilizing 1% agarose gel electrophoresis to assessment the size produces for PCR . The gel was tainted with 10mg/mL of 4 µL bromide ethidium (Sigma, USA) and it run at 80v for 1.5h. A single band observe at the position desired on ultraviolet light transellumintor (Cleavar, UK); bands were photogrephed utilizing documentation gel method (Cleaver, UK). A 100bp and 50bp ladder (Bionaer, Korea) was utilized to quantity the Mwut. of amplified produces [6].

Table (1): Primers utilized in the study

type Primer	Primer target	Primer sequence	Amplicon size	Reference
Flic-d	Flic-d	F- ACTCAGGCTTCCCGTAACGC R-GGCTAGTATTGTCCTTATCGG	763	(6)
blaTEM-1	blt	F-CCCCTATTTGTTTATTTTTC R-GACAGTTACCAATGCTTA	962	(6)

Table (2): PCR program of *flic-d* and bla TEM-1 primers that apply in the thermocycler

Gene	Primary denaturation	Number of cycles	Deneturation	Annaeling	Extansion	Final extansion
Flic-d	95 Cº for 5minute	35	95 Cº for 30sec.	55 Cº for 30sec.	72 Cº for 40minute	72Cº for 5minute.
blaTEM-1	94 Cº for 5 minute.	32	94 Cº for 1 minute	51 Cº for 1 minute	72 Cº for 1minute.	72Cº for 7minute.

Results and Discussion

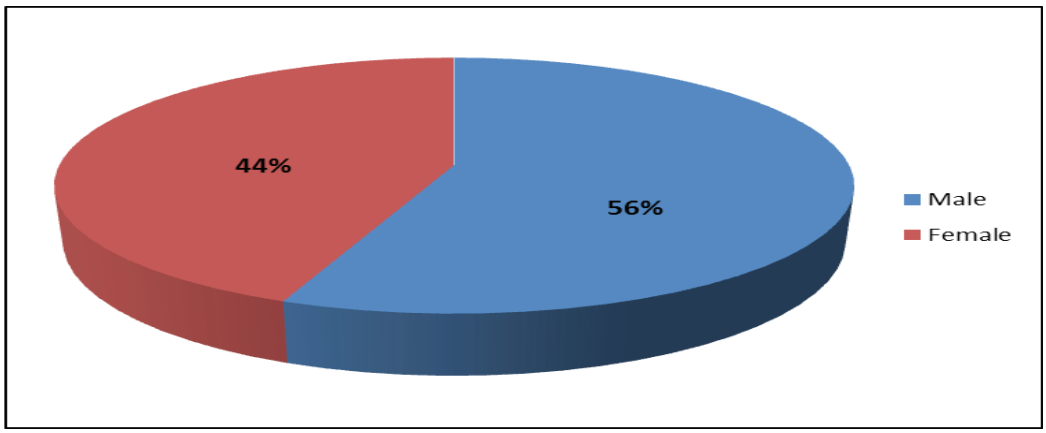
This study was conducted on 40 sample from typhoid blood of doubtful patients through the interval since Dec.2015 to Sep.2016. indicated the results that clinical sample were 40 sample.

The diagnosis clinical depended on the existence, of several symptoms like nausea , headache ,anorexia , fever and vomiting , discomfort abdominal with constipation and diarrhea for 6-18 days.

In 40 patients, 30 (75%) were male and 10(25%) were female as in figure(1). Males were found to be more infected than females. The result is similar to extra results of study [7] and Diyala [8]. This might be because 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. .

Butler (2001) the utmost exposition of male to polluted, food and water out - side the home may be part

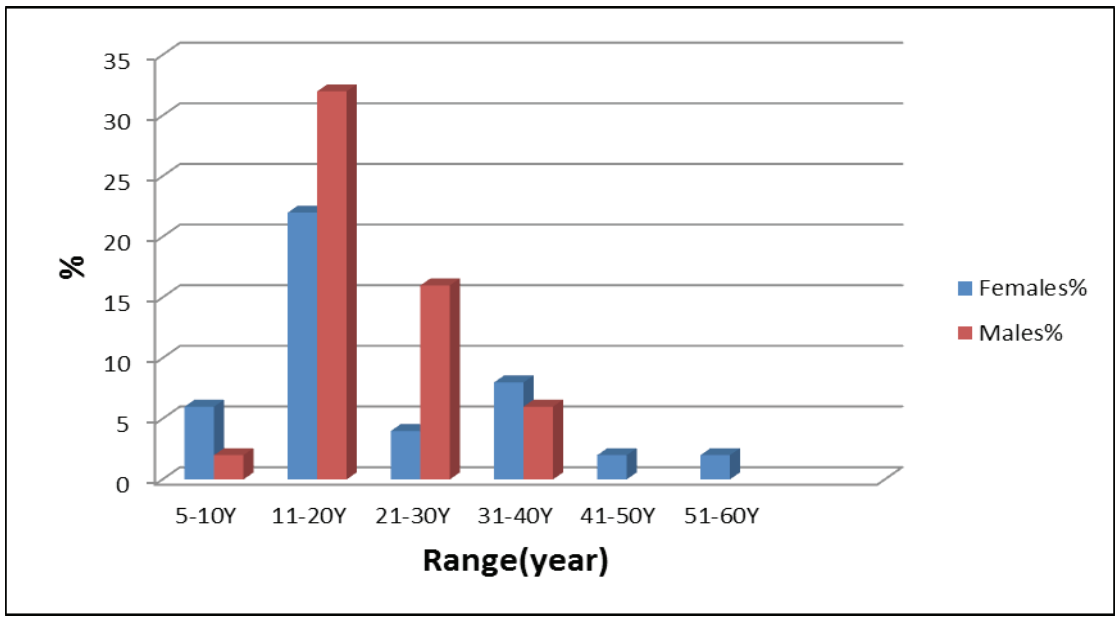
of higher rate of contagion between this population[9]. Fever typhoid is an contagion caused foremost via S. typhi and is conveyed by the oral route or the consumption of polluted, eggs , poultry , meat and milk [10].



Figure(1): Distribution of infected patients according to sex

The patient's age ranged from (5-60) years old. The age scattering patients appear in figure (2). The top incidence between about (41-50) and (51-60) age group 2% and 2% respectively, while the top incidence was between the (11-20) age group (54%) and (21-30) age group (20%).The disease affects all ages, 8(8%) of the study specimens was in the age group of 5-10 lead to *S. typhi* contagion between children due to the immature immune method .

These results are similar to the previous studies of [11;8]. This perhaps due to the mobility, exhaustion of unsanitary food and water in colleges and school [12] While few cases were in age groups (41-50) and (51-60) years old related to frequent boosting of immunity .



Figure(2):Distribution of infected patients according to age and gender

***Salmonella typhi* confirmation use PCR**

The technique of the Polymerase chain reaction for S. typhi clinical isolates detected with product 763 bp that exemplify the fliC gene as appear in fig. (3) .In theis study, used PCR to reveal fliC gene as 30(75%) isolates positive result .This result is associated with Khan et.al.,(2012) who found due to 80 cases doubtful ty fever phoid , detect (fliC-d) via PCR in 56 (70%) cases which agreement at similar effect of study done in Bangladash wherever PCR was positive in 88.7% of cases doubtful

fever typhoid .The rate positivity of PCR is 65%- 71.9% in several studies [13]. A flagellum has 3 basic kind: the farthermost and longestkind is a filament which included about 20,000 subunits protein of a single protein called (Flic) with a Mwut. of 50 to 60 KDa [14]. The gene *fliC-d* encode for the synthesis of H (flagellar) antigen. This antigen form the basis of classification for Salmonella via Kauffman– White scheme [15-16]. in the study useing utmost gene flagellin as a molecular technique for discovery of Styphi in clinical sample. The method alternative wherever Via B region is targeted give the result positive false caused by the existence of this sequence in S. Paretyphi . Flagelle are thin, appendages rigid of bacteria, and structures bactereal locomotive [17].

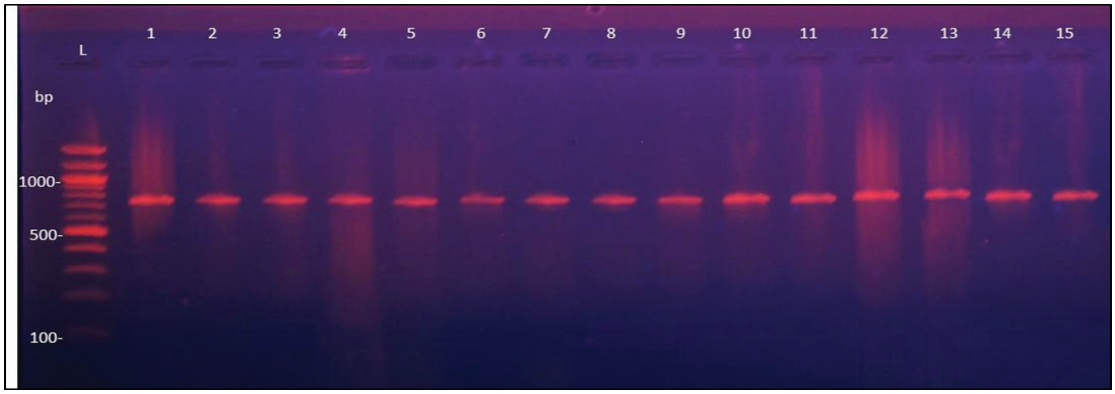


Figure (3): PCR amplificetion produces of *S.typhi* isolates that amplified with *fliC* gene primers with produce 763 bpLane (L), DNA size molecular marker (100-bp ladder), 20 specimen appear results positive with *fliC* gene

Molecular Detection of (ESBLs) Producing Isolates

S.typhi isolates were further investigeted to limit the occurrence and kinds of (ESBLs). However, only gene in the families of TEM was observed in the found study. The (*bla*TEM-1) was detected via PCR technique as in fig. (4). Revealed results the 16(40) isolates ,*S.typhi* transmit *bla*TEM-1 genes. Other study these genetic elements are either chromosomal and/or extrachromosomal in the form of plasmids. The present study was conducted to investigation of the presence antibiotic-resistance genes[18]. Ampicillin resistance in salmonellae may be due to enzyme βlactamase that breaks down the structural B- lactam of penicillin ring and derivatives the synthetic .The property of stability to many bacterial Blactamase increased with the later generation of Cephalosporins. These enzymes are most usually produced via Klebsiella spp and E.coli. but too occur in other bacteria gram negative like Salmonella, Citrobacter, Pseudomonas aeruginosa ,Proteus, Shigella dysentriae etc.. Though not all S. typhi isolates were ESBL reproducer but presence of TEM-1 (encoded via blaTEM-1) have been described to have clinical inclusion because the Blactamase is known, as the progenitor to many spectrum-extended Blactamases and inhibitor-resistant Blactamases .Mechanisms of resistance include not only production of ßlactamases, but also alterations in (PBPs) and reduced entry and efflux active of the antibiotic.

Although most of the strains of Salmonella , P. mirabilis, and Shigella so apted to ampicillin when utilizer in the early 1960s, and percentage increasing of the kind is resistant now. strains the resistant of Salmonella (plasmid mediated) had been recovered with increasing frequency in several the world. regrettably, ampicillin is hydrolyzed easily via spectrum broad ßlactamases and found with frequency increasing in isolates clinical of these bacteria gram-negative . In salmonellae, ampicillin resistance is maybe due to tem-β-lactamase [18].

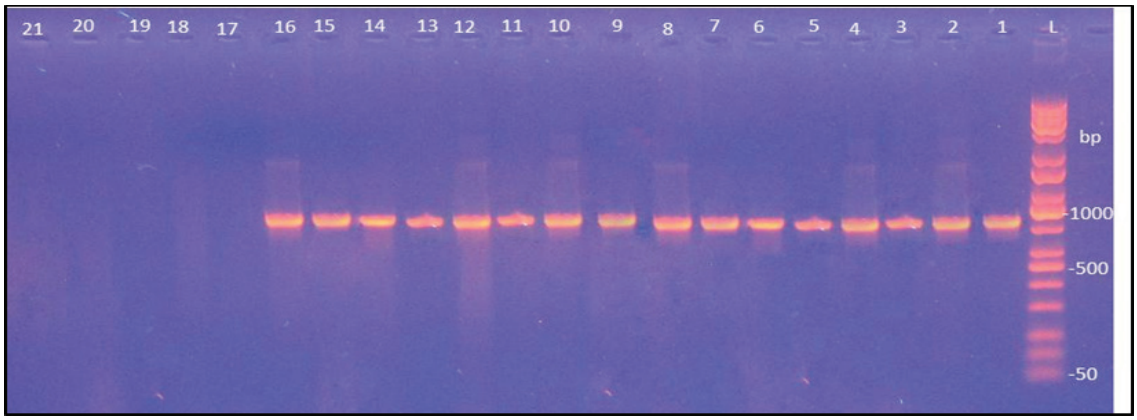


Figure (4): PCR products expansion of Styphi isolates that expand with blaTEM1 primers gene with produce 962 bpLane (L), DNA size molecular sign (50-bp ladder), Lanes (1 to16) appear the results positive with *bla*TEM1 gene, Lanes (17,18,19,20,21) show negative results with *bla*TEM1 gene.

Conclusions

1-*S.typhi* been recognized as a major public health problem,especially among typhoid patients.

2-High prevalence of ESBL producing *S.typhi* isolates were detected

3-The gene FliC-d was a specific gene for disclosure ofS. enterica serovar typhi among via the laboratories diagnostic.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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