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Detection of some virulence factors of *Salmonella typhi* isolated from patients' blood by PCR and Phylogenetic tree

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ABSTRACT

Typhoid fever is brought about by *Salmonella enterice serovar typhi*, which is a significant general medical issue in many developing nations. The severity the pathogenesis depends on *Salmonella's* possession of cytolethal distending toxin (CDT) and virulence factors such as fimbriae adhesions, which are important in the adherence, invasion and the development of typhoid fever, was as diagnosed serologically as well as diagnosis of *Salmonella typhi* causing these fever based on phenotypic and cultural characteristics. Therefore, the coding genes of CdtB protein and fimbriae were detected in molecular methods by PCR technique using special primers. while, the *fim* gene was 84.21% and *CdtB* gene was 100%. DNA sequencing was performed and this confirms the isolation obtained in our study. In addition, the phylogenetic tree was analyzed and registered at the gene bank site, where the sequence identity rate *fim* gene 99.26%, while sequence identity rate for *CdtB* gene was 99.31%.

Keywords: Salmonella typhi fim gene and CdtB gene, phylogenetic tree and genome sequencing.

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1. Introduction

Salmonella species enter their human by the intestinal area and irritated the mucous membrane of intestinal tract [1], the reason for typhoid fever, a fundamental, hazardous infection of people [2-6]. S.typhi is etiologic specialist, can be transmitted through tainted nourishment and water by means of the oral-fecal course. Yearly, more than 21 million cases and almost 200, 000 passing's are accounted for around the world [7]. The internal compositions of S.typhi, such as the fatty polysaccharides, pili and surface are influence the invasion factors of the bacteria [8]. The different kinds bacterial utilize comparative contamination methodologies because of the securing of assorted pathogenicity islands, a locale should convey qualities encoding at least one invasion factors for example adhesions, enzymes, and attacks, pathogenicity islands are found on the pathogen genetic material or on a plasmid and convey functional genes for DNA recombine, for example, integrase, transposase, or part of an insert element [9]. Multiple survival strategies allow S.typhi Caused typhoid fever disease outbreaks in different developed nations [10].

The Fimbriae are proteinaceous extracellular structures chiefly associated with attachment, an essential beginning advance for migration and passage into have cells, fimbriae have additionally been appeared by way of macrophages, intestinal ingenuity, biofilm development and bacteria collection in other Salmonella serovars [11-14]. The imperfection in swimming might be expected through abatement in development level or By over-expression of fimbriae fim, S.typhi pathogenesis, can be a significant role and other fimbriae and invasion factors, such as flagella, LPS, it can be controlled [15]. Bacteria S.typhi also contains of cytolethal distending toxin, the (CDT) consists of three subunit [16]. CdtA and CdtC structure a heterologous B subunit this is vitality for the conveyance of CdtB, the dynamic or A subunit [17].

The structure of act for toxin is sensibly surely known, on conveyance to have cells by CdtA and CdtC, the dynamic subunit CdtB is distributed to the center where it causes genome harm [18,19]. The CDT is coded through operon made out of the cdtA, cdtB, and cdtC genes [20,21]. The ongoing assurance of the whole nucleotide succession of the genomes of double single strains of Salmonella enterica serovar Typhi (S. typhi),

it has caused typhoid fever [22,23]. The CdtB toxin is a recently renowned invasion factors of Salmonella enterica subsp. serovar Typhi [24], quite represent wide range of other enterobacteriaceae pathogens , host cellular inebriated by CdtB show a DNA damage response (DDR) characterized by irreparable cells harm, through tireless DDR prompting cellular death by apoptosis [25], given the meaning of the role of invasion factors and toxins in cause of typhoid fever disease, that main focus of this research was to isolate and diagnose S.typhi strains from supposed blood, investigate the existence of fim gene and coding gene for cdtB protein, as well as examine the DNA sequence for confirmatory diagnosis and phylogenetic tree analysis.

2. Materials & Methods

Clinical specimens and identification of bacteria

During the period from May 2018 to February 2019, 86 blood specimens were collected at General Teaching Hospital in AL-Diwaniya city and suspected outpatient's patients for typhoid fever. The specimens were transplanted on blood also, macConkey agar that brooded at a temperature of 37°C for 24-48 hours, every single bacterial state were initially diagnosed on phenotypic, cultural characteristics and biochemically [26,27], as well as the discovery of the typhoid antibodies in blood specimen by widal examination [28].

Molecular Identification

DNA extraction from *S.typhi* isolates by DNA purification kit (Geneaid , USA). DNA preparation were then analyzed via electrophoresis thereof 1.5% agarose gel. PCR be used to amplify the entire sequences of the genes studied in this research. The specific primers (Bioneer company, Korea) utilized for the expansion to these genes were shown in (table 1).

The PCR mixtures contained: Top DNA polymerase 1U, dNTP (dATP, dCTP, dGTP, dTTP) each:250mM, Tris-HCL (pH 9.0) 10mM, KCL 30mM, $MgCL_2$ 1.5mM. and polymerase chain reaction (PCR) thermocycling conditions were shown in (table 2).

Table 1. The primer sequences of virulence gene & toxin in this study.

Primers		Sequence	Amplicon
16SrRNA	F	CCTGGACAAAGACTGACGCT	523bp
	R	CGCTTCTCTTTGTATGCGCC	
S.typhi			
fimA	F	GTGAGCGGCGGTACTATTCA	451bp
	R	TAAAGGTGGCGTCGGCATTA	
CdtB	F	TAAGTGGTACTGCCGGTGTG	508bp
	R	GTAGGTGCGAGTACGGCTAC	

Table 2. PCR thermocycling conditions

PCR steps	Temperature (^{o}C)	Time Period	return
Initial Denaturation	95	5 min.	1
Denaturation	95	30 sec.	
Annealing	58	30 sec.	
Extension	72	1min.	30 cycle
Final extension	72	5min.	1
Hold	4	forever	-

DNA Sequencing and Analysis

The isolates were sent from the reaction products of *16S rRNA* gene to confirm the diagnosis of *S.typhi* through the analysis the phylogenetic tree, the results were compared and analyzed using NCBI BIAST.

3. Results

The results of the current study for widal test showed a positive result for 44 patients out of 86 total blood specimens of clinically suspected patients with typhoid fever, also showed 26 (59%) isolation of *S.typhi* depending on phenotypic and culture characteristics. While the results of the current study PCR technique showed 19 isolates of *S.typhi* possessed the *16S rRNA* gene (Fig.1).
The results of our study some virulence factors and toxins, it was found that most of isolates carried the *fim* gene was at rate (84.21%,16/19) (Fig.2) and *CdtB* gene was (100%, 19/19) (Fig.3).
DNA Sequencer:
The results of the analysis showed the gene sequence of *16S* rRNA between local *salmonella enterica* subsp. *enterica serovar typhi* isolates and (NCBI-Gene bank) BLAST *salmonella enterica* subsp. *enterica serovar typhi* isolates, the sequence identity rate was (99.26%) for *fim* gene (Table 3), either sequence identity rate for *CdtB* gene was (99.31%) in (Table 4). Our study also showed the phylogenetic tree analysis of *fim* gene and compared it with other isolates deposited in the (NCBI-Gene bank) as shown in (Fig. 4,5), while the result of the analysis of the other *CdtB* gene is shown in (Fig. 6,7).

Table 3. Local and NCBI BLAST *Salmonella enterica* subsp. homology sequence identity *Enterica serovar typhi* isolates

Local isolate	Genbank accession number	Homology sequence identity		
		Identical isolate	**Genbank accession number**	**Identity**
Salmonella enterica subsp. *enterica serovar Typhi* isolate	MN839770	*Salmonella enterica* subsp. e*nterica serovar Typhi* strain 311189-291186	CP029894.1	99.26%

Table 4. Local and NCBI BLAST *Salmonella enterica* subsp. homology sequence identity *Enterica serovar typhi* isolates

Local isolate	Genbank accession number	Homology sequence identity		
		Identical isolate	**Genbank accession number**	**Identity**
Salmonella enterica subsp. *enterica serovar Typhi* isolate	MN839769	*Salmonella enterica* subsp. *enterica* strain FSL M8-0480	KX810912.1	99.31%

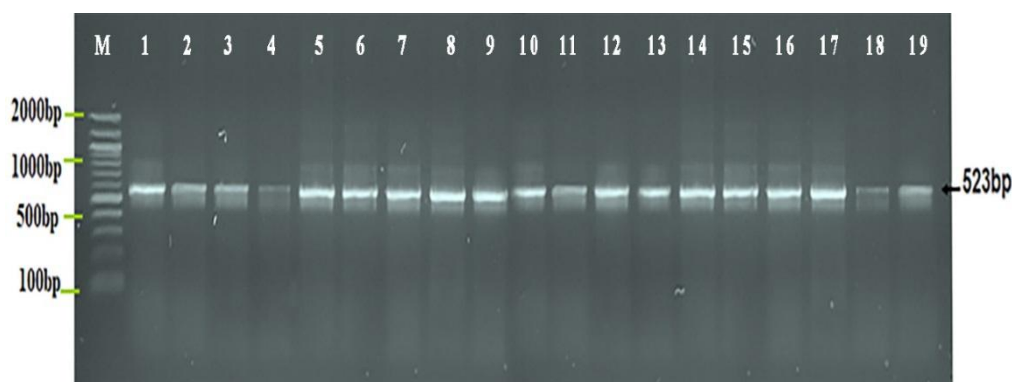

Figure 1. Agarose gel 1.5 percent electrophoresis, the *16SrRNA* gene PCR result isolates of *Salmonella typhi*. Where M: Marker (100-2000bp), lane (1-19) positive PCR (523bp) PCR producer size amplification.

Figure 2. Agarose gel 1.5 percent electrophoresis, the *fimA* gene PCR result isolates of *Salmonella typhi.* Where M: Marker (100-2000bp), lane (1-6) and (8-17) positive PCR (451bp) PCR producer size amplification

Figure 3. Agarose gel 1.5 percent electrophoresis, the *CdtB* gene PCR result isolates of *Salmonella typhi*. Where M: Marker (100-2000bp), lane (1-19) positive PCR (508bp) PCR producer size amplification

Figure 4. Multiple sequence alignment analysis of major pilin protein (*fimA*) gene partial sequence between local isolation and different isolates in the NCBI-Genbank for *Salmonella enterica* subsp. *enterica serovar Typhi* according to ClustalW alignment test

Figure 5. Phylogenetic tree analysis based on the partial sequence of major pilin protein (*fimA*)gene in local *Salmonella enterica* subsp. *enterica serovar Typhi* isolates that use for genetic analysis

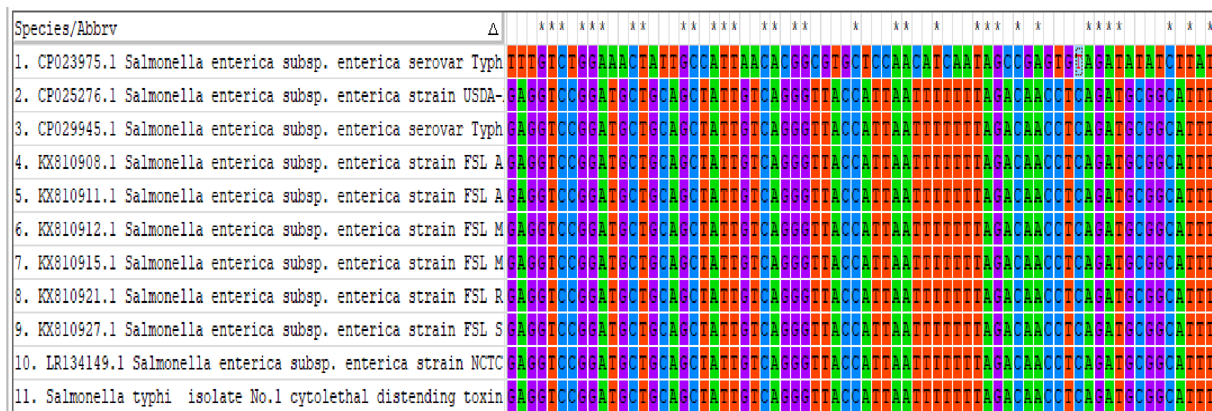

Figure 6. Multiple sequence alignment analysis of cytolethal distending toxin B (CdtB) gene partial sequence between local isolation and different isolates in the NCBI-Genbank for *Salmonella enterica* subsp. *enterica serovar Typhi* according to ClustalW alignment test

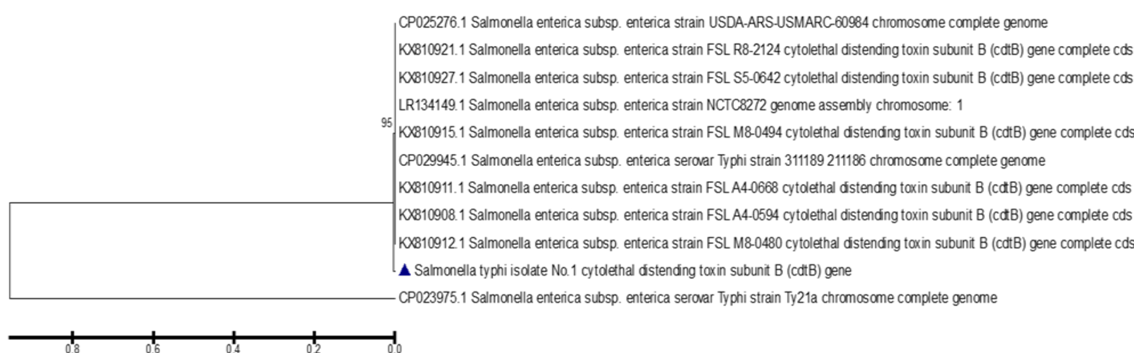

Figure 7. Phylogenetic tree analysis based on the partial sequence of cytolethal distending toxin B (*CdtB*) gene in local *Salmonella enterica* subsp. *enterica serovar Typhi* isolates that use for genetic analysis

4. Discussion

Typhoid fever causes estimated (5-30%) deaths in people in developing countries, the World Health Organization (WHO) estimates that between 16 and 17 million cases happen each year approximately 600,000 death-rate vary from district to district, but may be reach high as 5-7% although the usage of suitable antibiotics drugs [29]. Typhoid fever is also a major disease among young children and young adults in Nigeria, due to lack adequate facilities and misuse antibiotics [30,31]. These studies are consistent with our current study, showing that all *S.typhi* isolates were carried of *fimA* gene about 84.21% and *CdtB* genes about 100% as shown in (Fig.2 and Fig.3) respectively that enable them to developed symptoms of typhoid fever. Karine *et al.*[32] show athat all *S.typhi* fimbriae were better expressed in minimal broth that had six genes from within 12 chaperone-usher fimbriae that display extracellular structure by electron microscopy and show that when overexpressed only fimbriae had an effect on motility [32]. Fimbriae are tiny hair-like protein compositions on the cell surface that play about a dangerous role in the invasion by letting bacteria to happen interact with specific receptors of host cells [33].

As for the multiple sequence alignment analysis of major pilin protein fimA in our current study were showed the nucleotide similarity and substitution mutation in *fimA* gene nucleotide sequences as in (Fig. 4), also in our research, phylogenetic tree analysis based on the partial sequence of the main pilin protein reveals that the evolutionary distances are determined using the total composite likelihood model of the UPGMA tree (MEGA 6.0 version) as shown in (Fig. 5). In addition, there is a similarity in the sequence of nitrogenous bases of the *fimA* gene between the local and global isolation deposited in the (NCBI-Gene bank) where the proportion of high conformity of this gene at a rate of (99.26%) as shown in (table 3). Where the study proved Yap *et al.* [34], MLST data revealed that there was no guide of bias in the spatial and time distribution of STs,

suggesting that human migration rates across continents were very high to diffusion equal genotypes through people populations.
Human pathogens causing Cdt contain: an oral pathogen, a genital pathogen responsible for sexually transmitted cancroid, stomach pathogens, and cancer pathogens [35-37]. CDT consists of three proteins, CdtA, CdtB, and CdtC, CdtB being enzyme active or A secondary unit, having DNase I-very active, while CdtA and CdtC serve as heterogeneous B secondary units mediate the transmission of CdtB to host cells [38].
The *CdtB* gene has been detected in isolates from the blood of patients who have typhoid fever in our current study at a high rate of 100%, our study also found that multiple sequence alignment analysis of cytolethal distending toxin B were showed the nucleotide similarity and substitution mutation in *cdtB* gene nucleotide sequences as in (Fig.6), even analysis of the phylogenetic tree based on partial sequences of CdtB toxin as shown in (Fig.7). In addition, there is a similarity between identical and local isolation of the sequence of nitrogen bases of the *CdtB* gene at a rate of 99.31% as shown in (table 4).
That our study corresponds with Rivera *et al.* [38] where he showed that CdtB-islet coding the cytolethal distending toxin B is an invasion factor that not only one currently present in *Salmonella enterica* subsp. *Enterica serovar typhi* (*Salmonella typhi*) and hundreds of Gram negative bacterial as well as non-typhoidal *serovars Salmonella* (NTS).
There is a comparison in previous studies with all another bacteria that express Cdt, *Salmonella enterica* serotype *Typhi* (*S.typhi*) limited genes that code CdtA and CdtC [39-41].
S.typhi genome sequence has gave a wide look into its evolutionary history and phylogenetic analysis indicates this bacterium is powerfully monomorphic and may by entered recently the human enumeration [42-44]. The *S.typhi* chromosome has predicted a high than number of pseudogenes, an indicator that human-host adaptation cycle leads to a reduction in its genome [45,46]. These observations suggest that *CdtB* plays a role as an important *Salmonella* spp. of virulence attribute [47].

5. Conclusion

In conclusion, the current study showed that typhoid fever caused by *S.typhi,* which was isolated from infected patients; most of them were carriers of virulence factors that increase the pathogenicity of the fever, as well as the presence of typhoid toxin. This plays an active role in the severity of the bacteria's disease, which is the human carrier in his blood lead to damage the body cells by the coding gene of toxin and fimbriae proteins. The rate of *fimA* gene and *cdtB* gene is high, which has an impact on the phenotype and genetic pattern of bacteria.

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