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SEQUENCING OF THE *SDIA1* AMPLICONS AMONG *SALMONELLA TYPHI* ISOLATED FROM PATIENTS WITH TYPHOID FEVER, IRAQ

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Abstract

In this study, (100) blood sample were collected from patient suspected with typhoid fever how were visits the Al-hashimiya, Hilla Teaching Hospital and Morjan Teaching Hospital in Babylon province (Iraq) during the period November (2018) to February (2019). Identification was done by cultural and biochemical tests, and finally identification by Vitek2. Results showed that positive were (16) isolates of *Salmonella enteric serovar typhi* by Vitek2 (16%) from the (100) blood. Molecular detection of Vi gene (*sdiA*) was done for (16) *Salmonella typhi* isolates and the results showed that (15) isolates have this gene (93.75%). The positive results for (*sdiA*) virulence were detected by the presence of (274) bp band compared with allelic ladder. Within this locus, five samples were included, which had shown to amplify a coding portion of *sdiA1* genetic sequences within *S. enterica serovar typhi*, which is abbreviation for (suppressor of cell division inhibition). NCBI BLASTn engine has shown extremely high sequences similarities between the sequenced samples and the intended *SdiA1* target sequences. By comparing the observed DNA sequences of these local samples with the retrieved DNA sequences (Gene Bank acc. CP029646.1), the approximate positions and other details of the retrieved PCR fragments were identified.

Key words : *Salmonella enteric serovar typhi*, PCR technique, Sequencing of the *sdiA1* amplicons.

Introduction

The quorum-sensing system in bacteria is a well-known regulatory system that controls gene expression in a cell density-dependent manner. A transcriptional regulator (*LuxR* homologue), signal synthase (*LuxI* homologue) and autoinducer (acyl homoserine lactone) are indispensable for this system in most Gram-negative bacteria (Ahmer, 2004). Bacteria have mechanisms by which they sense their own population density and regulate their behavior accordingly, termed quorum sensing (QS) (Ahmer *et al.*, 1998). QS System controls a diverse range of cell density-dependent factor, such as antibiotic production, biofilm formation, and pathogenicity (Schauder and Bassler, 2001). A common mechanism of QS among the gram-negative bacteria is the synthesis and detection of a diffusible molecule of the N-acylhomoserine lactone (AHL) (Kleerebezem *et al.*, 2001). Many pathogens, such as *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*, adjust the expression of virulence factors required for pathogenicity via AHL-mediated QS systems (Smith and Iglewski, 2003). In gram-negative bacteria, the classical QS system is the *LuxR*/*LuxI* system, in which *LuxI* is the signal synthase, while *LuxR* is the signal receptor. *Escherichia coli* and *Salmonella typhi* colonize and proliferate in the intestine of animals in the presence of large numbers of other microbial species. Although no AHL-producing strains of *E. coli* or *Salmonella* have been identified, both have a *LuxR* homologue termed *SdiA* (Ahmer, 2004). Surprisingly, AHLs have not been found in the gastrointestinal tract of healthy mammals, with the exception of the bovine rumen (Edrington *et al.*, 2009). The sequencing of DNA is another PCR-based molecular method in which the nucleotide bases along a DNA strand are determined. The advent of rapid DNA sequencing methods has greatly accelerate biological and medical research and discovery (Hughes *et al.*, 2010). There are two different methods used in the sequence analysis chemical cleavage and dideoxy chain termination (Sanger sequencing method), Sanger sequencing was most frequently used which based on enzymatic DNA synthesis (Khan *et al.*, 2012). The obtained result is compared with reference sequences through the basic local

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alignment search tool (BLAST) or other database Third-generation DNA sequencing get possible method to completely sequence a bacterial genome in a few hours and identify some types of methylation sites along the genome as well. Sequencing of the bacterial genome is now a standard procedure, and provide the information from tens of thousands of bacterial genomes (Ahmer *et al.,* 1998). Sequencing of the bacterial genomes has significantly improved our understanding about the biology of many bacterial pathogens as well as identification of novel antibiotic targets (Zhang *et al.,* 2000). Bioinformatics is an interdisciplinary field that develops methods and software tools for understanding biological data and analyses of biological queries using mathematical and statistical techniques (Patel *et al.,* 2015). The common uses of bioinformatics include the identification of candidate genes and nucleotides (SNPs); such identification is made for better understanding the genetic basis of disease, unique adaptations, desirable properties or differences between populations. It has become an important part in experimental molecular biology, bioinformatics techniques and aid in the comparison of genetic and genomic data and more generally in the understanding of evolutionary aspects of molecular biology (Ahmer *et al.,* 1998). The advent of genome sequencing coupled with advances in bioinformatics analysis to model genome data, promises invaluable insights into bacterial pathogens, including, their evolution, ecology, pathogenesis, and the design of related therapeutic interventions (Guo, 2000).

Aim of study

To detected a Sequencing of *sidA* gene that related to quorum sensing and biofilm formation in some isolates of *Salmonella enteric serovar typhi*.

Materials and Methods

Patients and clinical specimens

A total of (100) samples were collected from blood with typhoid fever who attended different hospitals during the period from November (2018) to February (2019) in Al- Babylon. Blood sample was collected from patients, (10) ml of fresh venous blood samples were collected from suspected typhoid patients by sterile syringes which delivered into special screw cupped of culture bottle containing (100) ml of brain heart infusion broth and incubated at (37°C) for at least (3) days placed in bact/ alert 3D apparatus for a week. If positive sample, each specimen was inoculated using direct method of inoculation on culture of selective media namely MacConkey, Blood, XLD and SS agar then inoculated at (37°C) for (18-24) hours. After incubation period, the culture performed idols, catalase, oxidase, kligler, uearase biochemical test and final diagnosis by Vitek compact 2. After complete diagnosis, storage the pure colony in brain heart broth with glycerol in deep freezing.

Ethical Approval

A valid consent was achieved from each patients before their inclusion in the study.

Identification of bacteria

Colonial morphology and microscopic examination

A single colony from each primary positive culture on blood, MacConkey and nutrient agar and identify it depending on its morphological properties (colony shape, size, color, borders, and texture) and exam it by light microscope after being stained with Gram's stain. After examination it, biochemical tests were done on each isolates to complete the final identification according to Baron *et al.,* (1994); Collee *et al.,* (2006) and McFadden, (2000).

Serological characterization of isolates

Widal test: It was used for detection of typhoid antibodies in blood samples. The fresh blood samples were tested for presence of typhoid antibodies by using O and H antigens as follows:

1. One ml of blood was centrifuged at (10,000) rpm for (5) min and serum was separated for serology.
2. A single drop (50 µl) of each O and H antigen was applied separately on a clean glass slide.
3. One drop of serum separated from blood was mixed well with each antigen.
4. The observations were made after (2) minutes.
5. Clear agglutination was recorded as positive according to Saleh, (2013).

GN-ID with VITEK-2 Compact

This system consists of personal computer, reader/ incubator made up of multiple internal components including: card cassette, card filler mechanism, cassette loading processing mechanism, card sealer, bar code reader, cassette carousel and incubator, in addition to transmittance optics, waste processing, instruments control electronics and firmware. The system was equipped with an extended identification database for all routine identification tests that provide an improved efficiency in microbial diagnosis, which reduces the need to perform any additional tests, so that safety for both test and user will be improved. All the following steps are prepared according to the manufacturer's instructions. Three ml of normal saline were placed in plane test tube

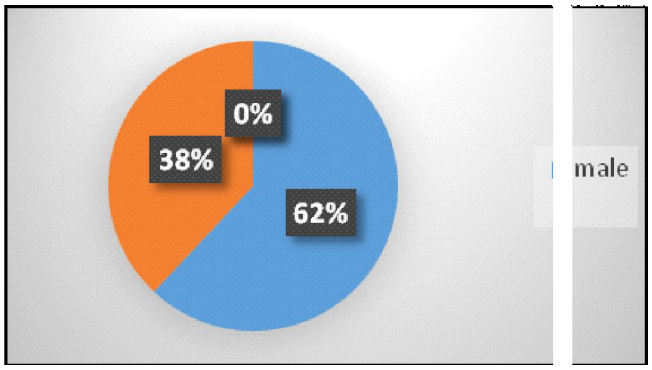

Fig. 1: Distribution of infected patients according to sex.

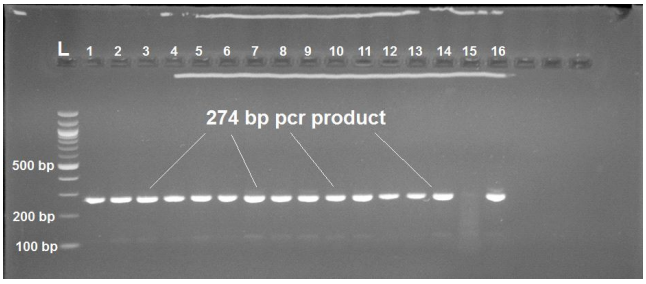

Fig. 2: Agarose gel electrophoresis (1.5%) of PCR amplified of *sdiA* gene (274)bp of *Salmonella Typhi* for (60) min at (100) volt. in this Figure show (1,2,3,4,5,6,7,8,9,10,11,13,14,16) isolates was positive results except the (15) isolates was negative result for *SdiA* gene.

and inoculated with a lopefull-isolated colony. Insert the test tube into dens check machine for standardization of colony to McFarland is standard solution (1.5 x 108 cell/ml). The standardized inoculums were placed into the cassette and a sample identification number entered into the computer software via barcode. The VITEK-2 card type then is read from barcode placed on the card during manufacture, and the card, thus, connected to the sample ID number. Then the cassette was placed in the filler module. When the cards were filled, the cassette was transferred to the reader/ incubator module. All subsequent steps were handled by the instrument, the instrument; controls the incubation temperature, optical reading of the cards and continually monitors and transfers test data to the computer for analysis.

Molecular study

Extraction of Genomic DNA

Genomic DNA was extracted by using a commercial extraction system (Genomic DNA Favorgen Kit).

1. Transfer bacterial cells (up to 1 x 109) to a 1.5 ml microcentrifuge tube and Centrifuge for (1) min. at 14-16,000 x g then discard the supernatant.
2. Add (200) µl of FAGT Buffer then re-suspend the cell pellet by vortex or pipette. Incubate for 5 minute at room temperature.
3. Add (200) µl of FAGB Buffer to the sample and vortex for (5) seconds and incubated at (70ºC) or until the sample lysate is clear, and during the incubation, invert the tube every (3) min. At this time, pre-heat the required Elution Buffer (for step 5DNA Elution) in a (70ºC) water bath.
4. Add (200) µl of absolute ethanol (96-100%) to the sample and mixed by vortex for (10) seconds. (If precipitate is appears, break it up as much as possible with a pipette). Then place the FAGB Column in a (2) ml collection tube and transfer mixture (including any insoluble precipitate) to the FAGB column and centrifuge at (14000rpm or 10,000 x g). Discard the (2) ml Collection tube containing the flow-through and then place the FAGB Column in a new (2) ml collection tube.
5. Wash FAGB column with 400 µl of W1 Buffer. Centrifuge at (14000 rpm or 10000 x g for 30 seconds); discard the flow-through then place the FAGB column back in the (2) ml collection tube.
6. Add (600) µl of Wash Buffer (with ethanol) to the FAGB column then centrifuge at (14,000 rmp or 10000 x g) for 30 seconds, discard the flow-through. Place the FAGB Column back in the (2) ml collection tube and centrifuge again for (3) min. at (14,000 rmp or 10000 x g) to dry the column matrix.
7. Place the dry FAGB Column to new (1.5) ml micro centrifuge tube, and add (100) µl of pre-heated Elution Buffer or TE to the membrane center of FAGB column. Stand FAGB column for 3-5 min or until the buffer is absorbed by the membrane and centrifuge at (14,000 rmp or 10000 x g) for (30) seconds to elute the purified DNA. Store the DNA fragment at (4 ºC) or – (20 ºC).

Table 1: Primer sequence that used in present study.

Type of gene	Sequ. of gene	Product	Reference
SdiA	AATATCGCTTCGTACCACGTAGGTAAACGAGGAGCAG	274bp	Reza *et al*., 2018

Table 2: Amplification Conditions of genes were used by PCR.

Gene	InitialDenaturation	No. ofcycles	Denaturation	Annealing	Extension	Final extinction
SdiA	94 Cº for 5 min	35	94 Cº for 30 sec	60 Cº for 30 sec	72 Cº for 30sec	72 Cº for 30 sec

Table 3: Biochemical test to *S. typhi.*

Result	Test
-	Oxidase test
-	Indole production
+	Methyl red
-	Voges –proskauer
	Simmons Citrate
+	H2 S production +
-	Urea hydrolysis -
+	Catalase test +
alk/acid	TSI
+	Motility

Polymerase Chain Reaction Assay

Detection of *sdiA1* genes by PCR assay

The composition of the PCR mixture was prepared in total volume (20) µl for each gene which done separately as in (Table 1).

PCR Thermocycling Conditions

The PCR tubes were placed on the PCR machine and the right PCR cycling program parameters conditions were installed as in table 2 (Bunyan and Obais, 2018; Bunyan *et al.*, 2018).

Results and Discussion

This study was conducted on (100) specimens from

Table 4: The position and length of the 274 bp PCR amplicons used to amplify the *SdiA* gene within the *Salmonella enterica* serovar *Typhi* genetic fragment (GenBank acc. no. CP029646.1). the highlighted sequences referred to the position of the forward and reverse primers respectively.

Amplion	Referring locus sequences (5′ - 3′)	length
SdiA DNA sequences	*GTAGGTAAACGAGGAGCAGCGTAAACTGCTACGGGAGAAAGATAAAA AGCCCAGCGCCCGGTTCGGCAACATCACACACTGGGTTACGCCTCTGCG TAATCCGAAACGCTGGGCGGCATCCCACATCGCCTGCGCTTCATGAAAT AGCACGTCATCCCAATGTAAATGACCCTGCCTGAAATTTTCCGGCTTTA ATACCGGATCAATCGCGAAATAGTTTTCGGACTGATAATGCGTTACCCA CGCCTGAGGATAAGTGGTACGAAGCGATATT*	274 bp

The highlighted sequences refer to the reverse and forward primers, respectively.

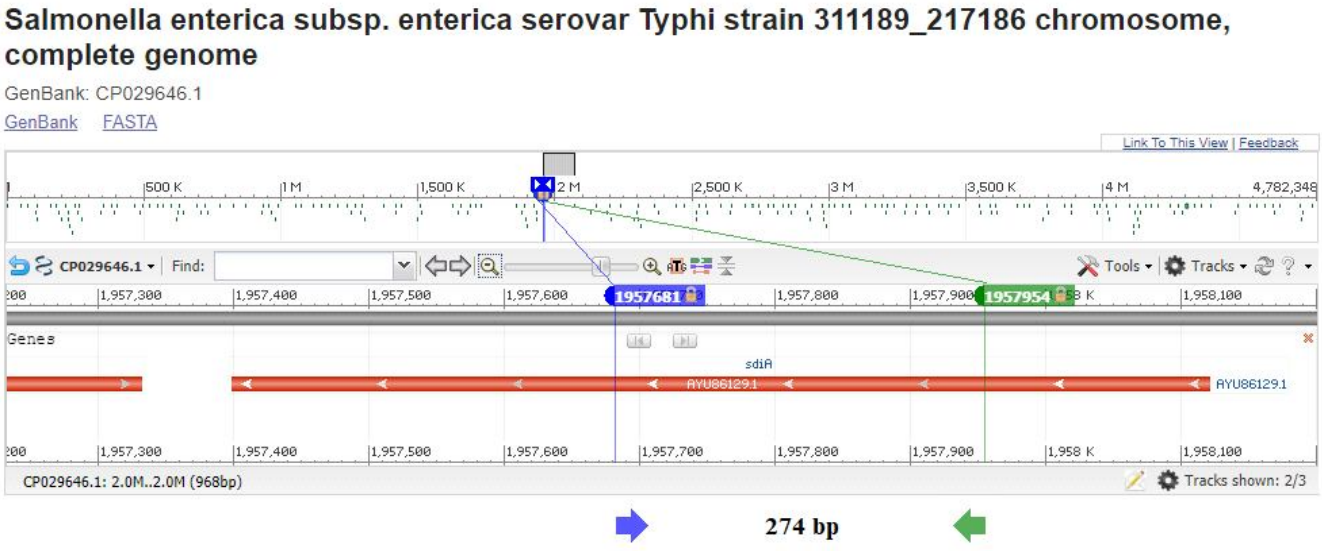

Fig. 3: The exact position of the retrieved 274 bp amplicon that partially covered the *SdiA1* genetic sequences within *S. enterica* serovar *Typhi* DNA sequences (GenBank acc no. CP029646.1). the green arrow refers to the starting point of this amplicon while the cyan arrow refers to its end point.

Table 5: The pattern of the observed SNPs in the identified *SdiA* DNA sequences in comparison with the NCBI referring sequences of 274 bp amplicons (GenBank acc. no. CP029646.1), in which, the annotation of all observed mutations were described.

No.	Mutation	Sample No.	position in the PCR amplicon	Position in the reference genome CP029646.1	Type of mutation	SNP Summary
1	T>G	S3, S4, S5	131	1957811	Ser114Ser (silent)	g.1957811T>G
2	G>T	All samples	217	1957897	N75K (missense)	g.1957897G>T
3	T>G	S2, S4, S5	248	1957928	Q65P (missense)	g.1957928T>G

blood samples, during the period from November (2018) to February (2019). The results indicated that clinical specimens were distributed into (23) specimens as *S. typhi* and (77) specimens as other bacteria, all these specimens were cultured on different media. The clinical diagnosis depended on the presence of some symptoms such as fever, headache, anorexia, nausea and vomiting, abdominal discomfort with diarrhea or constipation for (6-18) days. Out of (100) patients, 62(62%) were male and 38(38%) were female as shown in Figure (1). Males were found to be more infected than females, these results is similar to other results of studies in AL-Musaib (Saha *et al.*, 1996). The reasons may due to 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 (Salazar and McSorley, 2009). Michael and Al-Wan, (2008) expressed his opinion that greater exposure of male to contaminated food and water out-side the home might be region of higher rate of infection among this population. Typhoid fever is an infection caused primarily by *S. typhi* and is transmitted through the fecal oral route by the consumption of contaminated meat, poultry, milk and eggs.

Identification of *Salmonella typhi*

Serologically test

The blood specimens were positive by using Widal test. The Widal test, which measures antibody responses $\geq$160 titer to *S. Typhi* H and *S. Typhi* O antigens occur in the end of the first week of illness. The Widal test gave (100%) positive results for *S. typhi* while in blood culture gave (23%) positive result. misleading results using Widal test may keep one away from the true diagnosis because of cross-reaction of antigen from other infections with Salmonella antibody (Joshi *et al.*, 2016). An erroneous interpretation of rapid diagnostic tests delays the treatment of actual infection and increases morbidity. Increased request for Widal test as a quick diagnosis of typhoid fever has produced exaggerated results, since typhoid fever and malaria often show mimicking symptoms even in laboratory diagnosis. It is therefore recommended that the assumingly high incidence of the disease using Widal test will be greatly reduced if blood culture technique is routinely adopted as a baseline for the diagnosis of typhoid fever (Prasanna, 2011). Widal used to measure agglutinating antibodies against H and O antigens of *Salmonella typhi*. However, the major drawback of the Widal test is its cross reactivity with some other bacteria of the same genus. Blood culture, a more practical but less sensitive alternative is often used. This has its own lapses as it takes (2-3) days opposed to the quick diagnosis using Widal. As a result, diagnosis may appear delayed or over looked and patients without typhoid may receive unnecessary and inappropriate antimicrobial treatment (Nwafia and Nwafia, 2015).

Morphological characterization

The bacterial isolates obtained from clinical samples were identified initially according to cultural morphology, microscopic characteristics and biochemical tests. From those isolates, the cultural identification of *S. typhi* was depended on the colonial morphology. Since the colonies of *S. typhi* were grown on blood agar appears smooth white colonies and pale like shaped and smooth colonies when grown on the MacConkey agar, indicated that *S. typhi* is unable to ferment lactose sugar and showed pink colour colonies with black centre on XLD agar. Microscopically S. *typhi* appeared Gram-negative bacilli. The results of biochemical tests that recorded in table 3 were considered as a complementary of the initial identification of *S. typhi* isolates. The isolates confirm to general characteristics, isolates were negative for oxidase test, indole production, urease production and Voges Proskauer, simmon citrate utilization and positive to, H_2S production and catalase test and methyl red test. The *S. typhi* isolates showed production of hydrogen sulphide and no gas production in TSI. In triple sugar iron slants, both the slant and bottom turned into red and yellow colour respectively (indicating the fermentation of glucose alone

and no production of acid in the butt. All the results (morphology and cultural) were identical with Baron *et al.*, (1994); Collee *et al.*, (2006) and McFadden, (2000). The final identification was performed with the automated VITEK-2 compact system using GN-ID cards, which contained (47) biochemical tests and one negative control well. The results demonstrate that only (16) isolates from blood as *S. typhi* with ID message confidence level ranging between (95-99%).

Table 6: The *in silico* analysis of the observed nonsynonymous mutations in cell-division regulator protein using several bioinformatics tools.

SNP	SIFT		PROVEAN		Poly-Phen-2		SNAP2	
Q65P	0.18	Tolerated	-2.273	Neutral	0.003	Benign	-17	Neutral
N75K	0.13	Tolerated	-4.682	Deleterious	0.001	Benign	24	Effect

Fig. 4: DNA sequences alignment of five local samples with their corresponding reference sequences of the (274) bp amplicons of the *SdiA* locus within the *Salmonella enterica* serovar *Typhi* DNA sequences. Each substitution mutation was highlighted according to its position in the PCR products. The symbol "ref" refers to the NCBI referring sequence, "S1-S5" refer to the samples 1 to 5, respectively.

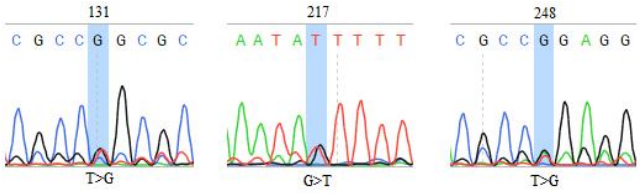

Fig. 5: The pattern of DNA chromatogram of the (274)bp amplicons of the *SdiA* locus within the *Salmonella enterica* serovar *Typhi* DNA sequences. Each observed substitution mutation was highlighted according to its position in the PCR products.

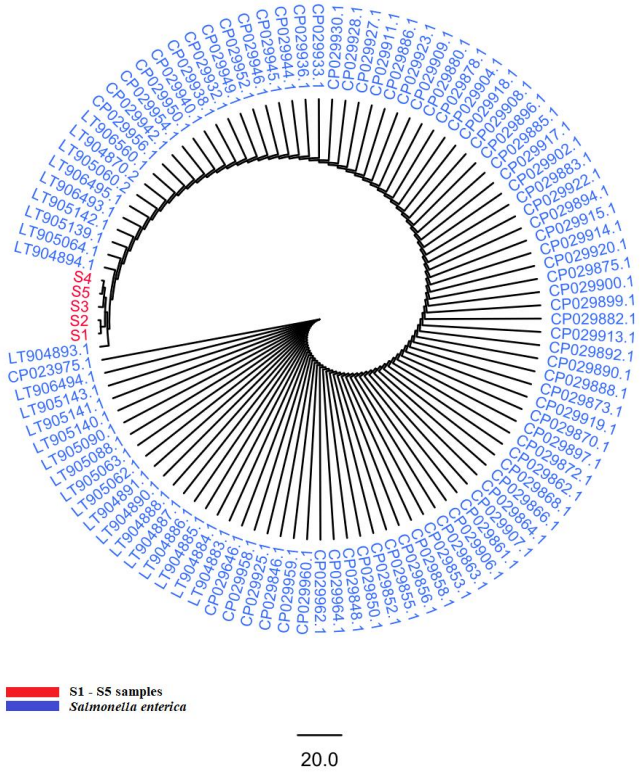

Fig. 6: The comprehensive phylogenetic tree of the 274 bp variants of *Salmonella enterica* serovar *Typhi* local isolates. The red arrows refer to the sequenced S1-S5 variants, while the cyan color refers to the relative *Salmonella enterica* serovar *Typhi* DNA sequences. All the mentioned numbers referred to Genbank acc. no. of each referring species. The number "20.0" at the bottom of the tree refers to the degree of scale range among the comprehensive tree categorized organisms.

Molecular study of *Salmonella typhi*

Detection of *(sdiA)* gene

Molecular detection of Vi gene (*sdiA*) was done for (16) *Salmonella typhi* isolates and the results showed that (15) isolates have this gene (93.75%). The positive results for (*sdiA*) virulence were detected by the presence of (274) bp band compared with allelic ladder as shown in Figure (2). *SdiA* is the first example of a bacterial

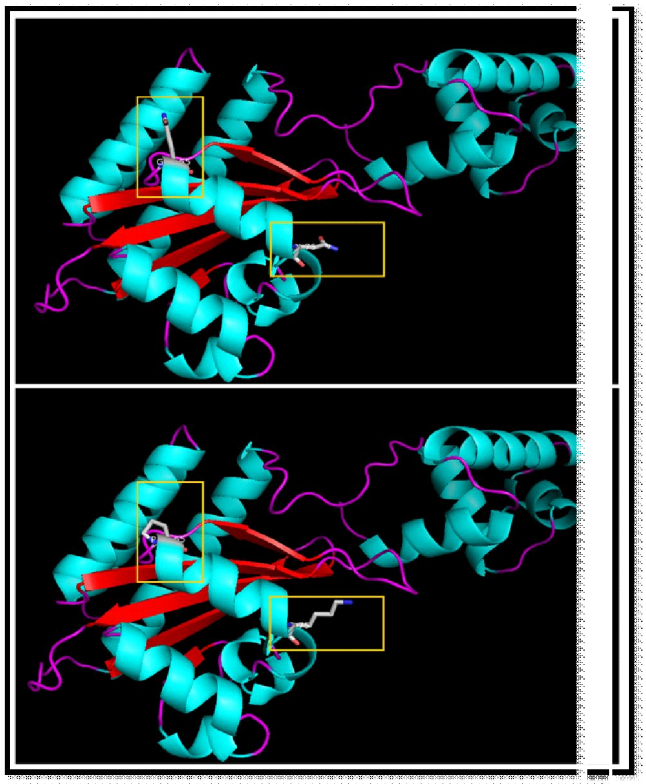

Fig. 7: The *in silico* visualization of the observed missense mutations in the cell-division regulatory protein. Both amino acid substitutions were highlighted before mutations (A) and after being mutated (B). the squares and arrows indicated the amino acids positions in which the missense mutations occurred.

receptor that exclusively detects the signals AHLs (N-acylhomoserine lactone) of other microbial species. The *sdiA* gene lies in centisome (42) region of the *Salmonella* chromosome (far enough from the unstable centisome (63) region) just upstream of *uvrC* and *sirA* (Ahmer, 2004). *sdiA* does not regulate the virulence associated type III secretion systems of *Salmonella*. Therefore, *sirA* appears to be a major regulator of *Salmonella* intestinal virulence, whereas *sdiA* regulates accessory factors that may contribute to intestinal survival or colonization and other genes of unknown function. The *sirA* gene has also been used for PCR detection of *Salmonella* ssp. but yielded nonspecific bands for non-*Salmonella* strains (Nwafia and Nwafia, 2015). *sdiA* is only known to activate genes that would help a single bacterium to adhere to host tissues, presumably because the presence of AHL producing bacteria indicates that a particular host environment has been reached (Ahmer, 2004). Consequently, *sdiA* as a response regulator of gene transcription has very conservative domains. Each element in a quorum sensing system has the freedom to evolve greater complexity or specificity without compromising the overall system

function. These characteristics (conservation and specificity) render the quorum sensing genes ideal targets for primers design (Guo, 2000).

Sequencing of the *sdiA1* PCR amplicons

Within this locus, five samples were included, which had shown to amplify a coding portion of *sdiA1* genetic sequences within *S. enterica serovar typhi*, which is abbreviation for (suppressor of cell division inhibition). The sequencing reactions indicated that the exact identity after performing NCBI blastn for these PCR amplicons (Zhang *et al.*, 2000). Concerning the supposed (274) bp PCR amplicons of the *sdiA1* gene, which encodes a cell-division regulatory protein (Ahmer *et al.*, 1998). NCBI BLASTn engine has shown extremely high sequences similarities between the sequenced samples and the intended *sdiA1* target sequences. By comparing the observed DNA sequences of these local samples with the retrieved DNA sequences (Gene Bank acc. CP029646.1), the approximate positions and other details of the retrieved PCR fragments were identified as shown in Fig. (3). After positioning the 274 bp amplicons' sequences within the *SdiA* DNA locus, the details of its sequences were highlighted as shown in (Table 4). The alignment results of the (274) bp samples revealed the presence of thee mutations that distributed variably in the analyzed samples in comparison with the referring reference *sdiA* genetic sequences. The positions of the observed mutations that localized with respect to the referring *sdiA* DNA sequences within *S. enterica serovar typhi*. Genomic DNA sequences were shown in Fig. (4). The sequencing chromatogram of each observed substitution mutation as well as its detailed annotations was documented as shown in Fig. (5). Three mutations were observed in bacterial isolates, namely g.1957811T>G, g.1957897G>T, and g.1957928T>G that were distributed amongst the analyzed bacterial samples. To sum up all the results obtained from the sequenced (274) bp fragments, the exact positions of the observed variations were mentioned in the NCBI reference sequences as shown in (Table 5).

Construction of phylogenetic tree of the *SdiA1* PCR amplicons

The currently constructed comprehensive tree indicated the presence of almost only one species allover (100) scanned of (274) bp of S1-S5 variants. It was found that all studied S1-S5 variants were occupied one position within the tree. The presented sequences were found to belong to *S. enterica serovar typhi*-related sequences. The current phylogenetic tree has provided an inclusive evidence regarding the confirmed identity of S1-S5 variants. Despite the unique characterization of S variants that originated from the presence of three substitution mutations (g.1957811T>G, g.1957897G>T, and g.1957928T>G), no deviation from *S. enterica serovar typhi* was revealed. However, all S1-S5 variants were clustered in one position within this comprehensive tree due to the observed insertion mutations. It is obviously observed in the presently constructed comprehensive phylogenetic tree as almost all the observed sequence-related species belonged to *S. enterica serovar typhi* species. As one species was resolved in the current tree, this notion provided a further indication of the absolute identity of the sequences S1-S5 studied isolates. Thus, this cladogram-based comprehensive tree provided an inclusive tool on the high ability of such selected PCR genetic fragment to efficiently identify *S. enterica serovar typhi* isolates. Add to that, our phylogenetic analysis has observed an extremely high detection specificity with regard to *sdiA*-based phylogenetic protocols. Accordingly, the currently observed PCR-sequencing protocol, as well as phylogenetic tools, have clearly indicated the relatively related similarity amongst all analyzed *S. enterica serovar typhi* isolates. In conclusion, by relying on DNA sequencing that followed by phylogenetic tree strategies, all five *S* strains were definitely found to belong to *S. enterica serovar typhi* isolates.

***In silico* prediction of the observed missense mutations**

The observed SNPs were further analyzed to identify whether it substitutes its corresponding position in the resulting encoded cell-division regulatory protein. Regarding Q65P, all the utilized *in silico* tools had indicate the non-deleterious effect of this missense mutation on the 3-dimentional structure of the altered protein. Conversely, some of *in silico* tools, including PROVEAN and SNAP2, had shown clear deleterious effect of N75K on protein structure and function, while both SIFT and Poly-Phen-2 tool had shown a non-deleterious effect of the same mutation on the altered protein (Table 6). This point of discrepancy could be attributed to the differences of the algorithm each tool relied on in its interpretation (Patel *et al.*, 2015). More in-depth computational data was provided through constructing a virtual 3-D structure to the protein of interest. The 3-D model of this protein was constructed using RaptorX protein modeling tool and visualized in PyMOL. The observed Q65P and N75K missense mutations were inserted in the native sequence of the protein and its consequences were checked using several computational tools. PyMOL was used to locate both Q65P and N75K mutations on protein 3-dimensional structure and for analyzing both native and mutant structures in the cell division regulatory protein of interest, which is made of 240 amino acid residues. So, the relatively deleterious Q65P, the non-deleterious N75K amino acid substitution were visualized in the 3-D structure in both native protein as well as its mutant counterpart.

However, the FT site tool had indicated no potential role for both missense mutations in inducing any possible alteration in the resulting protein. Therefore, the observed deleterious effect of D117N was caused such deleterious effects not by make any alteration in the binding with its receptor. It is well established that the *sdiA* gene product, cell-division regulatory protein, involved in the inhibition of the cell division of *Salmonella enterica serovar typhi* (Ahmer *et al.,* 1998). Thus, any deleterious effect of this protein would potentially have a negative impact on the inhibition of such cell division. Therefore, the observed deleterious effect of the K146N would possibly imply some impact on the cell division of the cells that have an altered cell-division regulatory protein. The D117N mutation may be involved in the alteration in the cell-division regulatory protein. Consequently, the metabolic inhibitory mechanism that mediated by this protein would potentially be inhibited. Accordingly, the mutant *Salmonella enterica serovar typhi* bacterial cells presumably would halt cell division thereafter.

Conclusions

1. Genotypic detection by PCR revealed the presence of *sidA* gene was have relationships with their pathogenicity.
2. Discovery of new strains of *S. typhi* by sequencing technology as belong to *S. enterica serovar Typhi*. This study is the first in Iraq.
3. Recorded Iraqi sequence in gene – bank \ NCBI, USA.

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