Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 2, 2021, Pages. 675 - 685
Received 20 January 2021; Accepted 08 February 2021.

Molecular Detection of Some of the Salmonella Typhi Virulence Genes Isolated in the Province of Babylon/Iraq

Jihad N. Abid Albanwawy[1], Lamees A. Abdul-Lateef*[2]

[1] Microbiologist at Alazizya General Hospital,Wasit Health Director, Iraq.

[2] PhD, Microbiology, Department of Microbiology, College of Medicine, University of Babylon, Iraq.

*Corresponding Author: Lamees A. Abdul-Lateef

Jihad5775@gmail.com

Abstract

Typhoid fever remains a most important global health problem in low- and middle-income countries.The severity the pathogenesis depends on Salmonella's possession of several virulence factors, encoded on *Salmonella* pathogenicity islands (SPIs). In current study 34 *S. Typhi* isolates were collected from patients suffering from typhoid fever who attended to Imam Al-Sadiq Teaching Hospital, and private medical clinics in Babylon province during the period from July to November 2020.Identification was done by cultural and biochemical tests, and finally identification by Vitek2 system. In current study, simple PCR assay was used to detect the existence of genes usually associated with virulence of *Salmonella Typhi*; five virulence-related genes detected in this study were; *ctdB, tviA, sipA/sspA, spiC, and orfL,* by using specific primers. Thirty-two isolates (94.1%) out of total 34 isolates showed positive results for *tviA* (typhi Vi) gene, while other virulence genes were detected in all (100%) isolated strain.This gene could be effective in the invasiveness of the*S. Typhi*strain. The results obtained from this study will help to understand the spread of *S. Typhi* virulence genotypes in Iraq.

INTRODUCTION

*Salmonella*represents a broad genus of global public health significance, and is the important cause of foodborne diseases responsible for thousands of deaths worldwide (Lee *et al.*, 2015). The *Salmonella* genusincludes Gram-negative, facultative anaerobic bacteria.*Salmonella* are small rod-shaped bacteria, 0.2-1.5×2-5 μm, non-sporeforming bacilli,belong tothe family Enterobacteriaceae (Alzwghaibi *et al.*, 2018), generally motile by peritrichous flagella with exception for*Salmonella enterica* subspecies. *enterica* serovars *pullorum* and *gallinarum*(Ellermeier and Slauch, 2006).

In order to overcome the pathogenic process, *Salmonella*owns many virulence strategies,used to interact with host defense mechanisms. Virulence genes essential forinvasion, and those essential for intracellular survival are clustered in large chromosomal DNA regions, designated Salmonella pathogenicity islands (SPIs), which are well-defined as large gene cassettes located within the Salmonella chromosome that translatefactors haveresponsibility for establishing specific interactions with the host, In particular, associated with bacterial virulence(Hyeon *et al.*, 2017).

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 2, 2021, Pages. 675 - 685
Received 20 January 2021; Accepted 08 February 2021.

Cytolethal distending toxins (CDTs) are important virulence factors produced by*S. Typhi. CtdB* gene, unique to S. Typhi serotype, which is encoded on SPI- 11. The toxin is mainly expressed when *S. Typhi* is intracellular and is located within *Salmonella* containing vacuole,and released into the extracellular space (Johnson, *et al.*, 2018).The cytolethal distending toxin cdtB gene, coding for toxins that induce apoptosis of infected cells (Ben Hassena *et al.*, 2021).After export, typhoid toxin is actively engaged in the intoxication of infected and uninfected cells by autocrine and paracrine pathways.(Johnson, *et al*., 2018).

The*tviA* gene encodes a regulatory protein that plays an important role in organizing the expression of Vi antigen, flagella, and a number of genes necessary for host invasion,(Santander, *et al.*, 2008).Vi polysaccharide capsular antigen is encoded by the viaB locus in the 134 kb Salmonella pathogenicity island 7 (SPI-7). The viaB locus consists of ten successive genes and consists of three functional segments: *tviA*, *tviBCDE* and *vexABCDE*. *TviA* production is a regulatory protein, that acts as an activating agent of the Vi antigen by binding upstream region of the *tviA* promoter, whereas *tviBCDE* and *vexABCDE* are needed for biosynthesis and export of the Vi antigen, respectively.(Virlogeux *et al.*, 1995; Zhang *et al.*, 2018).In response to osmolarity, *SalmonellaTyphi* regulates genes essential forexpression of Vi capsular antigen in the opposite direction to those essential for motility and invasion. TviA suppressed expression of genes that coding for flagella, andtype III secretion systemassociatedwith invasion through suppression of the flagellar regulators *flhDC* and *fliZ*, follow-onreduced invasion, motility, and reduced expression of *FliC*(Hu *et al.*, 2017).

The sipA gene plays an important role for both the invasion, and elicitation of intestinal inflammation, especially the type III secretion system that injects effector proteins in to the host enhancing the activity of bacterial invasion and stimulate intestinal inflammation.The *SipA* protein is needed to comprise a complex in the eukaryotic membrane that is considered necessary for the translocation of the remaining effectors into the host cell cytoplasm.(Holt *et al.*, 2011).The SpiA effector is an actin binding protein that improves the efficacy of uptake by promoting actin polymerization. This effector plays an important role in the invasion, and promoting actin polymerization that causes membrane ruffling and bacterial entry into the intestinal epithelium(Lin *et al.*, 2011), binds actin and improves entry efficiency by helping to promote actin polymerization and inhibiting filament disassembly(Brawn, *et al.*, 2007).

*SpiC*pathogenicity island 2 secreted effector protein is a virulence factor that encoded within SPI-2, could be secretedinto the macrophage cytoplasm by the Spi/Ssa T3SS2 (Wang *et al.*, 2019). This protein is transported by the SPI-2 TTSS to the cytosol of macrophages, where it interacts with the host proteins such as TassC (Protein NipSnap homolog 3A, target for Salmonella secreted protein C) (Lee *et al.*, 2002) and Hook3 (The human hook microtubule tethering proteins) (Shotland *et al.*, 2003) to modify intracellular trafficking.The *spiC* gene's function is to interfere with intercellular membrane trafficking in such a way that it is altered, preventing proper cellular function.(Uchiya and Nikai, 2008).SpiC prevents SCVs from interacting with late endosomes and lysosomes, as well as transferrin endocytosis and recycling.(Sun *et al.*, 2019). This disruption defends pathogens by counteracting the bactericidal contents of the cell, such as reactive nitrogen and oxygen species.(Kaur and Jain, 2012).

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 2, 2021, Pages. 675 - 685
Received 20 January 2021; Accepted 08 February 2021.

Finally, the orfL virulence gene is found in Salmonella Pathogenicity Island 4 (SPI-4) and is involved in adhesion, auto transportation and colonization. The orfL gene, which is necessary for intra-macrophage survival and possibly carries a system involved in the secretion of toxins (LEGBA *et al.*, 2017). The orfL virulence gene is involved in the production of toxins that cause immune cells apoptosis, and it is necessary for *Salmonella Typhi* survival in macrophage.(Gassama-Sow *et al.*, 2006).

Materials & Methods

A total of (130) samples were obtained from blood of patients with typhoid fever who attended to Imam Al-Sadiq Teaching Hospital, and private medical clinics in Babylon province during the period from July to November 2020. Identification was done by cultural and biochemical tests, and finally identification by Vitek2 system.Fresh venous blood samples (5 ml) were directly injected intospecial screw cupped of culture bottle (45 ml) BHI broth, and incubatedfor at least (7)days at (37°C), regularly blood cultures wereexamined to check the turbidity and color change that referred to microbial growth.Ten µl(loop full) from the inoculatedand incubated samples were streaked on XLD agar, and SS agar plates. Identification of *S. Typhi* were done depending on the colonial morphology, biochemical, and conformational diagnosis by VITEK2 compact system.A total of 34 (26.1%) *S. Typhi* isolates were obtained from 130 patients with clinically suspected typhoid fever.After complete diagnosis, the pure colonies stored insterilizedBrain Heart Infusion broth with glycerol in deep freezing.

Aim of study

Detection offive virulence encoding genes; *ctdB, tviA, sipA/sspA, spiC, and orfL*in*S. Typhi* isolates, from typhoid patients, by using specific primers.

Ethical Approval

The research was carried out in agreement with the ethical standards outlined in the Declaration of Helsinki. Before sampling, each patient or his parents gave their verbal consent. The investigation's requirements were strictly adhered to, particularly when it came to confidentiality. Furthermore, this study was kept under wraps, patients were given the option to participate, and verbal consent was obtained before the data collection process began. The sampling procedure, and the subject data and consent form have been reviewed and approved by the local ethics committee (at College of Medicine University of Babylon).

Identification of bacteria

Colonial morphology and microscopic examination

Based on colony morphological properties (colony form, color, size, Margene, and texture), A single colony of each primary positive blood culture, XLD agar, and was identified and analyzed under a light microscope after being stained with Gram's stain. Following that, biochemical tests on each isolate were performed to complete the final identification.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 2, 2021, Pages. 675 - 685
Received 20 January 2021; Accepted 08 February 2021.

GN-ID with VITEK-2 Compact

This device consists of a personal computer, a reader/incubator with various internal components such as a card filler mechanism, card cartridge, a cassette loading processing mechanism, a bar code reader, a card sealer, a cassette trolley, and an incubator, as well as transmittance optics, waste processing, instrument control circuitry, and firmware. The device came with an expanded identification database for all routine diagnosis tests, resulting in increased productivity in microbial diagnosis and a reduction in the need for additional tests, improving test and user safety. Almost all the steps that follow are done according to the manufacturer's orders. A lope full-isolated colony was inoculated with 3 ml of normal-saline in a plane test tube. For standardization of the colony to McFarland is standard solution (1.5 x 108 cell/ml), place the test tube into a denscheck system. The uniform inoculums were inserted in the tape, and a barcode was used to insert thenumber of sample identification into the computer program.The VITEK2 card type was then read from the barcode inserted on the card during the manufacturing process and the card is therefore connected to the sample ID number. The cassette was then inserted into the filler module. The cassette was transferred to the reader/incubator module once the cards were filled. The instrument was in charge of all subsequent measures, including controlling the incubation temperature, optical card reading, and continuously monitoring and transferring test data to the machine for analysis(Pincus, 2010).

DNA extraction

Genomic DNA of studied *S. Typhi* was extracted by using a Genomic DNA purification kit supplied from Geneaid company, UK.

Primer design

The oligonucleotide primers used in this study were obtained from previous studies, as shown in **Table 1**.

Table 1: Virulence genes primers sequences with their amplicon size base pair (bp).

Genes	Primer sequence (5'-3')	Size bp	PCR conditions	Reference
cdtB F	TAAGTGGTACTGCCGGTGTG	508		(Shaheed *et al.*, 2019)
cdtB R	GTAGGTGCGAGTACGGCTAC			
tviA F	GTTATTTCAGCATAAGGAG	599		(Liaquat *et al.*, 2018)
tviA R	ACTTGTCCGTGTTTTACTC			
spiA/sspA F	GTTAAGTAATGTGCTGGACGGCCT	100		(Liaquat *et al.*, 2018)
spiA/sspA R	ACCCGATCCACACCAGGTTTATTC			

SpiC F *SpiC* R	CCTGGATAATGACTATTGAT AGTTTATGGTGATTGCGTAT	301		(Hughes *et al.*, 2008)
orfL F *orfL R*	GGAGTATCGATAAAGATGTT GCGCGTAACGTCAGAATCAA	323		(Sánchez-Jiménez *et al.*, 2010)

RESULTS AND DISCUSSION

This study was conducted on (130) blood and stool specimens, during the period from July to November (2020). The results showed that clinical specimens were distributed as S. Typhi to (34) specimens,and (96) specimens as other bacteria or gave negative results. The presence of symptoms include; fever, headache, anorexia, nausea and vomiting, abdominal pain with diarrhea or constipation for 6-18 days was required for a clinical diagnosis. A total of 34 (26.1%) *S. Typhi* isolates were obtained from 130 patients with clinically suspected typhoid fever.

Molecular study of Salmonella typhi virulence genes

In current study, simple PCR assay were used for detection the presence of genes usuallyassociated with virulence of *Salmonella Typhi.*

A specific primer was used to detect the *ctdB* gene at the molecular level. It was found that *cdtB* gene observed in 34 isolates (100%) of *S. Typhi* isolates with long length (508 bp) as shown in **figure (1).**The result of this study was compatible with the result obtained by AL-Oqaili, (2019) which confirmed that *ctdB* gene was found in 100% of *Salmonella Typhi* isolates. In the same vein, Thakur *et al.*, (2019) in their study reported that *ctdB* gene was found in all *Salmonella Typhi* isolates.Secretion of the typhoid toxin, which is encoded on SPI11, is unique to S. Typhi. When *S. Typhi* is intracellular macrophages and localized inside the Salmonella containing vacuole (SCV), the toxin is only expressed(Johnson, *et al*., 2018).The cytolethal distending toxin (*cdtB*) gene encodes toxin that cause infected cells apoptosis(Ben Hassena *et al.*, 2021). Typhoid toxin is unusual compared to other CDTs, in that *S. Typhi* lacks the CdtA and CdtC subunits, and instead, typhoid toxin is a complex composed of one CdtB molecule, one PltA molecule and multiple PltB molecules (Johnson, 2018). The CDT-dependent cell death due to an apoptotic response, either inepithelial, fibroblast,or lymphoblastoid mammalian cells (Pons, *et al*., 2019).Typhoid toxin is thought to play a role in the development of chronic S. Typhi infection, although further investigation is warranted by the underlying mechanism. These researches were indicate that typhoid toxin is responsible for the development of symptoms and the shift from acute to chronic typhoid fever and could be a potential target for improving these symptoms.(Johnson, et al., 2018).

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 2, 2021, Pages. 675 - 685
Received 20 January 2021; Accepted 08 February 2021.

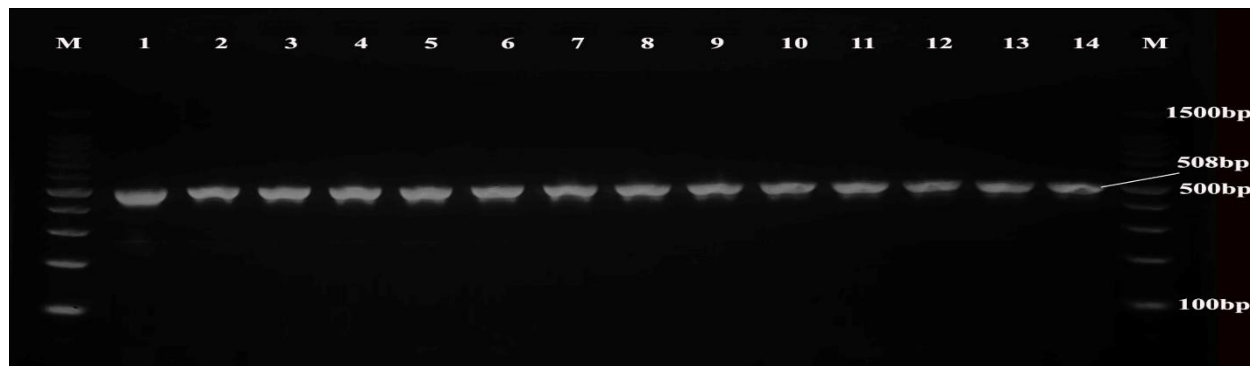

Fig. (1): Agarose gel electrophoresis of PCR product obtained with *Salmonella Typhi* using cdtB specific primers. lanes 1-14 represent the identified cdtB, Lane M represent 100bp DNA ladder. White lines represent PCR amplicon. the size of product is 508 bp for *cdtB* gene.

Molecular detection of *tviA* gene was also done by using specific primer. This study was found that *tviA* gene observed in 32 isolates (94.1%) of *S. Typhi* with long length (599 bp) as shown in **figure (2)**.Previous study conducted by Liaquat *et al.*, (2018) was closely corelated with our results, they confirmed that 89 % of *Salmonella Typhi* were carrying *Salmonella* pathogenicity island 7 (SPI-7) but some of them (11%) were deficient in SPI-7 associated genes (*tviA*) and other genes. These findings are in accordance with previous study obtained by Baker *et al.*, (2005), they found that 85 % of salmonella typhi isolates were positive for *tviA* whereas 15% were negative. Xie *et al.*, (2010)referred that, Regulation of Vi capsular antigen expression is essential for S. Typhi infection, such as suppression in the hyperosmotic intestinal tract to enhance invasion potential. Santander, *et al.*,(2008) also referred, S. Typhi is much more invasive into epithelial cells, but less resistant to macrophage killing under high osmolarity conditions. As a result, S. Typhi's Vi antigen is a negative factor for invasion but a positive factor for survival and duplication within macrophages. Previous study conducted by Valcheva *et al.*, (2015)suggested that after extended storage or frequent cultivation in laboratory culture media, Vi-negative strains will be the most common..

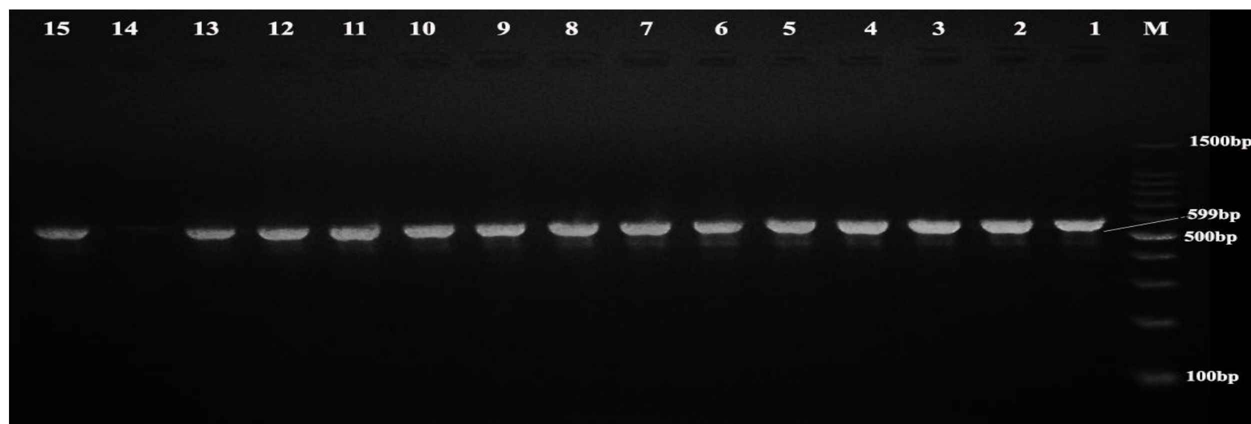

Fig. (2): Agarose gel electrophoresis of PCR product obtained with *SalmonellaTyphi* using *tviA* -specific primers. lanes 1-15 represent the identified *tviA*, in exception of Lane 14 (negative), Lane M represent 100bp DNA ladder. White line represents PCR amplicon.

Detection of (*sipA*) gene

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 2, 2021, Pages. 675 - 685
Received 20 January 2021; Accepted 08 February 2021.

Salmonella invasion protein (*sipA/sspA*) genewas investigated by PCR technique using specific primers for this gene. The result of this experiment indicates for positive amplification as shown in **figure (3).** It was found that *sipA* marker was observed in 34 isolates (100%), with long length 100 bp.The result of current study confirmed, 100% of *Salmonella Typhi* isolates was carrying *sipA/sspA gene.* This result was supported by previous study conducted by Bunyan and Obeis, (2019), they found that 100 % of S. Typhi isolates contained *sipA* gene.The *sipA* (also called *sspA*) gene play significant roles in both the invasion and the elicitation of intestinal inflammation, specifically type III secretion system that insert effector proteins into host cells to promote bacterial invasion and to stimulate intestinal inflammation. The *sipA* protein is thought to form a complex in themembrane of eukaryotic, that is essential for the other effectors to be translocated into the cytoplasm of the host cell.(Bunyan and Obeis, 2019).

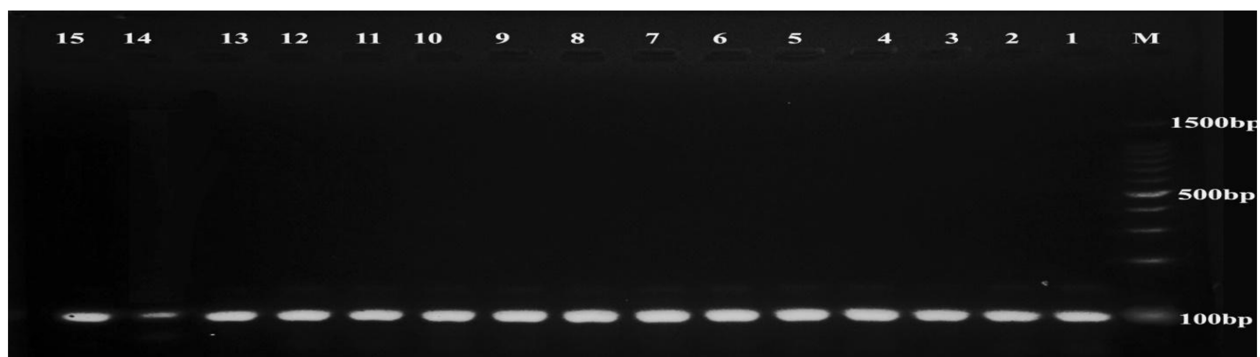

Fig. (3): Agarose gel electrophoresis of PCR product obtained with *SalmonellaTyphi* using *sipA/sspA* - specific primers. lanes 1-15 represent the identified *sipA/sspA* genes 100 bp, Lane M represent 100bp DNA ladder.

SpiC gene was also studied by specific primer. It was found that *spiC* gene detected in 34 isolates (100 %) out of total isolates of *S. Typhi* with long length (301 bp) as shown in **figure (4)**.We observed that all *Salmonella Typhi* isolates (100%) were carried *spiC* gene. The SpiC gene was studied because it is complicated in the interaction with the intercellular membrane and trafficking, which prevents proper cellular function. These results are in line with previous studies performed in Cochin, India by Parvathi *et al.*, (2011) and confirmed that 100% of typhoidal *Salmonella* isolates were carried *spiC* gene.Parvathi *et al.*, (2011)werereported, *spiC* is one of the genes in the spiCAB structural units of a type III secretion system that encoded on SPI-2 that supports Salmonella survive, and replication within cells.Just one SPI2 gene (spiC) has been shown to encode a protein that functions as an effector. SpiC has been shown to prevent SCV fusion with lysosomes, homotypic endosome fusion, and transferrin recycling(Beuzón *et al.*, 2000). Furthermore, the type III secretion mechanism encoded within SPI-2 translocates this protein into the cytosol of Salmonella-infected macrophages, and interacts with the proteins of the host. including TassC and Hook3, which are involved in cellular trafficking. SpiC, on the other hand, interacts with SsaM, an SPI-2-encoded protein, and is necessary for the translocation of SPI-2 effector proteins to the target cells, according to some studies.(Uchiya and Nikai, 2005).

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 2, 2021, Pages. 675 - 685
Received 20 January 2021; Accepted 08 February 2021.

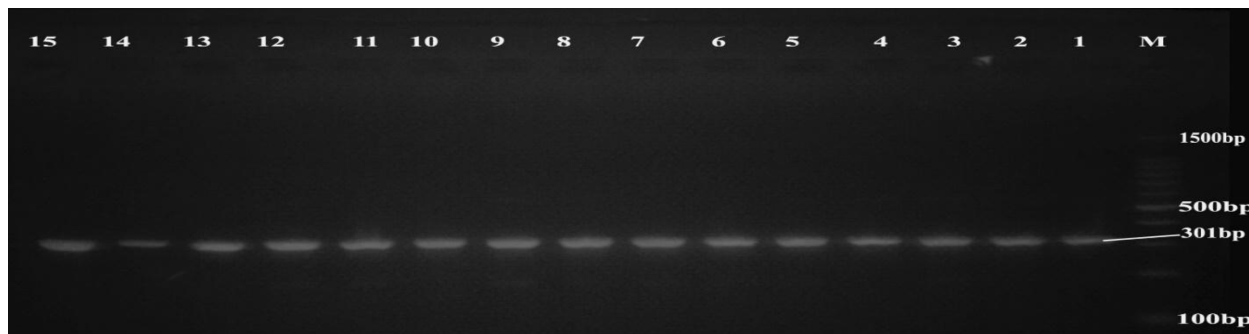

Fig. (4): Agarose gel electrophoresis of PCR product obtained with *Salmonella* strains using *spiC* - specific primers. lanes 1-15 represent the identified *spiC* gene 301 bp, Lane M represent 100bp DNA ladder. White line represents PCR amplicon.

Moreover, molecular detection of *orfL* gene was done using specific primer. It was found that *orfL* gene detected in 34 isolates (100%) of *S. Typhi* isolates with long length (323 bp) as shown in **figure (5)**.These results clearly indicated that total number of clinical isolates of *S. typhi* were positive for *orfL* gene. The presence of *orfL* among *all S. typhi* was in agreement with previous research conducted by Liaquat *et al.*, (2018) who reported the presence of *orfL* gene 100% among *S. Typhi* isolates collected in Pakistan from 2004 to 2013.

Within SPI-4 six ORF that are regulated by a single operon, and have a role in the early contact with the intestinal epithelium as well as long-term perseverance. The SPI-4 locus is approximately 27 base pairs in size. SPI-4 contributes to numerous potential virulence factors, including the putative type I secretion system, and Sic E, which are complicated in the process of adhesion to epithelial cells, though the role of SPI-4 in Salmonella virulence is still not clear. SPI-4 was reported to be conserved across Salmonella serovars(Singh *et al.*, 2018).

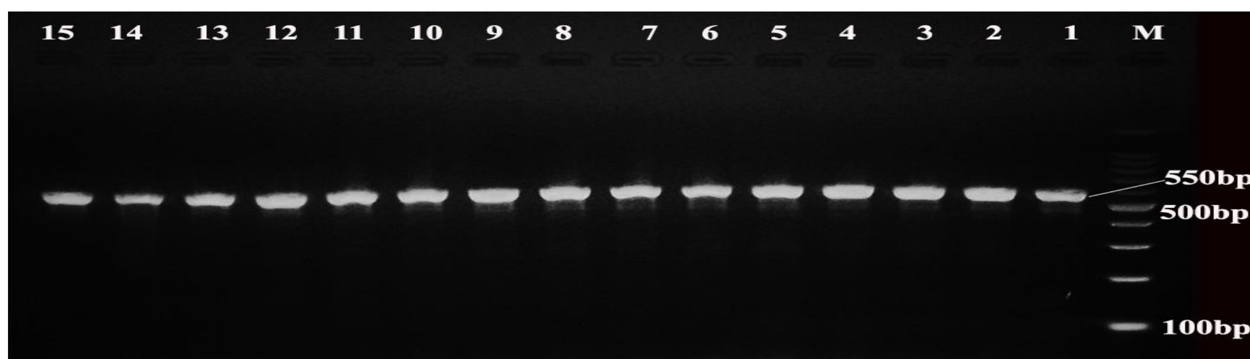

Fig. (5): Agarose gel electrophoresis of PCR product obtained with *SalmonellaTyphi* using *orfL*-specific primers. lanes 1-15 represent the identified *orfL* gene 323 bp, Lane M represent 100bp DNA ladder. White line represents PCR amplicon.

CONCLUSIONS

In conclusion, the present research demonstrated that typhoid fever caused by S. Typhi, which was isolated from infected individuals; most of themhad virulence factors which increased the pathogenicity of the fever, as well as the presence of typhoid toxin. This plays a part in the seriousness of the typhoidal disease, as the human carrier in his blood causes the coding gene of toxin and invasion proteins to kill the body cells. The prevalence of virulence genes among *S. Typhi* is high, which influences bacteria's phenotype and genetic pattern.This gene could be effective in the invasiveness of the *S. Typhi* strain.

REFERENCES

1. AL-Oqaili, N. A. D. (2019) ‘Detection of some virulence factors of Salmonella typhi isolated from patients’ blood by PCR and phylogenetic tree’, *Periodicals of Engineering and Natural Sciences*, 7(4), pp. 1915–1923. doi: 10.21533/pen.v7i4.941.
2. Alzwghaibi, A. B. *et al.* (2018) ‘Rapid molecular identification and differentiation of common Salmonella serovars isolated from poultry, domestic animals and foodstuff using multiplex PCR assay’, *Archives of Microbiology*, 200(7), pp. 1009–1016. doi: 10.1007/s00203-018-1501-7.
3. Baker, S. *et al.* (2005) ‘Detection of Vi-negative Salmonella enterica serovar typhi in the peripheral blood of patients with typhoid fever in the Faisalabad Region of Pakistan’, *Journal of Clinical Microbiology*, 43(9), pp. 4418–4425. doi: 10.1128/JCM.43.9.4418-4425.2005.
4. Beuzón, C. R. *et al.* (2000) ‘Salmonella maintains the integrity of its intracellular vacuole through the action of SifA’, *EMBO Journal*, 19(13), pp. 3235–3249. doi: 10.1093/emboj/19.13.3235.
5. Brawn, L. C., Hayward, R. D. and Koronakis, V. (2007) ‘Salmonella SPI1 Effector SipA Persists after Entry and Cooperates with a SPI2 Effector to Regulate Phagosome Maturation and Intracellular Replication’, *Cell Host and Microbe*, 1(1), pp. 63–75. doi: 10.1016/j.chom.2007.02.001.
6. Bunyan, I. A. and Obeis, A. A. (2019) ‘Molecular study about some genes among salmonella typhi isolates un Hilla / Iraq’, *International Journal of Drug Delivery Technology*, 9(2), pp. 264–271. doi: 10.25258/ijddt.9.2.24.
7. Ellermeier, C. D. and Slauch, J. M. (2006) ‘The Genus Salmonella’, in Dworkin, M. et al. (eds) *The Prokaryotes: Volume 6: Proteobacteria: Gamma Subclass*. New York, NY: Springer New York, pp. 123–158. doi: 10.1007/0-387-30746-X_7.
8. Gassama-Sow, A. *et al.* (2006) ‘Characterization of virulence factors in the newly described Salmonella enterica serotype Keurmassar emerging in Senegal (sub-Saharan Africa)’, *Epidemiology and Infection*, 134(4), pp. 741–743. doi: 10.1017/S0950268805005807.
9. Ben Hassena, A. *et al.* (2021) ‘Virulence and resistance genes profiles and clonal relationships of non-typhoidal food-borne Salmonella strains isolated in Tunisia by whole genome sequencing’, *International Journal of Food Microbiology*, 337, p. 108941. doi: 10.1016/j.ijfoodmicro.2020.108941.
10. Holt, P. S. *et al.* (2011) ‘The impact of different housing systems on egg safety and quality’, *Poultry science*, 90(1), pp. 251–262.

11. Hu, X. *et al.* (2017) 'Vi capsular polysaccharide: Synthesis, virulence, and application', *Critical Reviews in Microbiology*, 43(4), pp. 440–452. doi: 10.1080/1040841X.2016.1249335.
12. Hughes, L. A. *et al.* (2008) 'Characterisation of Salmonella enterica serotype Typhimurium isolates from wild birds in northern England from 2005-2006', *BMC Veterinary Research*, 4, pp. 1–10. doi: 10.1186/1746-6148-4-4.
13. Hyeon, J.-Y. *et al.* (2017) 'Genomic variation of Salmonella Typhimurium and dynamics of epidemics'.
14. Johnson, R. (2018) 'Investigating the globally dominant Salmonella Typhi haplotype H58 – an insight into the pathogenesis of typhoid fever'.
15. Johnson, R., Mylona, E. and Frankel, G. (2018) 'Typhoidal Salmonella: Distinctive virulence factors and pathogenesis', *Cellular Microbiology*, 20(9), pp. 1–14. doi: 10.1111/cmi.12939.
16. Kaur, J. and Jain, S. K. (2012) 'Role of antigens and virulence factors of Salmonella enterica serovar Typhi in its pathogenesis', *Microbiological Research*, 167(4), pp. 199–210. doi: 10.1016/j.micres.2011.08.001.
17. Lee, A. H., Zareei, M. P. and Daefler, S. (2002) 'Identification of a NIPSNAP homologue as host cell target for Salmonella virulence protein SpiC', *Cellular microbiology*, 4(11), pp. 739–750.
18. Lee, K. M. *et al.* (2015) 'Review of Salmonella detection and identification methods: Aspects of rapid emergency response and food safety', *Food Control*, 47, pp. 264–276. doi: 10.1016/j.foodcont.2014.07.011.
19. LEGBA, B. *et al.* (2017) 'Pathogenicity, Epidemiology and Virulence Factors of Salmonella species: A Review', *Notulae Scientia Biologicae*, 9(4), pp. 460–466.
20. Liaquat, S. *et al.* (2018) 'Virulotyping of Salmonella enterica serovar Typhi isolates from Pakistan: Absence of complete SPI-10 in Vi negative isolates', *PLoS Neglected Tropical Diseases*, 12(11), pp. 1–20. doi: 10.1371/journal.pntd.0006839.
21. Lin, D. Y. *et al.* (2011) 'Biochemical and structural studies of a HECT-like ubiquitin ligase from Escherichia coli O157: H7', *Journal of Biological Chemistry*, 286(1), pp. 441–449.
22. Parvathi, A. *et al.* (2011) 'Comparative virulence genotyping and antimicrobial susceptibility profiling of environmental and clinical salmonella enterica from Cochin, India', *Current Microbiology*, 62(1), pp. 21–26. doi: 10.1007/s00284-010-9665-7.
23. Pincus, D. H. (2010) 'Microbial identification using the bioMérieux VITEK® 2 system', *Encyclopedia of Rapid Microbiological Methods*, pp. 1–32.
24. Pons, B. J., Vignard, J. and Mirey, G. (2019) 'Cytolethal distending toxin subunit B: A review of structure-function relationship', *Toxins*, 11(10), pp. 1–20. doi: 10.3390/toxins11100595.
25. Sánchez-Jiménez, M. M. *et al.* (2010) 'Distribution of pathogenicity islands among Colombian isolates of Salmonella', *Journal of Infection in Developing Countries*, 4(9), pp. 555–559. doi: 10.3855/jidc.670.
26. Santander, J. M., Roland, K. L. and Curtiss III, R. (2008) 'Regulation of Vi capsular polysaccharide synthesis in Salmonella enterica serotype Typhi', *Journal of infection in developing countries*, 2(6), p. 412.
27. Shaheed, L. *et al.* (2019) 'Comparative studies between using antimicrobial effect with silver

nano particles effects for e. coli', *Plant Archives*, 19(MIC), pp. 1872–1876.

28. Shotland, Y., Krämer, H. and Groisman, E. A. (2003) 'The Salmonella SpiC protein targets the mammalian Hook3 protein function to alter cellular trafficking', *Molecular Microbiology*, 49(6), pp. 1565–1576. doi: 10.1046/j.1365-2958.2003.03668.x.
29. Singh, Y. *et al.* (2018) 'Virulence System of *Salmonella* with Special Reference to *Salmonella enterica*', *Salmonella - A Re-emerging Pathogen*. doi: 10.5772/intechopen.77210.
30. Sun, N. *et al.* (2019) 'Screening and Identification of Key Genes for the Survival and Multiplication of Salmonella typhimurium in the Host', pp. 1–36. doi: 10.21203/rs.2.18938/v1.
31. Thakur, R. *et al.* (2019) 'Prophylactic potential of cytolethal distending toxin B (CdtB) subunit of typhoid toxin against Typhoid fever', *Scientific Reports*, 9(1), pp. 1–12. doi: 10.1038/s41598-019-54690-1.
32. Uchiya, K. I. and Nikai, T. (2005) 'Salmonella pathogenicity island 2-dependent expression of suppressor of cytokine signaling 3 in macrophages', *Infection and Immunity*, 73(9), pp. 5587–5594. doi: 10.1128/IAI.73.9.5587-5594.2005.
33. Uchiya, K. I. and Nikai, T. (2008) 'Salmonella virulence factor SpiC is involved in expression of flagellin protein and mediates activation of the signal transduction pathways in macrophages', *Microbiology*, 154(11), pp. 3491–3502. doi: 10.1099/mic.0.2008/021667-0.
34. Valcheva, V. *et al.* (2015) 'Molecular Characterization of Salmonella Typhi Isolated from Typhoidial Humans', *Medical Journal of Babylon*, 12(3), pp. 646–652.
35. Virlogeux, I. *et al.* (1995) 'Role of the viaB locus in synthesis, transport and expression of Salmonella typhi Vi antigen', *Microbiology*, 141(12), pp. 3039–3047.
36. Wang, Y. *et al.* (2019) 'Controversy Surrounding the Function of SpiC Protein in Salmonella: An Overview', *Frontiers in Microbiology*, 10(August). doi: 10.3389/fmicb.2019.01784.
37. Xie, X. *et al.* (2010) 'Expression of tviA is transiently repressed by Hfq in Salmonella enterica serovar Typhi at hyperosmotic stress', *Microbial Pathogenesis*, 49(1–2), pp. 54–57. doi: 10.1016/j.micpath.2010.03.011.
38. Zhang, Ying *et al.* (2018) 'Reciprocal Regulation of OmpR and Hfq and Their Regulatory Actions on the Vi Polysaccharide Capsular Antigen in Salmonella enterica Serovar Typhi', *Current Microbiology*, 75(6), pp. 773–778. doi: 10.1007/s00284-018-1447-7.