Journal of Al-Nahrain University Science Vol.١٧ (٤), December, ٢٠١٤, pp.١٥٤-١٦١

Gene Expression of Heat Shock Protein (hsp٩٠) Using RT-PCR for Local Isolate of *Salmonella*

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Abstract

Six bacterial isolate were obtained for the genus *Salmonella* from the central health laboratory ministry of health Baghdad. Then these isolated were identified serologically by antisera test, from statins serum Institut (SSI) using the agglutination reaction (Antigen-antibody complex). The hsp٩٠ gene was identified for the studied isolates by specific primers for gene amplification using PCR technique to include nucleotide sequence up and down stream gene. Results of electrophoresis on agarose gel revealed that the approximate size of the gene is about ١٦١bp.The intensity of gene expression hsp٩٠ for *Salmonella Typhimurium* measured by CT when the bacterial isolate was exposed to different temperatures (٣٠,٤٠,٤٥,٥٠ and ٥٥) ºC using qRT-PCR result showed a regular increasing of gene expression with temperature, untile a maximum intensity observed at ٥٠ ºC. However the intensity at ٥٥ ºC was statistically insignificant. The impact of UV light at ٣٢٠nm on gene expression for hsp٩٠ on the *Salmonella Typhimurium* has been also studied and the results showed significant increase in the gene expression, compared to the normal growth temperature and to the internal positive control(Reference gene) ١٦sRNA.

Keywords**:** hsp٩٠. SPI. *Salmonella* serovars.

Introduction

Salmonella typhi is an important intracellular pathogen. Among the more than ٢,٣٠٠ closely-related *Salmonella* serovars bacteria recognized, *S. typhi* is the only one that is pathogenic exclusively for humans, in whom it causes typhoid or enteric fever [١].

The genome of *S. typhi* is approximately ٥ million base pairs (bp) long and codes for some ٤,٠٠٠ genes of which more than ٢٠٠ are functionally inactive**.** Different strains may also harbor extrachromosomal DNA in the form of plasmids which usually carry virulence or antibiotic resistance genes. The genes for virulence factors cluster in pathogenicity islands (PI) is integrated in to the bacterial chromosome. Non-pathogenic related species of *Salmonella* do not have PIs. PI genes expression is generally limited to specific host compartments [٢].

The genetic control of *Salmonella* virulence is not fully known. However, both plasmid and chromosomal genes are involved. Many of the virulence genes of *S. enteric* are located on pathogenicity islands of the chromosomes, referred to as '*Salmonella* pathogenicity islands' (SPI).

These genes are believed to have been acquired by *Salmonella* from other bacterial species through horizontal gene transfer [٣]. Many efforts have been made to find effective vaccines against *Salmonella* infections, especially in cattle and poultry but also in swine. However, due to the complicated pathogenesis of *Salmonella* infection, no significant breakthrough has been achieved [٤]. Vaccines to control *Salmonella* infections, especially inactivated vaccines, are in use all over the world, Increasing numbers of live vaccines have been developed but most of them are not yet authorised.

Vaccination can play an important role in intervening against *Salmonella* in high-prevalence herds [٥]. HSPs are classified into different families on the basis of their apparent molecular size, structure and function. Those families include HSP١٠٠ and higher molecular weight (MW), HSP٩٠, HSP٧٠, HSP٦٠ (chaperonin) and small HSP [٦]. As already presented, HSPs promise to be a suitable vaccine candidate for use in humans because of their ability to induce a memory T-cell response and having the ability to induce strong immune response even in the absence of adjuvants. Another important feature that HSPs share is that they can directly activate cytotoxic T-lymphocytes without the assistance of T helper cells, which might be

very useful in eliciting immune response even in immunocompromised individuals [٧].

There are a number of techniques used to evaluate the amount of mRNA expression including Northern Blotting, cDNA arrays, in situ hybridization, RNase protection assays, and reverse transcription polymerase chain reaction (qRT-PCR)[٨]. Reverse transcription along with the polymerase chain reaction has proven to be a powerful method to quantify gene expression [٩]. Real-time polymerase chain reaction (qRT-PCR) is rapidly becoming the new method for determining mRNA expression due to its capacity to use up to ١٠٠٠ times less RNA than other knows methods [١٠, ١١].

Aims of Study

The aims of this study were detecting the hsp٩٠ (HtpG) gene by amplifying it using the PCR technique from three isolate of *salmonella*: in addition the effect of some stress factors such as different temperature and ultraviolet ray and detecting the amount of expression by using qRT-PCR technique. The steps to achive the aim were:

١-collection of the local isolate belong to the genus of *salmonella* from the Central Health Laboratory and the isolates identification was repeated using same methods.

٢-using of specific primer for detection of heat shock protein (hsp٩٠) gene by means of PCR technique.

٣-DNA extraction from isolates under study with the installation of high purity optimum conditions for detection of (HSP٩٠) gene expression using qRT-PCR technique.

٤-Study the effect of some stress factors on the gene expression including the temperature at (٣٥ ºC, ٤٠ ºC, ٤٥ ºC, ٥٠ ºC and ٥٥ ºC) and UV ray.

Materials and Methods

Bacterial isolates, Media and Chemicals

Bacteria lisolates used in this study included S. *typhymurium*, S. *typhi*, S. *enteriadis*. *Salmonella* isolates were obtained from health laboratories diagnosed on the XLD and S-S agar, which appeared as black colony. The isolates of *Salmonella* were diagnosed in the health laboratories by the antisera from statins Serum Institute (SSI) and by using this test forms Antigen-antibody complexes (agglutination). *Salmonella typhimurium* used in RNA extraction for quantitative ereal time PCR were grown in ٥ml of BHI-broth (Bioneer, Company) for ١٨hrs.

DNA Extraction

The growth of bacterial isolates *S. typhimurium*, *S.typhi*, *S.enterdia* by incubation for ١٨hrs in BHI broth for extraction DNA using Exiprep ™ ١٦ plus to determine the gene Hsp٩٠ (HtpG). The cell pellet consisted of bacterial isolates after growing and centrifugation for ٥min at ٣٥ºCresuspend (up to ١*١٠^{٩} cells) in ٢٠٠ ul of resuspension buffer. Puncture the cartridge with the hole-punch tool to correspond with the sample number that used. The buffer cartridge was placed, elution tube rack and disposable tip rack on the setup tray, then the steps were completed by the device.

Amplification of HSPG ٩٠ gene

Primers: The primers were selected according to Osman *etal*., (٢٠١٢). These primers amplified the HSPG٩٠ gene the sequences of PCR primers as follows:

(HSP٩٠ F: ٥'-
TACGTTGACCATTGCCGATA -٣'
HSP٩٠R:٥'AGAAGCCTACGCCAAACTGA
٣'١٦sRNAF:٥'CTGTCGTCAGCTCGTGTTG
T٣'١٦sRNA R: ٥'-
CGTAAGGGCCATGATGACTT٣')

All primers were supplied by Alpha DNA Company, Canada.

Conventional PCR (reactions and programs) for detecting the gene hsp ٩٠ (HtpG):

Using ٢٥µL of PCR reaction, ١,٥ µL DNA template (١٠٠ ng/µL) was amplified using ١٢,٥ µL of *Go Taq*® green master mix ٢X (Promega, USA) and ٠,٣٣ µL of each primer of RM ١٧١ primers (١٠ pmol/µL) or ٠,٥ µL of each primer of P٣٥S primers (١٠pmol/µL),up to the final volume ٢٥ µL with nucleases free water. PCR programs were set on Lab net International thermal-cycler (Multigene TM Gradient Thermal Cycler, Korea); Table (١) describes the conditions of these programs [١٢].

Table (١)
The PCR programs conditions.

Steps	*Temperature (°C)*	*Time (min)*
Initial denaturation	٩٤	٥
Denaturation	٩٤	١
Annealing	٦٢	١
Extension	٧٢	١
Final Extension	٧٢	١٠

No. of cycles = ٤٠ cycles between initial denaturation and final extension.

Agarose gel electrophoresis

The PCR products and ١٠٠ bp DNA ladder bands (Promega, USA) were separated using ٢٪ agarose gel electrophoresis, then stained with ethidium bromide and visualized under the ultraviolet light (٣٢٠nm) [١٣]. The molecular size of PCR products were about ١٦١bp for HSP٩٠.

RNA Extraction and RT-PCR

After growing the *S. typhimurium* isolate in BHI by incubation overnight to determine the amount of gene expression,total RNA was extracted from *S. typhimurium* using RNA isolation kit according to the manufacturer's instructions. The absorbencies of RNA samples were checked at ٢٦٠ and ٢٨٠ nm for determination of sample concentration and purity using Nano drop. The ratio of A٢٦٠ to A٢٨٠ values is a measure of RNA purity [١٤]. The RNA concentration was adjusted to ١٠٠ng/µl. Total RNA was reverse-transcribed to cDNA using Super Script II reverse transcriptase (ACCU POWER Roket RT-PCR PreMIX).Total RNA (١٠µg) were mixed with ١ul of each primer and the total volume was made up to ٢٠ul by DEPC.

Gradient PCR for cDNA synthesis:

Each reaction mixture included ١٠ PCR sample mixtures and each sample was subjected to a different annealing temperature following a gradient temperature to determined the proper annealing temperature for each primer site to cDNA synthesis. Total RNA was reverse transcribed to cDNA using (ACCU POWER Roket RT-PCR PreMIX). Total RNA (١-١٢µl) was mixed with ١ul of each primer and the total volume was made up to ٢٠ul by DEPC. The professional gradient thermal cycler and the program was adjusted that as follows:١cycle at ٩٥°C for ٥ minute followed by heating to ٩٥°C for ٣٠ seconds, ٥٠-٦٢°C for ٣٠sec and ٧٢°C for ١ min, ٥٠-٦٠°C for ٣٠ sec. These three steps were repeated for ٣٥ cycles followed by final extension to ٧٢°C for ١ min for ١ cycle. The best annealing temperature was determined after visualizing the product on agarose gel.

Real-time PCR (reactions and program)

The Real-Time PCR reaction was performed using *Accu Power Green Star qPCR PreMix Kit* (Bioneer-Korea). This pre-mix pellet containing all required components for qPCR reaction with the exception of the DNA template and primers. Furthermore, this pre-mix containing SYBR Green dyes for monitoring the amplification process. For each reaction, ٠,٥ µL of each HSP٩٠ primers (١٠ pmol/µL) and ١,٥ µL of DNA (١٠٠ ng/µL) were added. The final volume was adjusted to ٢٠ µL with DEPC-distilled water. The Real-Time PCR program conditions for HSP٩٠ detection were carried out, initial denaturation, at ٩٥°C for ٥ min (١ cycle), followed by ٤٥ cycles of denaturation: at ٩٥°C for ٣٠ sec, annealing: at ٦٢°C for ٣٠ sec, extension: at ٧٠°C for ٣٠ sec and scan the fluorescent of SYBR Green dye. Subsequently, the PCR products exposed to melt by increasing ١°C every ٢ sec starting from ٦٠ up to ٩٥°C to making sure that products specificity All PCR runs, conventional PCR and Real-Time PCR, included negative and positive control samples. To detect any contamination, negative control reaction was set in each PCR experiment, a negative control reaction containing all components of the reaction without DNA template. On the other hand, a positive control was prepared to determine the effectiveness of the conditions of PCR reaction and program. A positive control reaction containing all components of the reaction with DNA template of standard sample.

Results and Discussion

Identification of *Salmonella* isolates

Salmonella Isolates were obtained from centralhealth laboratory and diagnosed in the first stepon the XLD and S-S agar, which

appeared as black colony. Then the diagnosis of isolates were completed by the antisera from statins serum Institute (SSI) using the agglutination reaction (Antigen-antibody complex).

To detect gene hsp٩٠ DNA extraction from (*S. typhimurium*, *S. typhi* and *S. Enterdis)*. by using the Exiprep ™ ١٦ plus Bacteria Genomic DNA kit (Bioneer company). This kit suitable for the extraction of genomic DNA from gram negative bacteria, gram positive bacteria and yeast. Gram positive bacteria and yeast need enzymatic digestion step with lysozyme to make spheroplast. Results showed high DNA concentration with ٣٥٠ ng/ µl, ٤٩٠ ng/ µl and ٣٧٥ ng/ µl for *S. Typhimurium*, *S.typhi* and *S.Enterdis* respectively. The high purity was determined by using the Nanodrope-ND ١٠٠٠. The absorbance at ٢٦٠/ ٢٨٠ gave an optical density ratio of ١,٦٥, ١,٧٥, and ١,٩١ respectively. Several amplification methods such as Uniplex PCR, Nested PCR and Real time PCR were used for detection of bacterial such as *Salmonella* species [١٥].

All tested samples of PCR showed positive results (band with size ١٦١bp), for HSP٩٠ in (Fig.(١)), this result is similar to those of previously published which used different species specific genes to detect gene expression in different Salmonella spp.[١٦]. Also to confirm the DNA integrity to be used in PCR experiments.

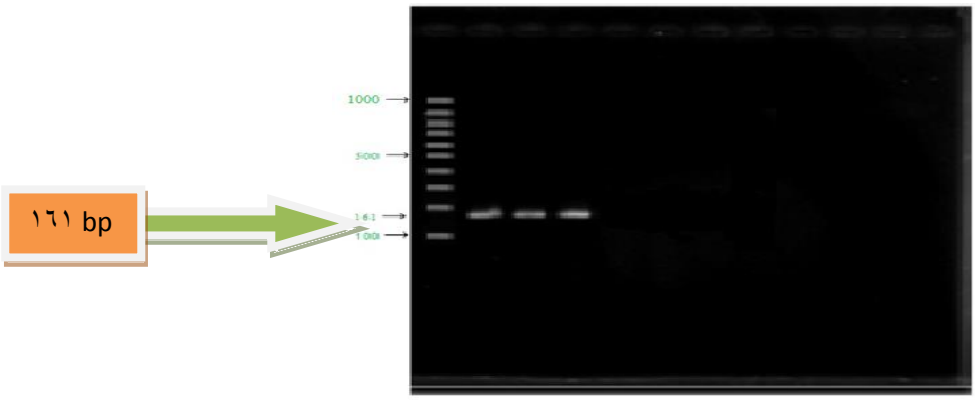

Fig. (١) PCR amplification of HSPG ٩٠. PCR products run in (٢٪) agarose gel using ٠,٥X TBE ٥V/cm for ٢hrs. Lanes M: ١٠٠bp DNA ladder; Lane ١: S. typhimurium, Lane ٢:S.Enteridis, Lane ٣: S.enteridis.

Heat Shock Protein ٩٠ Expression Profiling

Using the Quantitative Real time PCR, Fig.(٢) of the HSP٩٠ in *S. typhimurium* revealed a higher expression after exposure to different temperature at ٥٠ c compared to ٣٥, ٤٠, ٤٥, while the Duucan analysis showed in Fig.(٣) by ٢٩,٠٤ ± ٠,٥٧, ٣٠,٦٠ ± ٠,٧١, ٣١,٨٧ ± ١,٢٢and ٣٢,٦٧ ± ١,٤١ that showed in Table (٢).

Table (٢)
Quantitative analysis of the relative changes in heat shock protein ٩٠ (HSP ٩٠) expression levels using real-time quantitative PCR among different temperature of Salmonella typhimurium.

Temperature (°C)	*CT Value (Mean ± SD)**	
	Treated	*Controls*
٣٥	٢٩,٠٤±٠,٥٧^{B}	١٧,٩٩±٠,١٢^{C}
٤٠	٣٠,٦٠±٠,٧١^{AB}	١٧,١١±٠,٤٧^{C}
٤٥	٣١,٨٧±١,٢٢^{A}	١٧,٣٩±٠,٥٤^{BC}
٥٠	٣٢,٦٧±١,٤١^{A}	١٨,٦٦±٠,٠٩^{B}
٥٥	٣٢,٤٠±٠,٢٥^{A}	٢٢,٠٢±٠,١٩^{A}

* Different letters: Significant difference between means of Rows (Duncan test).

Gene-expression analysis is increasingly important in many fields of biological research. Understanding patterns of expressed genes is expected to provide insight into complex regulatory net works and will most probably lead to the identification of genes relevant to new biological processes, or implicated in disease. RT-PCR provides the simultaneous measurement of gene expression in many different samples for a limited number of genes and is especially suitable when only a small number of cells are available [١٥].

Fig.(٢) shows the melt-curve of the products specificity since SYBR Green Dye may bind to double strand DNA such as specific/non-specific PCR products or primer-dimmers [١٦]. If the PCR products were specified, they would have the same length; thus the melt peaks of these products were at the same temperature while the non-specific PCR products have different temperature depending on the product's length and other factors. These results came to confirm the results of conventional PCR and to detect the SYBR green kit effectiveness to be used in the future for routine work in detecting HtpG gene (hsp٩٠). This method is a rapid process for gene expression, where it gives a reliable result within a few hours without the need for agarose gel electrophoresis analysis [١٧].

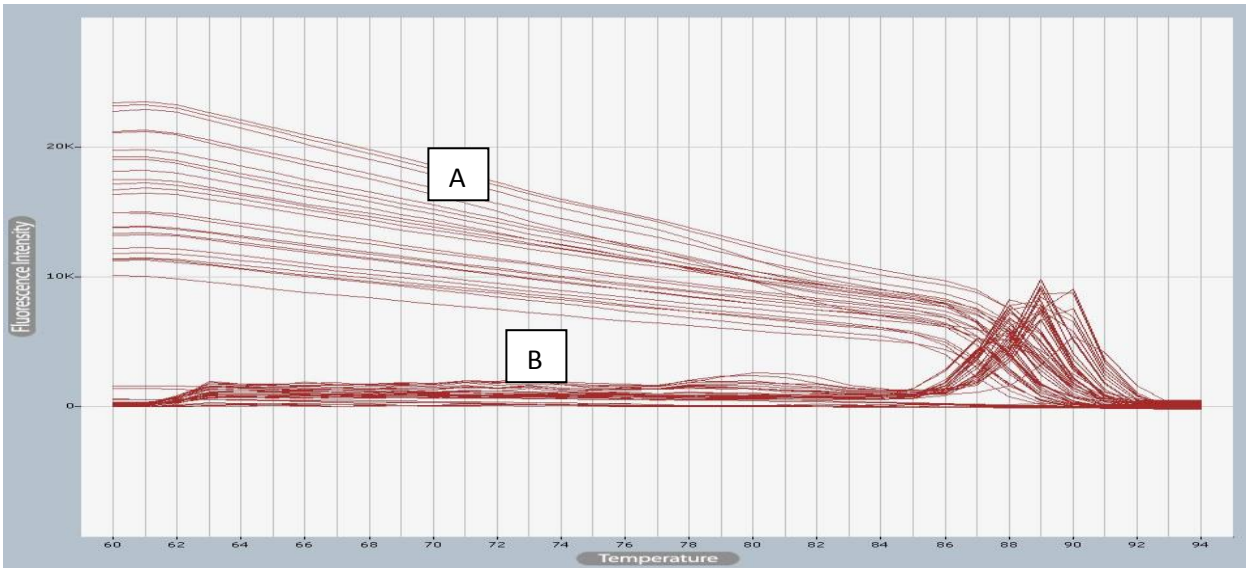

Fig.(٢) Graphs(A) of (hsp ٩٠) gene expression of real time PCR amplification –curve and (B) melt-curve of ١٦sRNA and stright curve negative control.

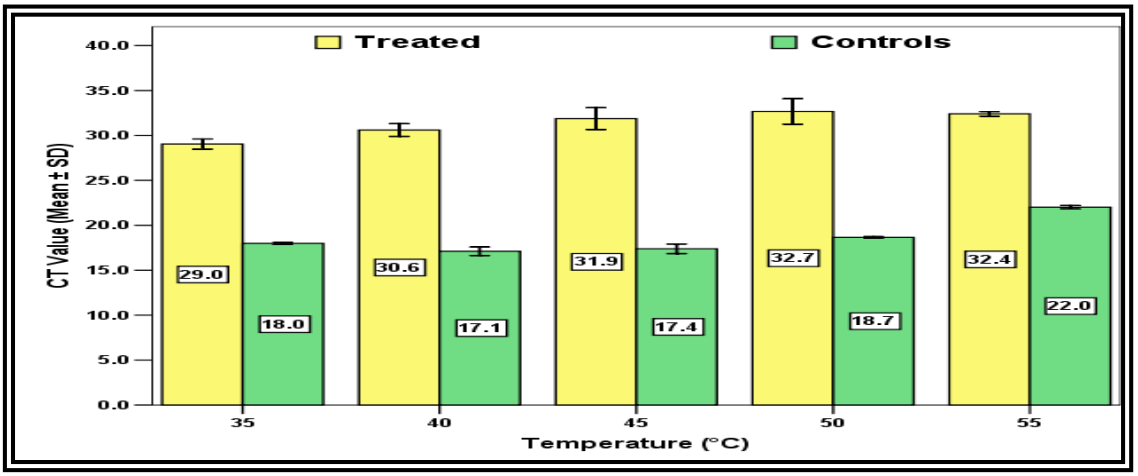

Fig.(٣) Relative gene expression of the treated and control in S.typhimurium for all treatment with temperature for detection of gene expression.
Yellow column: treated sample Hsp ٩٠.
Green column: control ١٦sRNA.

mRNA expression profiling quantification requires normalization and several variables need to be controlled in gene expression analysis [١٨].In the present study, QRT-PCR was used to examine differential expression profiles of some of NCBI identified proteins that are found to correlate virulence among *S.* enteritis disserovars in comparison to ١٦ sRNA as reference gene. QRT-PCR results obtained from measuring the differences in expression level among *Salmonella* pathogenicity [١٩]. This result gives a strong evidence of the specificity of these proteins to *S.* gallinarum which could increase the speculation of its role in host over adaptation and/or virulence among the poultry species. This coincides with the previous results [٢٠].

The *Salmonella* (SPI) ١ and ٢ are two major virulence determinants of *S. enterica*as they encode type III secretion systems (TIIISS) that form syringe-like organelles on the surface of Gram-negative bacteria and enable the injection of effector proteins directly into the cytosol of eukaryotic cells [٢١].

The effect of UV on the gene expression of *S.typhimurium.*

After exposure the of *S. typhimurium* broth to UV(٣٢٠nm) for ١٠min then extraction RNA by using the Gentrapuregen cell kit showed high RNA concentration with ٤٢٠ ng/ µl, with high purity which was determined by using the Nanodrope-ND ١٠٠٠. The absorbance at ٢٦٠/ ٢٨٠ gave an optical density ratio (١,٨) then converted to cDNA by Super Script II reverse transcriptase (Invitrogen). Then Run in RT-PCR the Ct average was ٣١,١١ compare with the average Ct value of the internal positive control (reference gene) the average Ct value ١٤,٤٨. This result mean UV induce hsp٩٠ (HtpG) gene and lead to increase gene expression compared to the normal growth temperature and the internal positive control. Fig.(٣) shows the gene expression after exposure UV.

While the Duncan analysis for gene expression after exposure *S. typhimurium* suspension to UV the result of Ct value ٣١,١٢ ± ٢,١١ and the internal positive control ١٤,٤٧ ± ٠,١٨ that showed in Table (٣) Fig.(٤) showed the results differences of HspG ٩٠ gene expression levels between UV treatment and ٣٥°C treatment showed the HSPG٩٠ gene expression levels difference between UV and ٣٥ °C. this result mean gene hsp٩٠ responds to UV stress and lead to increasing the level of gene expression of gene hsp٩٠ (HtpG).

Table (٣)
Quantitative analysis of the relative changes in heat shock protein ٩٠ (HSP ٩٠) expression levels using real-time quantitative PCR between ٣٥°C temperature and UV of Salmonella Typhimurium.

Temperature (°C)	*CT Value (Mean±SD)**	
	Treated	*Controls*
٣٥	٢٩,٠٤±٠,٥٧	١٧,٩٩±٠,١٢
٣٥+UV	٣١,١٢±٢,١١	١٤,٤٧±٠,١٨
	N.S.	٠,٠٣

Different letters: Significant difference (P≤٠,٠٥) between means of columns (Duncan test).

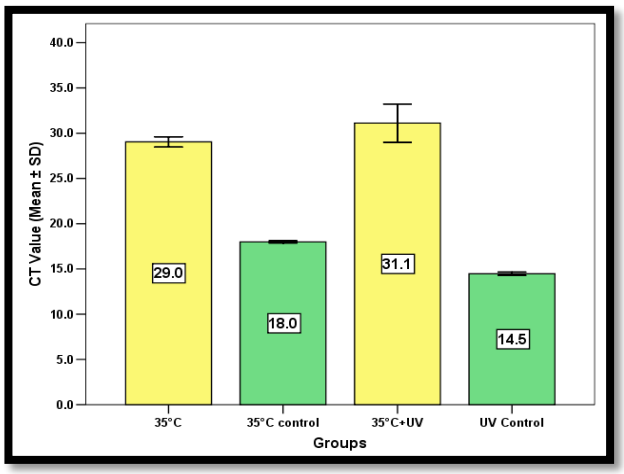

Fig. (٤) The relative gene expression between UV exposure and control (at ٣٥°C).

Conclusions: Based on the results of the present study, the followings conclusions were made: Amplified Htp Ggene of *S.typhimurium, S.typhi and S.enteridis* have a molecular weight of approximately ١٦١ bp with ١٠٠٪ identity as compared with HtpG sequence database found in NCBI Gen Bank. Detection of gene expression (HSP٩٠) of *S. Typhimurium* by qRT-PCR and identified the gene expression induction with increasing of elevated temperature. Based on there result it was found that ٥٠°C was the best temperature for hsp٩٠ gene expression. The same results was observed during exposure *S. typhimurium*

to UV light at ٣٢٠ nm the expression of hsp٩٠ gene was increased.

References

[١] Lian Zhang, X., Jeza, V.T. and Pan, Q. *Salmonella Typhi*: from a Human Pathogen to a Vaccine Vector. *Cellular Molecular* Immunology. ٥(٢):٩١-٩٧,٢٠٠٨.

[٢] Baker, S. and Dougan. G. The genome of *Salmonella enterica*serovar Typhi. Clin Infect Dis.٤٥: ٢٩-٣٣. ٢٠٠٧.

[٣] Van Asten, A.J.A.M and van Dijk, J.E. Distribution of 'classic' virulence factors among *Salmonella* spp. *FEMSImmunol. med. Microbiol.*,٤٤ (٣), ٢٥١-٢٥٩,٢٠٠٥

[٤] Chiu, C.H., Su, L. H., and Chu, C.*Salmonella enterica* serotype Choleraesuis epidemiology, pathogenesis, clinical disease, and treatment. *Clin. Microbiol. Rev.*, ١٧(٢), ٣١١-٣٢٢. ٢٠٠٤.

[٥] Haesebrouck, F., Pasmans, F., Chiers, K., Maes, D., Ducatelle, R. and Decostère, A. Efficacy of vaccines against bacterial diseases in swine.*Vet. Microbiol.*, ١٠٠ (٣-٤), ٢٥٥-٢٦٨,٢٠٠٤.

[٦] Pathan, M.M., Latif, A., Das, H., Siddiquee, G.M. and Khan, M.J.Z. Heat Shock Proteins and their clinical Implications. Veterinary World. ٣(١٢):٥٥٨-٦٠. ٢٠١٠.

[٧] Paliwal, P.K., Bansal, A., Sagi, S.S.K. and Sairam, M. Intraperitoneal immunization of recombinant HSP٧٠ (DnaK) of *Salmonella* Typhi induces a predominant Th٢ response and protective immunity in mice against lethal *Salmonella* infection. *Vaccine*, ٢٩: ٦٥٣٢-٦٥٣٩. ٢٠١١.

[٨] Giulietti, A., Overbergh, L., Valckx, D., Decallonne, B., Bouillon, R., and Mathieu, C. An overview of real-time quantitative PCR: applications to quantify cytokine gene expression. Methods, ٢٥:٣٨٦–٤٠١. ٢٠٠١.

[٩] Osman, K.M., Radwan, M.I., Zeinab, M.S., Bakry, M.A. and Hakim, A.S. Protein Expression Diversity amongst Different Serovars of *Salmonella enteric* Using Quantitative Real Time PCR Global Veterinaria, ٨ (٦): ٥٦٥-٥٧٣. ٢٠١٢.

[١٠] Bustin, S. A. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. J. Mol. Endocrinol, ٢٥:١٦٩-١٩٣. ٢٠٠٠.

[١١] Hashimoto, M., P. C., Chen, M. W., Mitchell, D. E., Nikitopoulos, S. A., Soper, and Murphy M. C. Rapid PCR in a continuous flow device. Lab Chip. ٤(٦): p. ٦٣٨-٦٤٥. ٢٠٠٤.

[١٢] Mahmood, T., Safdar, W., Abbasi, B. H. and Nagvi, N. S. An overview on the small heat shock proteins. African Journal of Biotechnology, ٩: ٩٢٧-٩٤٩. ٢٠١٠.

[١٣] Sambrook, J. and Russell, D.W. Molecular Cloning: A Laboratory Manual. ٣rd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. ٢٠٠١.

[١٤] Chadfield, M.S., Brown, D.J., Aabo, S., Christensen, J.P. and Olsen, J.E. Comparison of intestinal invasion and macrophage response of Salmonella Gallinarum (SG) and other host-adapted Salmonella enteric serovars in the avian host. Vet. Microbiol., ٩٢: ٤٩-٦٤,٢٠٠٣.

[١٥] John, M., McMenamy, M., Hjertner, B. and McNeilly, F.A. Uttenthal. Sensitive detection African swine fever virus using real-time PCR with a ٥_ conjugated minor groove binder probe. Journal of Virological Methods ١٦٨ (٢٠١٠) ١٤١–١٤٦. ٢٠١٠.

[١٦] Hapfelmeier, S., Ehrbar, K., Stecher, B., Barthel, M., Kremer, M. and Hardt, W.D. Role of the *Salmonella* Pathogenicity Island ١ Effector Proteins SipA, SopB, SopE and SopE ٢ in *Salmonella* enteric Subspecies ١ Serovar Typhimurium Colitis in Streptomycin-Pretreated Mice. Infect. Immun., ٧٢: ٧٩٥-٨٠٩. ٢٠٠٤.

[١٧] Vidhya, S.S., Ramanjini Gowda, P.H., Yogendra, K.N., Ningaraju,. M. and Salome, T. Detection of genetically modified cotton seeds using PCR and real-time PCR. Indian J. Biotechnol., ١١: ١٧٦-١٨١. ٢٠١٢.

[١٨] Rizzi, A., Sorlini, C. and Daffonchio, D. Practicality of detection of Genetically Modified Organisms (GMOs) in food. Agric. Biotech. Net., ٦: ١-٩. ٢٠٠٤.

[١٩] Osman, K.M., Ali, M. M., Radwan, M.I., Kim, H.K. and Han, J. Comparative Proteomic analysis on *Salmonella* Gallinarum and *Salmonella* Enteritidis exploring proteins that may incorporate host

adaptation in poultry. J. Proteomics, ٧: ٢٨١٥-٢٨٢١. ٢٠٠٩.

[٢٠] Dieye, Y., Ameiss, K., Mellata, M. and Curtiss, R. The *Salmonella* Pathogenicity Island (SPI) contributes more than SPI٢ to the colonization of the chicken by *Salmonella enterica* serovar Typhimurium. BMC Microbiol., doi: ١٠,١١٨٦/١٤٧١-٢١٨٠-٩-٣. ٢٠٠٩.

[٢١] Waterman, S.R. and Holden, D.W. Functions and effectors of the *Salmonella* pathogenicity island ٢ type III secretion system. Cell Microbiol., ٥: ٥٠١-٥١١,٢٠٠٣.

الخلاصة

تم الحصول على ٦ عزلات بكتيرية العائدة لجنس *salmonella* من مختبر الصحة المركزي/ وزارة الصحة بغداد. ثم بعد ذلك تشخيصها سيرولوجيا بواسطة اختبار الضد المصلي (antisera) الى الانواع *S.Typhimurium, S.typhi, S.Enteridis*. حدد جين الصدمة الحرارية (HSP٩٠) للعزلات تحت الدراسة باختيار بادئات متخصصة لتضخيم الجين المذكور باستخدام تقنية تفاعل تضاعف السلسلة (PCR) لتتضمن التتابعات النيوكليوتيدية الواقعة اعلى واسفل مجرى الجين. اظهرت نتائج الترحيل الكهربائي على هلام الاكاروز ان الحجم الجزيئي التقريبي لجين بروتين الصدمة الحرارية ٩٠ كان ١٦١ زوجا قاعديا.

تم تحديد شدة التعبير الجيني لجين HSP٩٠ العائد لعزلة *S.Typhimurium* مقاسا بتردد العتبة (CT) عند تعريض العزلة البكتيرية الى درجات حرارة مختلفة (٣٠،٤٠،٤٥،٥٠،٥٥)^{o}C وباستخدام تقنية تفاعل تضاعف السلسة الكمي (qRT-PCR) اظهرت النتائج زيادة مضطردة لشدة التعبير الجيني متوافقة مع الزيادة بدرجات الحرارة وصولا الى اعلى شدة عند درجة حرارة ٥٠^{o}C. بينما اظهرت الشدة عند درجة حرارة ٥٥^{o}C زيادة طفيفة غير معنوية احصائيا. كما تمت دراسة تأثير تعريض بكتريا *S.Typhimurium* الى الاشعة فوق البنفسجية بطول موجي ٣٢٠nm على شدة التعبير الجيني للجين hsp٩٠ واظهرت النتائج زيادة معنوية لشدة التعبير الجيني.