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INVESTIGATION OF VIRULENT AND TOXIN GENES EXPRESSION IN SOME *SALMONELLA* SEROVARS

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ABSTRACT: ***Samples collected from patient stool during their course of investigation at hospitals ministry of health, Egypt as well as solid and liquid food samples collected from local markets were examined for presence of Salmonella spp. Isolation and serological identification showed that 92.5% of collected samples contained Salmonella spp belonged to six serovars; Salmonella , enteritidis, Newport, derby, senftenberg and Virchow. These six serovars were checked by PCR for presence of three genes involved in virulence and toxin production, invA, avrA and stn. The gene invA was detected in all serovars, while avrA and stn genes were found in only 4 serovars. In this regard, S.typhimurium was found to harbour the three tested genes. When Salmonella typhimurium grew on normal synthetic medium and natural animal cells (Red blood; RBC and murine epithelial cells; IEC-6) and incubated separately at different temperatures 5, 25, 35 and 40 C for 60 h., they showed regular increase in growth rate with increasing temperature. Also, total RNA concentrations increased up to 24 h. then slightly decreased at each examined temperature specially at 25, 35 and 40 C, but the highest RNA concentration was observed at 40 C. indicating its preparedness in the form of metabolic and cellular activities to cope with temperature stress. Moreover, S. typhimurium seems to be more potent and showed increased ability to induce the expression of enterotoxin gene (stn) by the exposure of temperature elevation specially when grown on murine epithelial cells; IEC-6 comparing with synthetic media or red blood cells.***

Key words: ***Salmonella ; virulent genes; invA gene; avrA gene; enterotoxin gene (stn).***

INTRODUCTION

Gastrointestinal diseases of infectious origin usually arise upon ingestion of contaminated foods or water and can have a wide number of etiological agents, known as enteric pathogens (Kabir *et al*., 2012). Among them, the genus *Salmonella* is of particular clinical relevance in both developed and developing countries, where this pathogen is one of the most common causes of food-borne illness and is a major cause of diarrheal diseases, respectively (Kozak *et al*., 2013). *Salmonella enterica* is one of the most common causes of foodborne infection in human beings and still the main cause of acute diarrhea syndrome, (McClelland *et al*., 2001). *Salmonella enterica* serovar *Salmonella* is a Gram negative, facultative *Bacillus* and a leading cause of human gastroenteritis, which is often associated with non typhoid symptoms such as diarrhoea and abdominal pain (Krtinc *et al.,* 2010). In addition, result of usual occurrence of *Salmonella* organism in high concentration in wastewater and

its long survival period in moist soil, more over cattle grazing on wastewater irrigated pasture could be infected with this microorganism. Furthermore, people could take the infection when drinking milk or eating meat from such infected cattle (Taylor *et al.,* 1996). It has been reported that , about 46 countries including Egypt are using polluted water for irrigation purposes (UNHSP, 2008). However, the use of wastewater in irrigation involves many risks and negative impacts of great importance (Choukr-allah *and Hamdy* 2004). Among them the primary concern is to consumers using leafy green vegetables eaten uncooked and in raw salad dishes (Harris *et al.,* 2003). Nevertheless, fresh leafy green vegetables and their ready to eat salads are recognized as a source of food poisoning outbreaks in many parts of the world (Mercanoglu *et al.,* 2011). Detection methods must be able to detect both species and all serotypes, current methods for detecting *Salmonella* spp. require culturing, followed by a series of presumptive and confirmatory tests that take 5-7 days to complete, the widespread problems caused by *Salmonella* create the need for more rapid detection methods; however, the diversity of the organism has made it difficult to develop antibody-based or nucleic-acid-based methods that can detect every *Salmonella* serotype (FDA, 2015). *Salmonella* enterotoxin (Stn) is a putative virulence factor and causative agent of diarrhoea. It has been shown that the *stn* gene is specifically distributed only in *Salmonella* spp. irrespective of their serotypes (Moore and feist 2007). Biological activities of Stn are important to *Salmonella* virulence, especially acute gastroenteritis. It has shown an enterotoxic activity in a murine ileal loop model. Therefore, Stn is a *Salmonella* virulence factor and is responsible for the enterotoxicity of *Salmonella*. It is possible that Stn will play a pivotal role in special functions of *Salmonella* (Chopra *et al.,* 1999). Nucleic acid based techniques are being employed for the detection of various gene-encoded virulence factors *invA* and *avrA* genes that associated with *Salmonella* pathogenicity islands (SPIs) and *stn* gene involved in enterotoxin production. However, the distribution of these genes among various isolates obtained from biological sources is one of our aims This work allowed to know that in case of human infection the anti-biotherapy can be ineffective, and the *Salmonella* infection can be more severe. Identification of strains with high virulence potential and infectivity in the food at an early stage may help facilitate interventions reducing the risk of dissemination of epidemic strains.

MATERIAL AND METHODES

1. Samples collection and preparation

About 148 samples were collected from the Ministry of Health Laboratory and from the patients stool along their courses at Health Insurance Hospitals and others from local markets, Egypt. The patients' stool sampling were dole in sterile jars out the examined patients one day after sampling under the human stool collection instruction, each 5 grams of stool samples were mixed with 45 ml of buffered peptone water (BPW) and well homogenized to form uniform slurry (Vadivelu *et al*., 1989). Liquid samples were collected approximately 50 ml by using sterile plastic jar for soap, juice or potable water, each 5 gm were mixed with 45 ml of buffered peptone water (BPW) and well homogenized for bacteriological examination according to (APHA, 2015). For solid sampling, the homogenized 100 grams from the leafy greens or other solid food samples were taken by sterile gloved hand and directly transfer into sterile polyethylene bag,

each 5 ml were mixed with 45 ml of buffered peptone water (BPW) and well homogenized using stomacher lab blender for bacteriological examination according to Benjamin *et al*., (1989).

2. Isolation and detection of *Salmonella*

The pre-enrichment step were applied for the prepared samples collected from different places, *Salmonella* resuscitation done by re-inoculated of Luria broth (LB) medium by 1 ml of pre-enrichment culture media and incubated at 40ºC for 12 h. Then the cultures were streaked into modified *Salmonella Shigella* agar plates (Oxoid) and incubated at 40ºC for 24 h. After incubation, the black colonies on the plates were morphological examined and single colony was sub-cultured on xylose lysine deoxycholate XLD agar (Oxoid) at 40ºC for 24 h as purification step according to Koneman *et al*., (1992).

3. Serological examination of *Salmonella* serovars

All purified *Salmonella* isolates were serologicaly examined at Immunity and Serology Unit, Ministry of Health laboratory. The polyvalent and monovalent antisera were used for serotyping detection by slide agglutination technique according to Kauffmann white Scheme (Kauffmann, 1974). The identified serovars used in the current study were routinely cultivated at 40°C in a Luria broth (LB) and stored at -20°C as seed cultures. Later, the exponential phase cells (A_{600} = 0.3) were grown in a minimal E medium containing 0.4% glucose at pH 7.7, which was then exposed to various examined temperatures according to Lee *et al*., (2001).

4. Cultivation of *Salmonella* with animal cells

The *S. Salmonella* serovar as described previously was grown to the exponential phase on LB medium and used to infect animal cells. RBCs or IEC-6 cells (2×10^5) monolayer at a multiplicity of infection of about 1 to 10 as seeds to bacterial cells (2×10^6) and then incubated at the exposed temperatures during the period of investigation according to Liming *and Bhagwat (*2004).

5. Growth and analytical determination

Microbial growth determination, the cell density was measured by the cell dry weight (CDW) and measuring the density absorbance. A vacuum filtration (Buchner, Millipore® 0.2 µm, GR) were used for filtration, microbial filter disks were oven dried at 60°C for 24 h. After drying it were cooled in a glass desiccator, the filters were weighed to calculate the difference between the weight after and before filtration which represents as CDW. The wave length of 600 nm (visible light) were used for optical density measurements, this wave length were found to have the least amount of the growth medium (Fahim *et al*., 2012).

The total DNA and RNA determination

The protocol of DNA Genomic purification kit was applied using the wizard of (Promega Co., Appl Microbiol Biotechnol Madison, USA). The total concentration of DNA was measured by A_{260} direct method (1 ABS = 1 $mg.ml^{-1}$), the absorption at 260 nm was measured using NanoDrop UV-Vis spectrophotometer (Thermo Fisher Scientific ^{TM}). The purified DNA was directly used for PCR amplification or stored at -20°C. While, the total concentration of RNA was measured by A_{230} direct method (1 ABS = 1 $mg.ml^{-1}$), the absorption at 230 nm was measured using NanoDrop UV-Vis spectrophotometer (Thermo Fisher Scientific ^{TM}). The purified RNA was directly used for PCR amplification or stored at -20°C (Switzer *et al*., 1979).

For molecular weight determination, the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) profiles for extra-cellular degrading enzymes were preformed, the gel made of (10%) polyacrylamide and 5% stacking gels. After electrophoresis run, the gels were stained with Coomassie brilliant Blue R-250 staining method and overnight de-stained by 45% methanol and 10% acetic acid (Pant *et al*., 2015). The gel was stained for 12 h in 0.1% coomassie brilliant blur and distained until bands were clearly observed. Gel bands were scanned and analysed using (Gel Doc Bio-Rad system, USA).

6. Molecular detection and expression quantification techniques

The Molecular detection of virulence potential genes involved in *Salmonella* serovars were investigated, the presence of three genes involved in virulence (*invA*, *avrA* and *stn* genes) were estimated in to six serovars of *Salmonella* typhimurium , enteritidis, newport, derby, senftenberg and Virchow from examined sources on account of their higher expansion.

The isolation of *Salmonella* genomic DNAs

DNA was isolated using Wizard®Genomic DNA Purification Kit from Promega (USA), the isolation depends on four steps; step (1) is performed by nuclei lysis solution to lyses cells and nuclei; step (2) digestion of the RNA by RNase; step (3) is to precipitate and remove the cellular proteins by salt precipitation, which leaving only genomic DNA in solution; step (4) is to concentrate the genomic DNA by isopropanol precipitation and dissolution in a TE buffer. PCR technique was prepared by mixing 12.5 µl of RedTaq PCR Master Mix (Takara) from Japan with 1.5 µl of each primer (20 pmol) and 50 ng of DNA template, the reaction was completed to 25 µl with (d. H_2O). The reaction was carried out in Eppendorff thermal cycler for 35 cycles under different temperature conditions depending on the investigated gene. PCR conditions and designed primers. All primers used in detection of virulence associated genes were listed and illustrated in (Table 1). Denaturation was performed for 5 min at 94°C for *invA* primers, for 10 min at 94°C for *avrA* and *Stn* primers. Hybridization at 55°C for 30 sec for invA, for 30 sec at 58°C for *avrA* gene and for 45 sec at 59°C for *Stn* gene. Elongation for 30 sec at 72°C except for 35 sec for *Stn* gene, a final extension was performed for 10 minutes at 72°C for all genes except for *invA* gene (for 7 minutes at 72°C) according to (Hussein *et al*., 2016).

Agarose gel electrophoresis, 1.5% agarose gel was prepared to separate PCR products. Gels were colored by addition of Gel Red 10 (µL/100ml) and were run in an electrophoresis unit using 0.5X TBE buffer pH 8.8 at 100 volts for 1.5 h, gels were photographed using gel documentation system.

Gel extraction after amplification, the extraction and purification of the different fragments were carried out by Gel extraction QIAquick kit from QIAGEN (Hildon, Germany), the QIAquick system is depend on spin-column technology with the properties of selective binding to silica-gel membrane. With each kit, special buffers are provided for DNA efficient recovery and contaminants removal, the silica-membrane bind DNA while contaminants pass through the column and are efficiently washed away, the purified DNA then is eluted with Tris buffer or water.

Table (1): Primers used in detection of virulence associated genes

Target gene	Primer sequence (5`-3`)	Product size (bp)	references
invA	GTGAAATTATCGCCACGTTCGGGCAA	284	murugkar *et al* (2003)
	TCATCGCACCGTCAAAGGAACC		
avrA	CCT GTA TTG TTG AGC GTC TGG	422	huehn
	AGA AGA GCT TCG TTG AAT GTC C		
	TTC TGC TCG CCG CTA TTC G		
Stn	TTG TGT CGC TAT CAC TGG CAA CC	617	oliveira *et al* (2003)
	ATT CGT AAC CCG CTC TCG TCC		
	TTC CGG ATC CTT CAT TGA AAA ATC CCT CT		
	TAGGTAAAGTACTGCCGGAACTG		

Extraction of RNA

The Ambion RNA-later kit (USA) was used for RNA extraction analysis. the storage of tissues can be indefinitely at -20°C or below, the cells are thawed on ice and then resuspended, the cells are centrifuged at -9 ºC for 1 min at 12000 g, after discard the supernatant and the pellet was resuspended in 50 µL of T10 E1 buffer and 10 mg/mL lysozyme and incubated at room temperature for 10 min. The equivalent of 250 µL of zirconium beads which were provided in the kit was added to the tubes, cells resuspended in 350 µL of RNA wiz (provided in the kit) were transferred into tubes with beads. The RNA wiz contains phenol, which allows fragilization of the cells and inhibits the RNases, the whole was homogenized 10 min by vortex to lyse the cells, the samples were then centrifuged at 4ºC for 5 min at 12000 g. After that, a volume of 200 µL was obtained and 200 µL of chloroform were added, then the mix was agitated during 30 s and incubated at room temperature for 10 min, samples were centrifuged at 4ºC for 5 min at 12000 g. The aqueous phase was obtained, 0.5 vol of ethanol 100% was added and the whole sample was stirred vigorously, sample was loaded on the column provided by the kit to purify the RNA. The treatment by DNase was started and the RNA dosage measured by Spectrophotometer (WPA LIGHT WAVE II). 2.5 volume of ethanol 95% was added to the RNA and stored at -80ºC., Revert Aid First Strand cDNA Synthesis Kit (K1621, Fermentas) was used to the RNA reverse transcription into cDNA, PCR analysis was performed by reverse transcription products as templates (Hussein *and fahim* 2017).

The Real Time-PCR mixture of 25 µl of total volume containing 1_X SYBR green Fluorescein Mix, 1 µl of cDNA and 250 nM of each primer. The PCR program were performed at 95 °C for 5 min, 35 cycles with 95°C for 20 S, 45 S at the annealing temperature depending on primer, 72°C for 40 S for 45 cycles. After amplification, a melt curve temperature was used to calculate the threshold cycles, results of RT-PCR were expressed as C_t (cycle threshold) values and relative gene expression was quantified using stn gene primers as previously reported (Hussein *and fahim* 2017).

RESULTS AND DISCUSSION

1. Prevalence of recovered Salmonella

One hundred and thirty seven *Salmonella* spp were isolated from 148 food and patients stools samples by percentage 92.5% of samples collected from the Ministry of Health Laboratory at Health Insurance Hospitals and local markets, Egypt. One hundred seventeen isolates were from one hundred stool samples, eighteen isolates were from forty eight food samples. The *Salmonella* isolates were serological identified to six serovars, *Salmonella* Typhimurium (109 isolates), Enteritidis (13 isolates), Newport (8 isolates), Derby (3 isolates), Senftenberg (2 isolates) and Virchow (2 isolate). However, the most *Salmonella* spp spreading in examined samples were the serovars of *S.* Typhimurium by percentage of 79.6% and eleven samples were found free from *Salmonella* by percentage of 8% according to these data were illustrated in Table (2).

The repeated isolates and serotypes diversity of *Salmonella* from samples in many investigation may reflect wide variance of pollution sources and could be assign to high probability human contact with animal stocking densities with subsequent the heavy uses of contaminated irrigated water. In addition, *S. typhimurium*, *enteritidis* and virchow serovars isolated from agricultural drains water in various studies which were found to be the most predominant among serovars of *Salmonella* from irrigated water such as Egypt, Southern Spain and Portugal (Dioniso *et al*., 2003). In Egypt, *S. typhimurium and enteritidis* serovars which isolated from cattle faeces were found the most common among all *Salmonella* serovars isolates from farm animals. While, the *Salmonella* isolated from cattle serovars are refer to the most dangerous role which could be played by cattle in salmonellosis dissemination to human and other animals (Moussa *et al*., 2013). Another investigation reported that, *S. typhimurium* serotype was the principle issue isolated from infected cattle and caused cereals contamination to by cattle farms when were used as grain stores, in addition that the cow's serovars, the target udder contamination with such microbes by pasture usually causes contamination of milk and others food-borne pathogens (Dwivedi *and Jaykus* 2011).

2. DNA-based detection of virulence associated genes in Salmonella isolates.

The molecular investigation of *Salmonella* serovars (typhimurium, enteritidis, newport, derby, senftenberg and Virchow) widely refer to that occurrence of *invA*, *avrA* and *stn* within the most virulence genes associated with *Salmonella* pathogenicity islands (SPIs), in the screened isolates with the ideal symptoms. The targeted virulence factors were examined *in Don't ced by Salmonella* isolates. The detection of *invA* gene by PCR was amplified a fragment of 284 bp of length in all examined *Salmonella* isolates and a fragment of 422 bp of length for *avrA* gene was amplified from *S.* Typhimurium, Enteritidis, Newport, and Senftenberg isolates. While *stn* gene PCR amplified 617 bp fragment in *S.* typhimurium, enteritidis, newport, derby and virchow isolates. The presence of higher prevalence virulence associated three genes was detected in all isolated *Salmonella* genes conclude *invA* and *avrA* genes which associated with *Salmonella* pathogenicity islands (SPIs), while the *stn* is involved in enterotoxin production which illustrated in left side of Figure (1).

Table (2): The prevalence of recovered *Salmonella* from different samples sources.

Samples			*Salmonella* serovars					
Source	Type	N°	Typhimurium	Enteritidis	Newport	Derby	Senftenberg	Virchow
1	Stool	57	56	7	4	0	0	2
2	Stool	43	42	6	2	0	0	0
2	Food	17	8	0	0	1	1	0
3	Food	31	3	0	2	2	1	0
Total		148/137	109	13	8	3	2	2
Percentage		92.5%	79.6%	9.5%	5.8%	2.1%	1.5%	1.5%

[1] Health Insurance Hospitals [2] Ministry of Health Laboratory [3] Local market

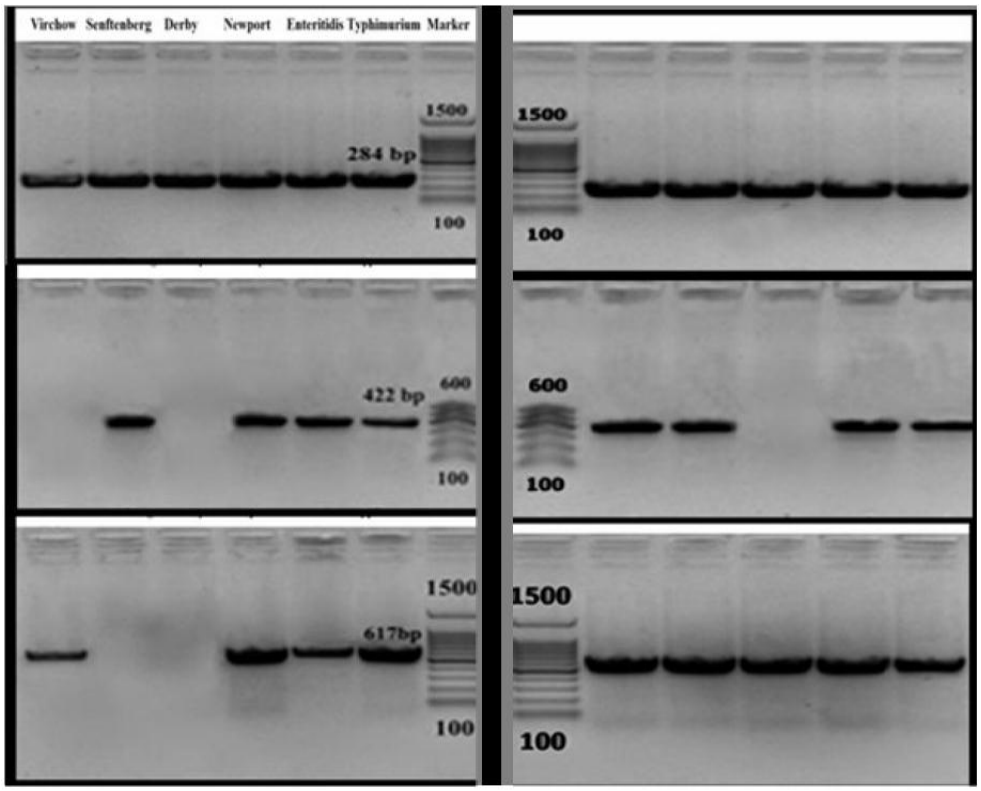

Fig (1): PCR amplification of *invA*, *avrA* and *stn* genes in examined *Salmonella* isolates (left) and *S.* Typhimurium serovar (right)

On other side, amplified *invA*, *avrA* and *stn* genes which involved in virulence associated factors with *Salmonella* (SPIs) were detected as overall values from *most S. Salmonella* isolates which illustrated in right side of Figure (1). Similar results, about the *stn* virulence gene was detected in all *S. Salmonella* isolated from different human sources, but it was detected in only 79 to 82 % of *S.* Typhi and Paratyphi A, respectively (Muthu *et al*., 2014). The occurrence of *invA* gene in whole *S. Salmonella* isolates was agrees with

those reported by (Osman *et al.* 2011) who found *invA* in whole serovars of *S. Salmonella* and enteritidis. While, *avrA* was found only in 51 % of *S. Salmonella* and 48 % of *S. enteritidis* isolates from various sources in Egypt. So, this investigation refer to widespread distribution of *invA*, *avrA* and *stn* virulence associated genes among the isolates which provided a strong evidence on virulent salmonellosis probability risks posed from various environmental sources. In addition, the diversity number of virulence markers distribution between screened *Salmonella* serotypes suggests that different topical species are likely to be responsible for different clinical syndromes in the host within those serovars. Also, to assess the virulence potential of isolated *S. Salmonella* serovars from the deferent sources, the presence of three amplified associated genes were detected in five of the most sever varying isolates of *S. Salmonella* serovars.

3. RNA-based investigation of associated genes in Salmonella isolates.

3.1. RNA quantification and growth recovery

The results of temperature elevation effect on *S. Salmonella* growth with time passing was determined for 0, 12, 24, 36, 48 and 60 hours, The quantification of total RNA concentration from *S. Salmonella* by regarding the individual temperature referred to increased in RNA from zero up to maximum concentration (9.82 mg/ul) at 40 c during the first 24 h of bacterial growth , than gradually decreased, However, the growth at 5°C has shown RNA considerable stability in in total RNA concentrations up to 1.1 µg/µl. The integrity between the total RNA concentrations and growth rates were calculated to achieve the increasing levels of RNA activity by temperature elevation during *S.* typhimurium growth as illustrated on Figure (2).

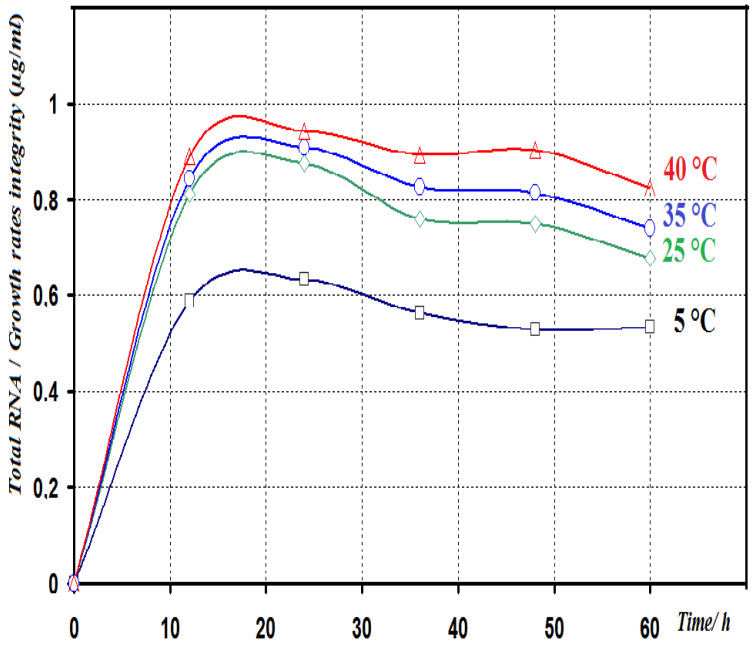

Figure (2): The recovery of temperature elevation effect on the integrity of *S. typhimurium* total RNA with its growth rates.

In fact, the ribosomal-RNA forms 82-90% of total bacterial RNA and represented the active fraction between the cellular activity and bacterial metabolic state in environmental samples. The mRNA levels refers to genes expressions process and the rates of overall turnover activity so, many authors hypothesize that the total RNA determination could be qualitatively indication to the growing mode and active of microbial cells or it just present in idle state (Deutscher, 2006). similarly, our quantification investigation established that the integration between growth rates and the total RNA could be a qualitative coursers of the metabolic activity and then genes promoter activities by comparing the deference growth conditions to speculate genes activity. On other side, the quantity of total RNA were proportionally correlated with the temperature exposure during growth. Whereas, rising total RNA concentrations were detected from *S.* typhimurium which growing at room, optimum and elevated temperature, respectively when compared with growing at low temperature. Also, we found that total RNA were steadily rising during 24 h of *Salmonella* growth, even though, there was decline in optical densities after 36 h following of bacterial growth at optimum and elevated temperature, the activates averages were increased approximately by 6.66 and then 7.69 % more than activates at room temperature, respectively. There was a positive correlation between the concentration of total RNA and *S.* Typhimurium growth in first 12 to hours which represented the exponential phase of bacterial growth curve, the continues increasing in total RNA concentration after 48 h at declined phase growth referred to *Salmonella* cellular activity prolonging and availability of protective environment, thus RNA content remained were more stable and extended active at ambient temperature. In addition that, reduction on cellular activity was observed at 5°C and complete metabolic disruption prevailed at -20°C, could be attributed due to the frozen conditions of cell contents as well as cell death, which can be attributed to cell death caused by frozen conditions of cell contents.(Data not shown)

3.2. QRT-PCR validation and relative gene expression

The gene expression of qRT-PCR amplification was quantified by analyzing the melting curve of *S.* typhimurium for *stn* genes which was a single peak, the analysed expression of reference gene (*stn*) was validated at various temperature exposures during exponential and stationary phases of the growth curve as shown in Figure (3).

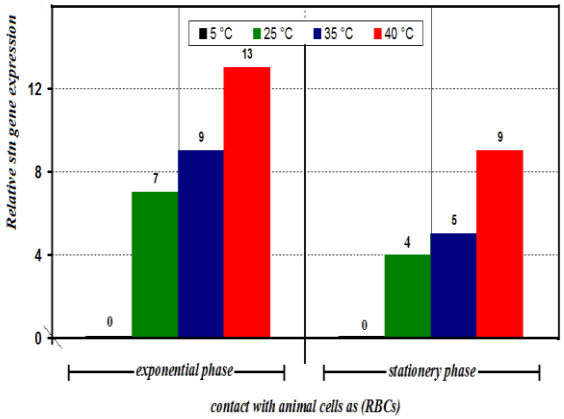

Figure (3): The effect of temperature on relative *S. typhimurium stn* gene expression.

The *stn* target gene differential expression wasn't detectable in expression pattern and did not show any significance difference at 5°C, this data confirmed that *stn* gene was consistent across the triggered by exposure to low temperature. Almost 4 fold up-regulation in *stn* target gene on the exponential phase, while an increasing by 2 fold was observed for it on stationary at room temperature. Also, considerable up-regulation was observed by 5 and 9 fold on exponential phase throughout at 35 and 40°C, respectively after that down-regulation was observed by 3 and 7-fold on stationary phase at 35 and 40°C, respectively during exposure period. The total RNA concentrations resulted from various temperature exposure of *S. Salmonella* were used to investigate *stn* gene expression. the presented data the differences among the expressed *stn* gene at low, room and elevated temperatures; which were significantly down regulated at low temperature and substantially up-regulated at room temperature, nevertheless it was significantly up-regulated at elevated temperature. Also, the expression pattern of *stn* gene of *S. Salmonella* at various temperature remained up-regulated on 12 to 24 h of growth (exponential phase) and my be elongated more than 24 h (stationery phase) thereafter down regulation was observed after 36 h (decline phase). The obtained results strongly referred to the induction of *S. Salmonella stn* target gene by exposture to the examined ambient temperature which could be contributing on pathogenicity vigour and virulent improvement, these results matched with those previously reported by (Lagha *et al*., 2012). However, the logically cause behind the static *stn* gene up-regulation could be returned to the high temperature induction by enhancing the promoter expression of *stn* gene in *Salmonella* (Bang *et al*., 2005).

3.3. QRT-PCR in vivo validation and relative gene expression

The in vivo gene expression of qRT-PCR amplification was quantified by analyzing the melting curve of *S.* typhimurium for *stn* genes when growing in contact with animal cells, On other hand, the analysed expression of *stn* reference gene (*stn*) was validated with various temperature exposures during exponential and stationary phases of growth curve by additions of red blood cells (RBCs) or a murine epithelial cell line (IEC-6) as shown in Figure (4).

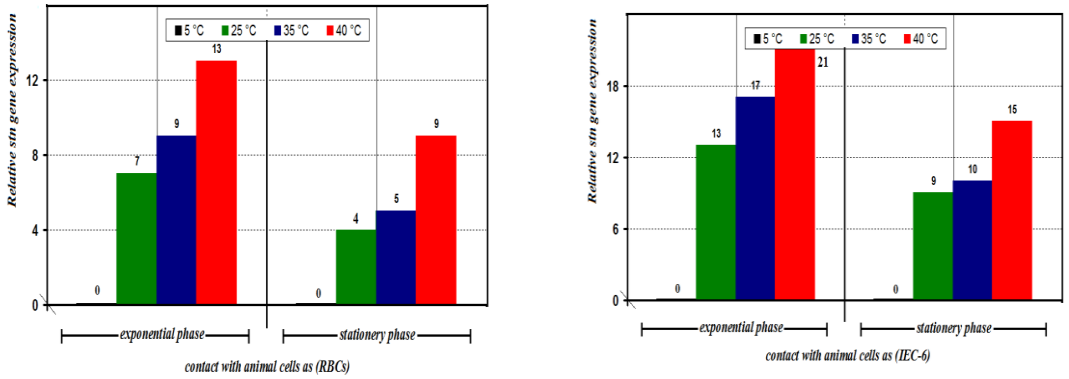

Figure (4): The effect of temperature on relative *S. typhimurium stn* gene expression by contact with animal cells as (RBCs) and (IEC-6)

The differential expression of *stn* gene wasn't detectible within the pattern of target and did not show any significance difference at 5°C, this data confirmed that *stn* gene was consistent across the triggered by exposure to low temperature. Seven fold up-regulation in *stn* genes during the exponential phase, whereas four fold up-regulation were obtained during stationary phase of the growth at room temperature. Considerable up-regulation was observed by 9 and 13-fold during exponential phase of growth throughout 35 and 40°C, respectively after that down-regulation was observed by 5 and 9-fold on stationary phase of growth at 35 and 40°C, respectively during exposure period. On other side, the differential expression of *stn* gene wasn't detectible within the pattern of target genes and did not show any significance difference at 5°C, this repeated data confirmed that *stn* gene was consistent across the triggered by exposure to low temperature. Almost 13-fold up-regulation in *stn* genes during the exponential phase, whereas 9-fold increase was observed for them on stationary phase of growth at room temperature. Also, considerable up-regulation were observed by 17 and 21-fold during exponential phase throughout 35 and 40°C, respectively after that down-regulation was observed by 10 and 15-fold on stationary phase at 35 and 40°C, respectively during exposure period. The same expression pattern of *stn* gene of *S*. typhimurium was the same when contact with animal cells as (RBCs) or (IEC-6) at various temperature as remained up-regulated on 12 to 24 h of growth (exponential phase) and my be elongated more than 24 h (stationery phase) thereafter down regulation was observed up to 36 h (decline phase) Figure (5).

The up-regulated expression values were significally increased when contact with (RBCs) and approximately duplicated when contact with (IEC-6) as indicator for the specificity of *Salmonella* with these type of animal cells which to be similar to the in nature vivo condition. In Earlier investigations concluded that, the method of mRNA isolation as technique for study transcriptional regulation was recently used to investigate whether the target *stn* genes are actually active in a native environment. However, all strains of *Salmonella enterica* carry the *stn* gene but little number of its serovars have been detected phenotypically to exaggerate the enteric toxin (Prager *et al*., 1995). Also, (Mekalanos, 1992) reported that, the *stn* gene was over-expressed after many years of starvation in seawater which in turn is able to contributing more vigour towards its virulent. While, the virulence genes expression might be based on appropriated environmental factors, but it absolutely inadequate under common laboratory cultivation in vitro conditions. However, it must be know that the specific virulence factors specification could be established inner the host under infection in vivo condition. Consequently, the *Salmonella* enumeration in contact with epithelial cells types of IEC-6 cells due to an augmentation in the enterotoxin productivity. And also achieved that, the target *stn* gene promoter expression need to epithelial cells specific induction signal.

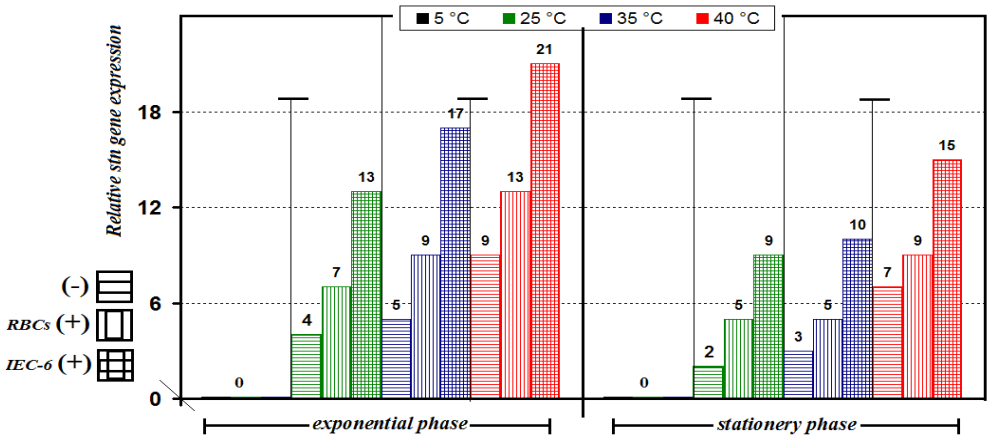

Figure (5): The effect of temperature on relative S. typhimurium *stn* gene expression with and without contact by various animal cells

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دراسات على منظمات التعبير الجينى لبعض بكتيريا التسمم الغذائى (السالمونيلا)

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الملخص العربي:

فحص التعبير الجينى لجينات الضراوة والتسمم فى بعض الانواع السيرولوجيه للسالمونيلا

تم اختبار وجود ميكروب السالمونيلا فى عينات البراز المأخوذه من المرضى اثناء وجودهم بمستشفيات وزارة الصحة (مصر) وكذلك فى عينات الأغذية الصلبة والسائلة المأخوذه من الأسواق المحلية .

تم عزل وتعريف ميكروب السامونيلا بطريقة سيرولوجيه حيث وجد ان ٩٢.٥ % من العينات التى تم فحصها كانت محتويه على ميكروب السالمونيلا وأظهر التعريف السيرولوجيى أن السالمونيلا المعزولة كانت منتمية إلى ٦ أنوع من جنس السالمونيلا وهم :

Salmonella – Enteritidis – Newport – Derby – Senftenberg – Virchow

وأظهر فحص جينات الضراوه تعريف PCR فى الستة أنواع من السالمونيلا أن الجين Inva و Avr و ASTL موجود فى كل الأنواع أما Avra و STN موجودة فى اربعة أنواع فقط وقد وجود أن ***S.Salmonella*** يملك هذه الجينات الثلاثة ، وعند تنمية السالمونيلا على البيئة الصناعية (LB.medium) وكذلك على الخلايا الحيوانية الطبيعية (خلايا الدم الحمراء والخلايا الطلائية لمعدة الفأر) وتحضينها على درجات حرارة مختلفة ٥ ، ٢٥ ، ٣٥ ، ٤٠ درجة مئويه وجد زيادة فى معدل النمو وكذلك فى تركيز RNA كلما زادت درجات الحرارة ، كما وجد أيضا فى التعبير الجينى للجين المسبب للتسمم STN كلما زادت درجة الحرارة ، وقد زاد التعبير الجينى للحد الأقصى عند ٤٠ درجه مئويه عند نمو الميكروب على الخلايا الطلائية بالمقارنة بنموه على البيئات الصناعية .

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English Summary

Studies on promoter expression of toxins genes in *Salmonella*

This work provides the evidence that considerable increase in *Salmonella* activity takes place within 24 h at ambient and above room temp up to 4٠°C. The temperature range for the growth of *Salmonella* spp. is 5 to 40°C, where the optimal temperature range lies in between 35 and 40°C. Exposure to high temperature, *Salmonella* Typhimurium serovar was found to be more sensitive. We provided the evidence that concentration of Salmonella total RNA indicates its preparedness in the form of metabolic and cellular activities to cope with environmental stress while in contact with epithelial cells (IEC-6) led to an increase in the production of toxin. Relative expression of temperature of toxin gene and virulent genes of *Salmonella* reveals both in terms of activation and repression of target genes in diverse expression modes depending on the cellular activity. *Salmonella* Typhimurium seems to be more potent and showed increased ability to induce the expression of enterotoxin gene (*stn*) by the exposure of temperature elevation.