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Research Article

Study on Heat Stress Response in *Salmonella* Typhimurium and *Salmonella* Enteritidis and its Impact on their Attachment to Dressed Broiler Skin Surface

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Abstract

Salmonella is an important food-borne pathogen associated with poultry. Prevalence of *Salmonella* was assessed in fecal swab samples collected from broiler birds of native farms in which 5% samples were found positive by conventional and PCR based methods. Out of 200 fecal swab samples 10 samples were *Salmonella*. Out of these *Salmonella* isolates, 4 were serotyped as *Salmonella* Typhimurium, 5 as untypable and 1 as rough strain. Survival of *Salmonella* Typhimurium and *Salmonella* Enteritidis was assessed at 30, 42 and 50°C for 24 h under laboratory conditions and results revealed that both serotypes survived at 50°C up to 24 h. Expression profiling of genes conferring survival and thermotolerance to these serotypes was assessed at high temperature using real-time PCR with transcripted RNA, after exposure at 42 and 50°C for 10 and 20 min in a water bath. Transcriptional profiling analyzed to study the relative expression of thermotolerance genes (*rpoE*, *rpoH*, *rpoS*, *htrA*, *uspA* and *uspB*) showed over expression of *rpoE*, *rpoH* and *htrA* genes at 5°C in both serotypes. Since *S.* Typhimurium exhibited higher expression of various genes when exposed to 50°C for 20 min, it may have better ability to respond to higher temperatures stress compared to *Salmonella* Enteritidis. Both serovars showed higher attachment to skin after exposure to temperature, which may lead to cross-contamination and foodborne illness. This study will be helpful for the poultry processors to design new intervention strategies for the effective destruction of such type of *Salmonella* cells on dressed poultry carcasses.

Key words: Broiler chickens, *Salmonella* Typhimurium, *Salmonella* Enteritidis, heat stress, gene expression, bacterial attachment, food safety

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INTRODUCTION

Salmonella is one of the most important pathogen of public health significance with poultry as an important reservoir (Mughini-Gras *et al*., 2014) and has been reported as the most common cause of food-borne illness by Food Safety and Inspection Service (FSIS), continues to be major concern to regulatory agencies and the food industry. S*almonella* spp. are causing 1.4 million food-borne illnesses, 15,000 hospitalizations and 400 deaths annually in the United States (Voetsch *et al*., 2004). As per the report of Centers for Disease Control and Prevention (CDC., 2015) among 6,565 (88%) serotyped *Salmonella* isolates in 2014, *S*. Enteritidis and *S*. Typhimurium were the most predominant serovars of *Salmonella*. Foods of animal origin, particularly poultry, cattle and pig are the major vehicles of diseases caused by food-borne pathogens (EFSA and ECDC., 2012; Dhama *et al*., 2013). Among all the foods, poultry accounted 17% food-borne illness outbreaks from 1998-2008 (CDC., 2013). Contaminated poultry meat and poultry products are the most important source for human food poisoning salmonellosis and in some cases it may be fatal (De Freitas *et al*., 2010; Kabir, 2010). Previous studies suggested that among more than 2600, *Salmonella* serotypes presently known, *S*. Enteritidis and *S*. Typhimurium account for the majority of cases of human salmonellosis (O'Regan *et al*., 2008).

Stress hardening and survival of bacterial pathogens at higher temperature has received a great attention of food processors for public health concern in recent years. Some earlier studies suggested that *Salmonella* cultured at higher temperatures prior to heating were more resistant than those grown at lower temperatures (Dega *et al*., 1972). Pre-exposure to sub-lethal temperature at 42°C was found to induce protection in bacterial pathogens leading to development of resistance against subsequent heterogonous stresses and imparted higher virulence in *Salmonella* Typhimurium (Sirsat *et al*., 2011). This adaptation in bacterial pathogens to environmental stresses lead to induced tolerance and stress hardening to cope with stressful conditions (Yousef and Courtney, 2003) resulting in better bacterial survival and tolerance in harsh environments (Wesche *et al*., 2009). Although studies addressing the stress hardening in bacterial populations have been reported (McMahon *et al*., 2007) with increased virulence, acid tolerance and antibiotic resistance, but there appears no study available to explain the cue of genes involved in imparting thermotolerance or thermal stress response during lethal heat stress. Further, it is also essential to assess the changes in attachment potential of such thermal stressed bacteria on dressed poultry skin surface as most the chickens are processed with intact skin.

In the current study, two predominant serotypes of *Salmonella* (Typhimurium and Enteritidis) in poultry (Jinu *et al*., 2014) were used to assess the gene expression scenario of heat stress related sigma factor (*rpoH*), alternative sigma factor *rpoE*, universal stress protein (*uspA* and *uspB*) and *htrA* (serine protease) genes on the exposure of 42 and 50°C for 10 and 20 min to understand the genomic trigger of thermotolerance. This pioneer study will be helpful to improve the knowledge of thermal stress tolerance in *Salmonella* at higher temperature typically used during thermal processing of poultry and effect of heat stress on its attachment. This study will be helpful to expand our knowledge of thermotolerance in pathogens during thermal inactivation at high temperature.

MATERIALS AND METHODS

Sample collection: A total 200 fecal swab samples of broilers from farms located in and around Bareilly region were collected in Buffered Peptone Water (BPW) and were brought to the laboratory under aseptic conditions and then processed for isolation of *Salmonella*.

Isolation of *Salmonella* Typhimurium: Isolation and identification of *Salmonella* Typhimurium from fecal swab samples (200) was carried out. After pre-enrichment of fecal swab samples in BPW, 1 mL aliquot of sample was transferred to 9 mL of tetrathionate enrichment (TT) broth and then incubated at 37°C for 24 h. After enrichment in selective broth, a loop full inoculum was streaked on Hektoen Enteric (HE) agar and the plates were incubated for 24 h at 37°C. Greenish colonies with or without black center were picked up and confirmed biochemically. Biochemical identification was carried out by inoculating the suspected colonies into Triple Sugar Iron (TSI), urea agar, lysine decarboxylase (LDC) broth, Simmon citrate agar, Lysine Iron Agar (LIA) slants and incubated at 37°C for 20-24 h. Isolates presumptively identified as *Salmonella* were sent to the National *Salmonella* Centre (NSC), IVRI, Izatnagar for confirmation and serotyping.

Confirmation of *Salmonella* Typhimurium with PCR: Genomic DNA of all the isolates was extracted by CTAB method as described by Wilson (1987). Detection of *Salmonella* Typhimurium was carried out with targeting the amplification of *fimA* and *stm* 4497 genes. The primers used in this study were found specific for serotype Typhimurium (Table 1) as confirmed by the results of serotyping. The PCR was performed in a thermal cycler (epgradient Mastercycler, eppendorf) with bacterial genomic DNA. The PCR products

Table 1: Details of primer pairs specific for the detection of *Salmonella* Typhimurium

Genes	Primers	Primer sequences	Annealing temperature (°C)	Product size (bp)
fimA	Forward primer	CCGGACGGCGCGACCTTCTC	60-68	86
	Reverse primer	GCGGTTGCCTTATAGCGTGCGGTA		
Stm 4497	Forward primer	CCGCCAATGGGGAGAGATCGTGT	60-68	128
	Reverse primer	GGGTAACGCCTGGCCGCTGGT		

Table 2: Annealing temperatures and amplicon sizes of heat resistance gene primers

Genes	Primers	Primer sequences	Annealing temperature (°C)	Amplicon size (bp)
rpoD	Forward primer	GGCACTGTTGAACTGTTGA	58	118
	Reverse primer	GCAGATAGGTAATGGCTTCC		
rpoE	Forward primer	ACCTGGTTGTATCGTATTGC	58	86
	Reverse primer	GCGTCTACATCACTGGAAG		
rpoH	Forward primer	AGATCGCCCTGGTAATGCAG	58	118
	Reverse primer	TAGCTTTAGCCCCTGTTGGC		
rpoS	Forward primer	GCCGTATGCTTCGTCTCA	58	127
	Reverse primer	TCTTGCGTGGTGTCTTCC		
htrA	Forward primer	CGCCAGCGTGATTAAAGTA	58	139
	Reverse primer	GGAGTCCGCCAGCTTAAT		
uspA	Forward primer	CGTTGATGTGAACTATTCTGAC	58	124
	Reverse primer	GTAGCCAGCGTTGGTAGA		
uspB	Forward primer	CCACCACCAAACCGCATAA	58	124
	Reverse primer	CGCCTGGTATGGTATATCTACG		

were analysed on ethidium bromide stained 1.5% agarose gel in Gel Documentation System (Alphaimager EP, Alpha Innotech, Cell Biosciences, California, USA).

Survivability and growth profiling of *S.* Typhimurium and *S.* Enteritidis at different incubation temperatures: To study the growth profile and survivability at high temperature, *Salmonella* Typhimurium isolated from broiler birds and standard *Salmonella* Enteritidis strain (E-2478) procured from the National *Salmonella* Centre (NSC), IVRI, Izatnagar were used. Briefly, 1 mL culture of each *Salmonella* serotype was inoculated to 100 mL of Luria Bertani broth and then grown at 30°C to mid-log phase (OD_{600} approx. 0.4). Each culture was divided in three parts and incubated at 30, 42 and 50°C for monitoring the growth spectro-photometrically (OD_{600}) as well as survivability at 4 hourly intervals till 24 h.

Heat stress treatment: To assess the effect of thermal stress on gene expression profile of *Salmonella* Typhimurium and *Salmonella* Enteritidis, overnight culture of both serotypes was freshly inoculated in 100 mL LB broth. At mid-log (OD_{600} approx. 0.4) phase, 6 mL culture of both serotypes was pelletized and redissolved in 12 mL PBS (pH-7.4). It was divided into 6 vials (2 mL each). A total of three incubation temperatures (30, 42 and 50°C) and two times (10 and 20 min) were used in the experiment. Each of the six vials was incubated at each temperature and time combination. The vial subjected to 30°C for 10 min was used as control while, vials subjected to 30°C for 20 min, 42 and 50°C for 10 min and 20 min were used as treatment. After heat treatment, each of the culture vials incubated at different time-temperature combination were centrifuged to obtain the pellets which were processed for RNA extraction.

Differential expression of genes conferring thermal resistance: Total RNA was extracted from *Salmonella* Typhimurium and *Salmonella* Enteritidis cultures grown under different heat stress treatments using Thermo Scientific GeneJET RNA Purification Kit (Thermo Scientific). The concentration and purity of total RNA was determined spectro photometrically in Biospectrophotometer (eppendorf). The cDNA synthesis was carried out with same amount of RNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) in a thermalcycler (epgradient Mastercycler, eppendorf) as protocol given by manufacturer. Final products were used as template for amplification of thermotolerant gene expression analysis with qRT-PCR. Specific primer pairs for *rpoE*, *rpoH*, *rpoS*, *htrA*, *uspA*, *uspB* and *rpoD* genes (Table 2) were designed using PrimerPlex2.5 (PREMIER Biosoft (PB), California, United States) software. Critical cycle threshold values were normalized using *rpoD* as a reference gene. Gene amplification and standard curve to determine PCR efficiency were carried out in a thermal cycler (Bio-Rad CFX96™ Real Time System). Relative expressions of target genes at each treatment with control (30°C for 10 min) were calculated by $2^{-\Delta\Delta Ct}$ method (Pfaffl, 2001) using PCR efficiency of primers.

Attachment study of normal and heat stressed *S.* Typhimurium and *S.* Enteritidis: To determine the effect of thermal stress on attachment of *S.* Typhimurium and

S. Enteritidis to dressed chicken surface, bacterial cultures of both serovars were subjected to 50°C for 10 min, prior to inoculation. Heat stressed population of both these serovars were inoculated on dressed chicken skin (5x5 cm) and culture without pre-exposure to heat was used as control. To enumerate the bacterial population present in inoculum, same inocula from heat exposed and normal *S.* Typhimurium and *S.* Enteritidis cultures were serially diluted and pleated on bismuth sulphite agar. After providing 30 min for bacterial attachment to dressed chicken skin inside biosafety II cabinet, skins were transferred in whirl pack bags containing 9 mL PBS (pH 7.4) and pummeled for 2 min. One milliliter of inocula from each bag was serially diluted with sterile 0.1 % peptone water and appropriate dilutions were surface plated onto agar dishes containing bismuth sulphite agar. All plates were incubated at 37°C for at least 48 h prior to counting colonies and results are expressed as CFU per sample.

RESULTS

A total 200 fecal swab samples were subjected to conventional culture method and PCR to detect the *Salmonella* Typhimurium. Conventional culture method revealed that out of 200 fecal swab samples, 5% (10) were found positive for *Salmonella*. Serotyping of suspected isolates revealed that out of 10 *Salmonella* isolates, 40% (4) were *Salmonella* Typhimurium, 5 were untypable and 1 was rough strain. Similarly, PCR detection of suspected isolates also confirmed that out of 10 *Salmonella* isolates, 40% (4) were *Salmonella* Typhimurium (Fig. 1).

Growth profiling of *S.* Typhimurium and *S.* Enteritidis at different incubation temperatures: To study the effect of heat stress on *S.* Typhimurium and *S.* Enteritidis growth, cultures were monitored spectrophotometrically at OD_{600} for 24 h at 4 h interval. *S.* Typhimurium and *S.* Enteritidis did not show any notable difference in their growth pattern at 30 and 42°C, while at 50°C both serovars showed marked decline in growth pattern as compared to growth at 30 and 42°C and both were found to survive at 50°C (Fig. 2).

Relative gene expression in response to thermal stress: The expression profiles of thermotolerance genes expressed under thermal stress conditions in *Salmonella* Typhimurium and *Salmonella* Enteritidis are presented in Fig. 3. As evident by expression profiles at 50°C for 10 min, *rpoE*, *rpoH* and *htrA* genes were significantly upregulated which indicated their major role to induce thermotolerance in

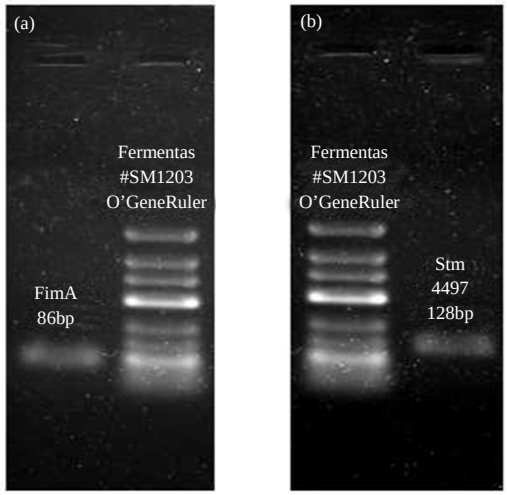

Fig. 1: Amplification of (a) *fimA* and (b) *stm* 4497 genes with the DNA of *S.* Typhimurium

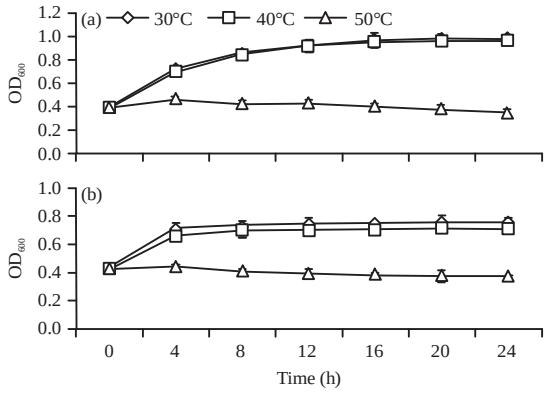

Fig. 2(a-b): Growth profiling of (a) *S.* Typhimurium and (b) *S.* Enteritidis at different temperatures

Salmonella Typhimurium and *Salmonella* Enteritidis, while *rpoS* gene did not showed any significant difference in expression with control at any temperature exposure used in this study. In *Salmonella* Typhimurium, *uspA* gene was significantly upregulated at 30°C for 20 min, 42°C (for 10 and 20 min) while, at 50°C (for 10 and 20 min) it was significantly downregulated. In case of *Salmonella* Enteritidis the expression of *uspA* gene was not significantly different from control. The *uspB* gene was significantly upregulated at 30°C for 20 min and 42°C for 20 min in *Salmonella* Typhimurium, while the expression of this gene did not show any significant difference from control in case of *Salmonella* Enteritidis (Fig. 3). Various genes showed differential expression pattern at different temperatures and time in *S.* Typhimurium and *S.* Enteritidis. Expression of *rpoE*, *htrA* and *uspB* genes

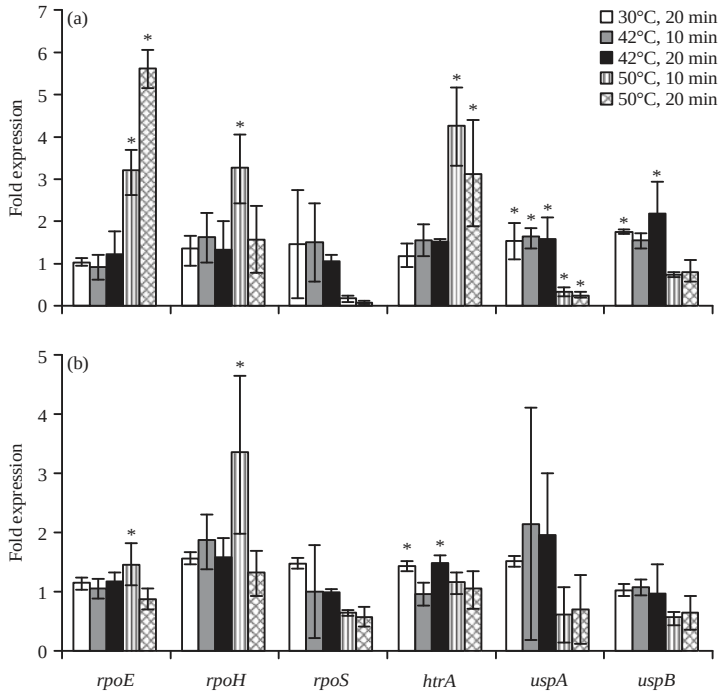

Fig. 3(a-b): Relative expression of stress-related genes in thermal-stressed (a) *S.* Typhimurium and (b) *S.* Enteritidis, *Significant difference with control

were higher in *S.* Typhimurium than *S.* Enteritidis at higher temperatures. Expression of *rpoH*, *rpoS* and *uspA* were almost comparable in both the serotypes. The overall gene expression scenario of stress related genes in both *Salmonella* serovars suggested the major role of *rpoE*, *rpoH* and *htrA* genes in providing thermal resistance to bacterial cells during high temperature stress conditions.

Attachment study of normal and heat stressed *S.* Typhimurium and *S.* Enteritidis: Skin attachment study was performed to assess the effect of pre-exposure of heat on attachment of *S.* Typhimurium and *S.* Enteritidis to dressed chicken skin. Pre-exposure to 50°C for 10 min elevated the attachment of *Salmonella* Typhimurium from 9.41-12.94% as compared to unstressed bacterial cells. Similarly, pre-exposure to 50°C for 10 min increased the attachment of *Salmonella* Enteritidis from 9.39-11.68% (Fig. 4). These findings suggest that pre-exposure of *Salmonella* to higher temperature resulted in increased attachment of bacteria to chicken surface.

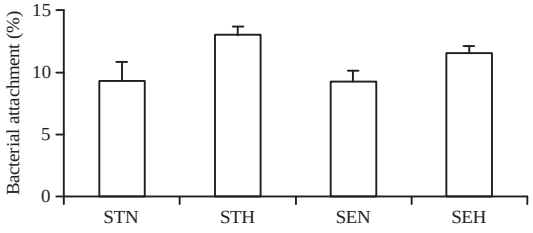

Fig. 4: Attachment of *Salmonella* Typhimurium (ST) and *Salmonella* Enteritidis (SE), Normal (N) and heat stressed (H) cells on chicken skin surface, bars represent standard errors of the means

DISCUSSION

Salmonella is a mesophilic bacterium and can survive and replicate in a temperature range of 15-45°C and is an important food-borne pathogen of public health significance. Contaminated poultry meat and meat products serve as important sources of human foodborne salmonellosis (Kabir, 2010). To assess the prevalence of *Salmonella* Typhimurium in broiler birds, fecal swab samples were collected from native broilers farms to detect them by conventional culture method and PCR. The results of prevalence study revealed that out of 200 faecal swab samples analysed, 10 (5%) samples were positive for *Salmonella*. Similarly, a recent study also reported 5.09% prevalence of *Salmonella* in chickens samples (Jinu *et al*., 2014). Further, the *Salmonella* presence in poultry birds and fecal samples in US has been found in the range of 5-100% which indicates wide variation in prevalence of *Salmonella* in poultry and fecal samples (Bailey *et al*., 2002).

Serotyping of the isolates in the present study revealed 4 isolates (40.0%) of *Salmonella* Typhimurium, 1 rough and 5 (50.0%) untypabale. The PCR based detection of isolates with serovar specific primers also confirm 4 isolates as *Salmonella* Typhimurium. *Salmonella* Enteritidis is also important serovar of poultry origin having great public health significance and it has been found to be predominant serovar in earlier studies carried out in poultry birds.

In this study, gene expression study of stress related genes of *Salmonella* under lethal heat stress conditions was carried out with these two predominant serovares of *Salmonella* in poultry. Growth profiling of *S*. Typhimurium and *S*. Enteritidis at 30 and 42°C did not show any notable difference in growth pattern, however, incubation at 50°C, a declined growth pattern was obtained in both serotypes, but both the serotypes survived up to 24 h, it could be due to the ability of pathogens to increase their survivability in stressful conditions, which are normally lethal to these bacterial populations. Mackey and Derrick (1986) also reported induced tolerance in *Salmonella* after a heat pre-shock at 48°C resulting in an increase in the induced thermotolerance of the bacteria. These sublethal stresses lead to the induction of stress-related genes and cause the surviving pathogen to be harder to subsequent stresses (Kwon *et al*., 2000) and induce the hardiness and ability to survive and thrive under temperature abused foods (Kwon *et al*., 2000). Some earlier studies have also reported survival of *Salmonella* at higher temperature of 76.6°C up to 10 h period and insufficient killing at 85°C after 2 h in specific conditions (McDonough and Hargrove, 1968). This ability of the pathogen has been described as stress adaptation, stress adaptive response, habituation, induced tolerance, acclimatization or stress hardening (Yousef and Courtney, 2003; Begley and Hill, 2015). These kinds of pathogens are potentially dangerous as these are much harder to be killed by normal destruction regimes and may pose a great threat for human health. Induction of thermotolerance in such surviving bacterial populations have been reported by earlier workers with higher D-values in bacteria previously exposed to a sub lethal heat stress as the D-values at 57.8°C was found to be increased by 1-3 min in *S*. Typhimurium exposed to sub-lethal heat (Bunnig *et al*., 1990).

The heat shock response is important during adverse conditions, like sudden temperature shifts and exposure to organic chemicals as well as under non-stress conditions (Hartl, 1996). In present study, exposure of *Salmonella* Typhimurium and *Salmonella* Enetritidis to 50°C induced the expression of *rpoE*, *rpoH* and *htrA* genes while, other genes were relatively down-regulated. Induced expression of these genes at high temperature is due to activation of *rpoE* and *rpoH* genes by the presence of unfolded proteins in the cell envelope (extracytoplasmic stress) and by unfolded cytoplasmic proteins/heat shock, respectively (Bang *et al*., 2005). These finding suggests that *rpoH* and *rpoE* are the main sigma factors which trigger the transcription of thermotolerance genes (Zhao *et al*., 2005). McMeechan *et al*. (2007) reported that *rpoS* and *rpoE* are linked with survival during starvation and osmotic stress conditions, while some studies suggested that during heat stress *rpoS* decrease its expression due to less availability of RNAP core enzyme as the maximal intracellular level of *rpoS* is only 30% that of *rpoD* (Piper *et al*., 2009). Further, this decreased expression of *rpoS* results in decreased expression of *rpoS* dependent genes, when *rpoD* is overproduced (Farewell *et al*., 1998). As the primary sigma factor, *rpoD* directs the transcription of many housekeeping genes (Ishihama, 2000) and is the most abundant sigma factor in *E. coli* with highest affinity for core RNAP (Jishage *et al*., 1996; Maeda *et al*., 2000). The *HtrA* has both protease and chaperone activity and these activities are reciprocally regulated by temperature *in vitro* (Spiess *et al*., 1999). In our study, this over expression of *rpoD* (reference gene) at 50°C resulted in relatively down-regulated fold expression of *uspA* and *uspB* genes, however, these genes were over expressed due to heat stress. In addition, this family also responds to a large variety of stress conditions, including starvation and exposure oxidants, metals and antibiotics etc., for the protection of bacterial DNA (Kvint *et al*., 2003). The key findings of gene expression analysis of this study suggested that *rpoE* and *rpoH* genes acts as a trigger of thermotolerance and *htrA* gene degrade the damaged and misfolded proteins which accumulate in the periplasm when the bacteria are exposed to certain stresses such as high temperature (Spiess *et al*., 1999) in both *Salmonella* Typhimurium and *Salmonella* Enteritidis at higher temperature stress during poultry processing. This may often cause stress hardening in pathogens to subsequent stresses (Yousef and Courtney, 2003) and bacterial survival in harsh environments (Wesche *et al*., 2009). This adaptation in bacterial pathogens to thermal stress may result in bacterial survival even after food processing.

Exposure of *Salmonella* to heat stress also resulted in increased attachment of bacterial pathogen to chicken carcass. Sirsat *et al*. (2011) also reported increased virulence and attachment of *Salmonella* Typhimurium to Caco-2 cells after pre-exposure of 42°C. In present study, exposure of 50°C to both *Salmonella* serovars resulted in increased attachment of pathogen on dressed chicken surface which might be due to increased demand of nutrients during heat stress condition for the higher expression of thermotolerance genes and necessary for survival of the pathogen.

CONCLUSION

The results of this study showed that *S.* Typhimurium as the predominant serovar of *Salmonella* in broiler chickens. Gene expression profiling of stress related genes in *S.* Typhimurium and *S.* Enteritidis indicated the major role of *rpoE*, *rpoH* and *htrA* genes during the exposure at 50°C. Further, pre-exposure to heat also induced the attachment ability in these *Salmonella* serovars. This study is important in widening the knowledge about the induction of stress response during thermal inactivation of a pathogen as imparting survivability and increased attachment ability to dressed skin surface thus, inviting concern to devise interventional strategies to eliminate such pathogens during processing of poultry meat for ensuring human health safety.

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REFERENCES

Bailey, J.S., N.A. Cox, S.E. Craven and D.E. Cosby, 2002. Serotype tracking of *Salmonella* through integrated broiler chicken operations. J. Food Prot., 65: 742-745.

Bang, I.S., J.G. Frye, M. McClelland, J. Velayudhan and F.C. Fang, 2005. Alternative sigma factor interactions in *Salmonella*: σ^E and σ^H promote antioxidant defences by enhancing σ^S levels. Mol. Microbiol., 56: 811-823.

Begley, M. and C. Hill, 2015. Stress adaptation in foodborne pathogens. Annu. Rev. Food Sci. Technol., 6: 191-210.

Bunnig, V.K., R.G. Crawford, J.T. Tierney and J.T. Peeler, 1990. Thermotolerance of *Listeria monocytogenes* and *Salmonella* Typhimurium after sublethal heat shock. Applied Environ. Microbiol., 56: 3216-3219.

CDC., 2013. Surveillance for foodborne disease outbreaks-United States, 1998-2008. Morbidity Mortality Weekly Rep., 62: 1-34.

CDC., 2015. Preliminary incidence and trends of infection with pathogens transmitted commonly through food-foodborne diseases active surveillance network, 10 U.S. Sites, 2006-2014. Morbidity Mortality Weekly Rep., 64: 495-499.

De Freitas, C.G., A.P. Santana, P.H.C. da Silva, V.S.P. Goncalves and M. de Aguiar Ferreira Barros *et al*., 2010. PCR multiplex for detection of *Salmonella* Enteritidis, Typhi and Typhimurium and occurrence in poultry meat. Int. J. Food Microbiol., 139: 15-22.

Dega, C.A., J.M. Goepfert and C.H. Amundson, 1972. Heat resistance of salmonellae in concentrated milk. Applied Environ. Microbiol., 23: 415-420.

Dhama, K., S. Rajagunalan, S. Chakraborty, A.K. Verma, A. Kumar, R. Tiwari and S. Kapoor, 2013. Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: A review. Pak. J. Biol. Sci., 16: 1076-1085.

EFSA and ECDC., 2012. The European union summary report on trends and sources of Zoonoses, Zoonotic agents and food-borne outbreaks in 2010. EFSA J., Vol. 10. 10.2903/j.efsa.2012.2597

Farewell, A., K. Kvint and T. Nystrom, 1998. Negative regulation by RpoS: A case of sigma factor competition. Mol. Microbiol., 29: 1039-1051.

Hartl, F.U., 1996. Molecular chaperones in cellular protein folding. Nature, 381: 571-579.

Ishihama, A., 2000. Functional modulation of *Escherichia coli* RNA polymerase. Annu. Rev. Microbiol., 54: 499-518.

Jinu, M., R.K. Agarwal, B. Sailo, M.A. Wani, A. Kumar, K. Dhama and M.K. Singh, 2014. Comparison of PCR and conventional cultural method for detection of *Salmonella* from poultry blood and faeces. Asian J. Anim. Vet. Adv., 9: 690-701.

Jishage, M., A. Iwata, S. Ueda and A. Ishihama, 1996. Regulation of RNA polymerase sigma subunit synthesis in *Escherichia coli*: Intracellular levels of four species of sigma subunit under various growth conditions. J. Bacteriol., 178: 5447-5451.

Kabir, S.M.L., 2010. Avian colibacillosis and salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. Int. J. Environ. Res. Public Health, 7: 89-114.

Kvint, K., L. Nachin, A. Diez and T. Nystrom, 2003. The bacterial universal stress protein: Function and regulation. Curr. Opin. Microbiol., 6: 140-145.

Kwon, Y.M., S.Y. Park, S.G. Birkhold and S.C. Ricke, 2000. Induction of resistance of *Salmonella* Typhimurium to environmental stresses by exposure to short-chain fatty acids. J. Food Sci. Chicago, 65: 1037-1040.

Mackey, B.M. and C.M. Derrick, 1986. Elevation of the heat resistance of *Salmonella* Typhimurium by sublethal heat shock. J. Applied Bacteriol., 61: 389-393.

Maeda, H., N. Fujita and A. Ishihama, 2000. Competition among seven *Escherichia coli* σ subunits: Relative binding affinities to the core RNA polymerase. Nucl. Acids Res., 28: 3497-3503.

McDonough, F.E. and R.E. Hargrove, 1968. Heat resistance of *Salmonella* in dried milk. J. Dairy Sci., 51: 1587-1591.

McMahon, M.A.S., J. Xu, J.E. Moore, I.S. Blair and D.A. McDowell, 2007. Environmental stress and antibiotic resistance in food-related pathogens. Applied Environ. Microbiol., 73: 211-217.

McMeechan, A., M. Roberts, T.A. Cogan, F. Jorgensen and A. Stevenson *et al*., 2007. Role of the alternative sigma factors σ^E and σ^S in survival of *Salmonella* Enterica serovar Typhimurium during starvation, refrigeration and osmotic shock. Microbiology, 153: 263-269.

Mughini-Gras, L., R. Enserink, I. Friesema, M. Heck, Y. van Duynhoven and W. van Pelt, 2014. Risk factors for human *Salmonellosis* originating from pigs, cattle, broiler chickens and egg laying hens: A combined case-control and source attribution analysis. PLoS One, Vol. 9. 10.1371/journal.pone.0087933

O'Regan, E., E. McCabe, C. Burgess, S. McGuinness and T. Barry *et al*., 2008. Development of a real-time multiplex PCR assay for the detection of multiple *Salmonella* serotypes in chicken samples. BMC Microbiol., Vol. 8. 10.1186/1471-2180-8-156

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res., 29: 2002-2007.

Piper, S.E., J.E. Mitchell, D.J. Lee and S.J. Busby, 2009. A global view of *Escherichia coli* Rsd protein and its interactions. Mol. BioSyst., 5: 1943-1947.

Sirsat, S.A., K.M. Burkholder, A. Muthaiyan, S.E. Dowd, A.K. Bhunia and S.C. Ricke, 2011. Effect of sublethal heat stress on *Salmonella* Typhimurium virulence. J. Applied Microbiol., 110: 813-822.

Spiess, C., A. Beil and M. Ehrmann, 1999. A temperature-dependent switch from chaperone to protease in a widely conserved heat shock protein. Cell, 97: 339-347.

Voetsch, A.C., T.J. van Gilder, F.J. Angulo, M.M. Farley and S. Shallow *et al*., 2004. FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. Clin. Infect. Dis., 38: S127-S134.

Wesche, A.M., J.B. Gurtler, B.P. Marks and E.T. Ryser, 2009. Stress, sublethal injury, resuscitation and virulence of bacterial foodborne pathogens. J. Food Protect., 72: 1121-1138.

Wilson, K., 1987. Preparation of Genomic DNA from Bacteria. In: Current Protocols in Molecular Biology, Ausubel, F.A., R.E. Brent, D.D. Kingston, J.G. Moore and J.A. Seidman (Eds.). John Wiley and Sons, New York, pp: 2.4.1-2.4.5.

Yousef, A.E. and P.D. Courtney, 2003. Basics of Stress Adaptation and Implications in New Generation Foods. In: Microbial Stress Adaptation and Food Safety, Yousef, A.E. and V.K. Juneja (Eds.). CRC Press, Boca Raton, ISBN-13: 9781420012828.

Zhao, K., M. Liu and R.R. Burgess, 2005. The global transcriptional response of *Escherichia coli* to induced σ^{32} protein involves σ^{32} regulon activation followed by inactivation and degradation of σ^{32} *in vivo*. J. Biol. Chem., 280: 17758-17768.