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REDUCTION OF *SALMONELLA* HAZARD IN CHICKEN FILLETS USING VARIOUS MICROBIAL INHIBITORS

GHADA M. MOHAMED[1] and HALA I. SCHARAWEE[2]
[1] Animal Health Research Institute, Assiut Regional Lab, Meat Hygein.
[2] Animal Health Research Institute, Serology Unit.

Received: 27 March 2016; **Accepted:** 29 April 2016

ABSTRACT

A total of 80 samples of chicken fillet were obtained from local markets with different sanitation levels in Assiut city to study the prevalence of *Salmonella* spp. The virulence factor(s) in isolated samovars were detected by PCR. The obtained results showed that the incidence of *Salmonella* spp was7.5%. The isolated *Salmonellae* could be serologically identified as *S.* Entetitidis and *S.* Typhimurium as (2.5%) for each, *S*V irginia and *S.* Finkenworder (1.25%) for each. Results of the virulence genes of *Salmonella* seovars shown that *Inv* A gene was detected in all examined serovars. All serovars were detected to harbor *Stn* gene except *S.* Finkenworder. Regarding *fim* H gene both *S.* Typhimurium serovars and one strain of *S.* Enteritids were positive only. Also this trial was carried out on chicken fillet to determine the effectiveness of using acidified sodium chlorite (ASC) and chlorine on the viability of *S.* Enteritidis by inoculating cubes of the chicken fillet with 10^6 cfu/g, of *Salmonella* Enteritidis and dipping in different concentrations of ASC (400,600 and 900 ppm) (leaving non treated sample as a control) then counted at the 0, 1, 2, and 4 hours of refrigeration storage for the viablecells of *S.* Enteritidis. The maximum reduction of *Salmonella* was (1.5 log cfu/g) occur immediately after treatment at (0h) with concentration 900ppm, while it was 1.1, and 1.3 log cfu/g in concentration 400 and 600 ppm, respectively. After the first hour of storage in refrigerator at (4C°), the reduction values were0.6, 0.9 and1.2 log cfu/g respectively. While, the reduction of *Salmonella* after the second hours were 0.4, 0.7 and 1.0 log cfu/g respectively. Finely when examination of samples after 4 hours, the reduction were 0.2, 0.5 and 0.9 respectively. On the other hand, when used different concentrations of chlorine (20,30 and 40 ppm) on inoculated cubes, then counted at 0, 1, 2, and 4 hours of refrigeration storage for the viable cells of *Salmonella* Enteritidis. The maximum reduction of *Salmonella* to the chicken cubes (3 log cfu/g, compared to control) was immediately occur after treatment (0h) with concentration 40ppm. While, it was 2.4 and 2.6 log cfu/g respectively at concentration 20 and 30 ppm. After the first hour of storage in refrigerator at (4C°), the reduction values were 1.5, 1.7 and 1.8 log cfu/g respectively. While after the second hours, the reduction was 1.4, 1.6 and 1.7 log cfu/g respectively. When examination of samples after 4 hours, the reduction was1.2, 1.4 and 1.5 respectively. None of these antimicrobial agents changed in smell or texture values of chicken breast fillet. Finally the residual of ASC and chlorine were Measured. The public health importance of the organism was discussed and the suggestive measures for control were outlined.

Key words: *Salmonella, Chicken Fillets, Microbial Inhibitors*

INTRODUCTION

Poultry meat and its products are very popular food throughout the world and no wonder since they are delicious, nutritious and considered as a good and cheap source of protein with good flavor and easily digested. They ranked first or second in foods associated with disease in most of the countries all over the world (Ibrahim *et al.,* 2014). There is no primary religious rescission on the consumption of poultry meat (Kim and Day, 2007). Poultry products are highly perishable, and depending on the processing condition, their spoilage varies significantly even under refrigeration (Patsas *et al.*, 2006). It can easily be contaminated with microorganisms because fresh meat is very suitable for microbial multiplication. Meat has high water activity, high in nutrients and readily utilizable low molecular weight substances and is a source of carbon and energy. As a result; fresh meat is a suitable substrate for bacterial multiplication (Hinton, 2000). The major bacterial contamination on chicken includes pathogens such as *Salmonella* spp. (Kim and Day, 2007).

Salmonellae are members of the *Enterobacteriaceae* family. They are gram-negative facultative anaerobic, non-spore-forming coccobacilli. Salmonellae are

Corresponding author: Dr. GHADA M. MOHAMED
E-mail address: ghada02468@yahoo.com
Present address: Animal Health Research Institute, Assiut Regional Lab, Meat Hygein.

usually motile except *S.* Gallinarum and *S.* Pullorum. The genus *Salmonella* has been divided into two species, *Salmonella* enterica with 6 subgroups (I, II, IIIa, IIIb, IV and VI) and *Salmonella* bongori (formerly subsp. V) (Brenner *et al.,* 2000).

Salmonellosis is a foodborne illness caused by infection with *Salmonella* spp http://kidshealth. org/parent/general/sick/germs.html. Not everyone who ingests *Salmonella* bacteria will become ill. Children, especially infants, are most likely to get sick from it (The Nemours Foundation, 2015). It depends upon the health status, age and immune system of the person (Mahajan *et al.,* 2003). However, the infective dose of salmonellosis is variable. Eating food contaminated with approximately 10^5 to 10^6 cells per gram of food causes an illness (Fehlhaber and Janetschke, 1992). A Salmonella infection generally causes nausea, vomiting, abdominal cramps, diarrhea, fever, and headache. Finally, the infection can cause other health problems, like meningitis and pneumonia (The Nemours Foundation, 2015).

Salmonella is one of the most important causes of food borne illness of known etiology in the world. These pathogens are usually present in chicken flocks during production and transferred to non-infected broiler chickens during the subsequent stages of transportation to processing plants, processing and retail (Ajok *et al.,* 2014). Poor hygienic condition during the sub stages of processing may be responsible for the significant increase in *Salmonella*. The sub stages include stunning, slaughter, scalding, defeathering, evisceration, washing, deboning and packing. Cross contamination was more likely to occur on contact with surfaces of the machine containing a higher bacterial load (Bada *et al.,* 2006). In addition, it has been noted that there is a greater population of bacteria on the breast than other edible portions of the chicken carcass making this an important site to control the organism (Kotula and Davis, 1999).

An analysis of a PCR technique to validate sensitivity and specificity 0f culture techniques for detecting *Salmonella* contamination in retail poultry meat was conducted. In the last time with the purpose of declaring the virulence factor (s) some authors reported that virulence of *Salmonella* is encoded with certain genes which can be detected by polymerase chain reaction (PCR). The location of the genes on chromosomes or plasmids has controversy character in published investigations (Osman, 2015).

The use of antimicrobial agents allows products to be microbial safe with the enhanced ability to extend shelf life. Recently, acidified sodium chlorite (ASC) has been suggested as an effective in reducing microbial contamination and its use has been approved by the United States Food and Drug Administration (USFDA) for the application on various food products (Sexton *et al.,* 2007 and kim *et al.,* 2009). ASC is considered a broad spectrum oxidative antimicrobial effective on pathogenic bacteria. It works by forming Oxychlorus antimicrobial intermediates as it comes into contact with organic matter (Kross, 1984). The Oxychlorine compounds forming chlorite, chlorate and chlorine dioxide after contact with food surfaces. Chlorine dioxide is either evaporated or reduced without residual traces (FSAN, 2003). Candian Food Inspection Agency Meat Hygiene Directive (2001) listed ASC approved microbial control agent in range of 500-1200 ppm for use on Poultry.

In addition, chlorine is the most frequently used antimicrobial intervention in commercial poultry processing due to its availability, low cost, and efficacy (Northcutt and Jones, 2004). In general, chlorine compounds are effective against Gram-positive, Gram-negative, and acid fast bacteria. The potential antimicrobial action of chlorine summarized in the following: its molecules penetrate the bacterial cell wall and react with key enzymes to prevent normal respiration and carbohydrate metabolism (Fabrizio *et al.,* 2002). Chlorine is permitted, the level do not normally exceed 50ppm, which results in a reduction in microbial load of around one log (Northcutt *et al.,* 2005).

In general, it must be emphasized that, decontamination treatments are able to reduce the contamination level but do not completely eliminate pathogens. Their effectiveness depends on the initial microbial load and treatment conditions. Regarding treatment conditions, there are many factors affecting the efficacy of these antimicrobials including concentration of the substance, time of exposure, temperature, pH and hardness of water, strength of bacterial adhesion to the carcasses, (Lechevalier *et al.,* 1988).

The purpose of the present investigation was designed to evaluate the prevalence of *Salmonella* spp. in chicken fillet, confirmation of the isolated strains by PCR with carrying out the virulence factor(s) in isolated organisms. Moreover, the effect of acidified sodium chlorite and chlorine in reducing the population of *Salmonella* with measurement their residues on chicken fillet were studied.

MATERIALS AND METHODS

1- Microbiological analysis of chicken fillet samples:

Collection and preparation of samples: (Jamshidi *et al*., 2008)

Eighty samples of chicken fillets were obtained from local markets with different sanitation levels in Assiut

city. Samples were transported to the laboratory immediately after collection in an ice box. The frozen collected samples were left to be thawed overnight in refrigerator, then they were analyzed bacteriologically.

Isolation and Identification of *Salmonella*:
Twenty – five grams of each sample were put into a stomacher bag containing 225/ ml buffered peptone water and homogenized using a stomacher then incubated at 37°C for 18hrs. One ml was transferred to 10ml selenite cystine broth and incubated for 20-24hrs. at 37°c. Plating was carried on XLD agar and incubated at 37 for 24h. Then examined for typical colonies of *Salmonella* (red with black center). Presumptive *Salmonella* colonies were confirmed by biochemical test (Indole, Methyl red, Voges proskauer, citrate, urease, (TSI) and sugar fermentation) (APHA, 1992).

Serological Identification of suspected *Salmonella* isolates:
Using rapid agglutination technique as described by (Minor and popoff, 2000). *Salmonella* antisera were obtained from DENKA SEIKEN Co.Lid, Tokyo, Japan.

2- Molecular analysis:
DNA Extraction

This part was done by Benha University, Faculty of Veterinary Medicine, Food Analysis Center.

Using QIA amp. Kit according to (Shah *et al.,* 2009) as shown in **Table 1:**

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
invA (F)	**5′** GTGAAATTATCGCCACGTTCGGGCA **′3**	284	Shanmugasamy *et al.* (2011)
invA (R)	**5′** TCATCGCACCGTCAAAGGAACC **′3**		
Stn (F)	**5′** CTTTGGTCGTAAAATAAGGCG **′3**	260	Makino *et al*. (1999)
Stn (R)	5′ TGCCCAAAGCAGAGAGATTC ′3		
fimH (F)	**5′** GGA TCC ATG AAA ATA TAC TC **′3**	1008	Menghistu (2010)
fimH (R)	**5′** AAG CTT TTA ATC ATA ATC GAC TC **′3**		

DNA amplification for the selected virulent genes:
As described by (Singh *et al.,* 2013).

3- Studying the effect of Acidified Sodium Chlorite and Chlorine on survivial of *Salmonella* Enteritidis in chicken fillet:
Bacterial Cultures and Inculum:
Salmonella Enteritidis was cultured in tryptic soy broth and cultures were incubated at 37°C for 24h. After incubation the number of cfu/ml was determined according to (FAO, 1992).

Aseptically obtained chicken cubes:
Frozen chicken fillet was thawed by overnight refrigeration, and slicedaseptically into approximately 1 × 1 × 1 cm pieces (1g weigh pieces).

The Antibacterial Agents: (Inatsu *et al.,* 2010).
Acidified sodium chlorite solution (ASC):
Acidified sodium chlorite solution (ASC) was prepared by mixing 0.5g/log sodium chlorite and 1g/L citric acid and the solution pH was 2.62 then allowed to activate for 10 minute. Then diluted with sterile distilled water to form different dilutions of 400,600 and 900 part per million (ppm).

Chlorine solution: (Nassar *et al.,* 1997).
This was prepared according to the instructions of the manufacturer: 14 g/m^3 of water, giving 1 ppm chlorine. Three concentrations of chlorine in water were used in this experiment: 20 ppm, 30 ppm and 40ppm. The concentration of each solution was tested with a photometer to confirm the amount of chlorine. The various treatments were prepared just prior examination and placed in sterile containers.

Assessment of microbial growth:
Analysis was conducted on the artificially contaminated chicken fillet within determined time of analysis after bacterial inoculation and refrigeration (at 4^{o}C for4 hours). Counting of bacterial load was applied for *S.* Enteritidis according (FDA, 2011). For

statistical analysis, average count of colonies on duplicate plates was transformed in to Log cfug-1.

4- Measurement of the residual ASC and chlorine: ASC and chlorine treatment residue analysis by High Pressure Liquid Chromatography (HPLC) (NZFSA, 2003).

RESULTS

Table 2: Incidence of *Salmonella* spp. in the examined chicken fillet samples

No.of examined samples	*Salmonella* serovares								Total	
	***S*. Enteritidis**		***S*. Typhimurium**		***S*. Virginia**		***S*. Finkenworder**			
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
80	2	2.5	2	2.5	1	1.25	1	1.25	6	7.5

P CR identification:

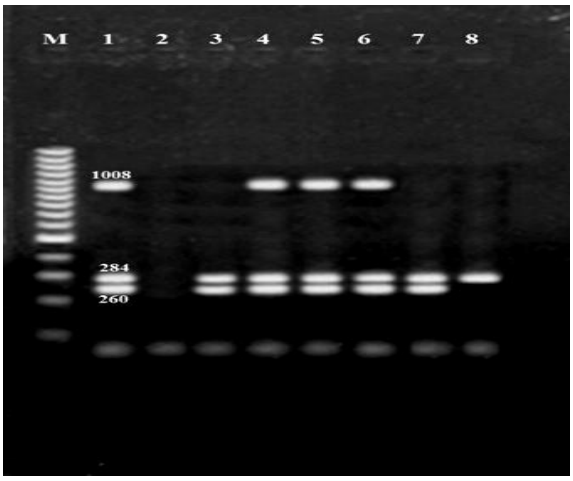

Fig. 1: Agarose gel electrophoresis of multiplex PCR of *stn* (260 bp), (260 bp), *inv*A (284 bp) and *fim* H (1008 bp) virulent genes for identification and characterization of *Salmonella* spp.

Lane M: 100 bp ladder as molecular size DNA marker.
Lane 1: Control positive *Salmonella* strain for *stn* and *inv* A and *fim* H genes.
Lane 2: Control negative.
Lane 3: Positive *S.* Enteritidis strain for *stn* and *inv* A genes.
Lane 4: Positive *S.* Enteritidis strain for stn and inv A and fim H genes.
Lanes 5 & 6: Positive *S.* Typhimurium strains for *stn* and *inv* A and *fim* H genes.
Lane 7: Positive *S.* Virginiastrain for *stn* and *inv* A genes.
Lane 8: Positive *S.* Finkenworderstrain for *inv* A gene.

Table 3: Effect of different concentrations of Acidified Sodium Chlorite on the viable count of inoculated *S.* Enteritidis in chicken fillet:

Sampling time	Control/log cfu	400ppm		600ppm		900ppm	
		C/log cfu	R/log cfu	C/log cfu	R/log	*C/log cfu	**R/log cfu
Zero	5.6	4.5	1.1	4.3	1.3	3.1	1.5
1 h	3.8	3.2	0.6	2.9	0.9	2.6	1.2
2 h	3.5	3.1	0.4	2.8	0.7	2.5	1
4 h	3.2	3	0.2	2.7	0.5	2.3	0.9

*C: Count
**R: Reduction

Table 4: Effect of different concentrations of chlorineon the viable count of inoculated *S.* Enteritidisin chicken fillet:

Samples time	Control	20ppm		30ppm		40ppm	
		C/log cfu	R/log cfu	C/log cfu	R/log cfu	C/log cfu	R/log cfu
Zero	5.6	3.2	2.4	2.9	2.9	2.6	3
1 h	3.8	2.3	1.5	2.1	1.7	2	1.8
2 h	3.5	2.1	1.4	1.9	1.6	1.8	1.7
4 h	3.2	2	1.2	1.8	1.4	1.7	1.5

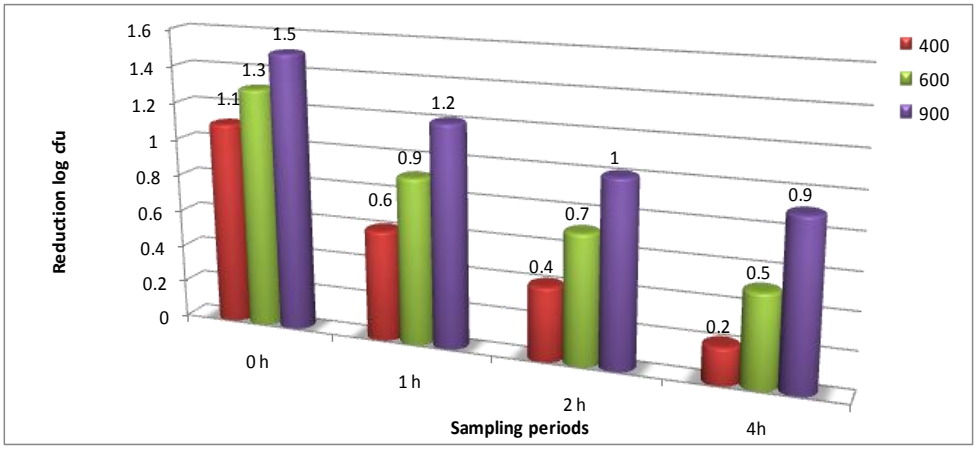

Fig. 2: Effect of different concentration of ASC on *S.* Enteritidisin chicken fillet

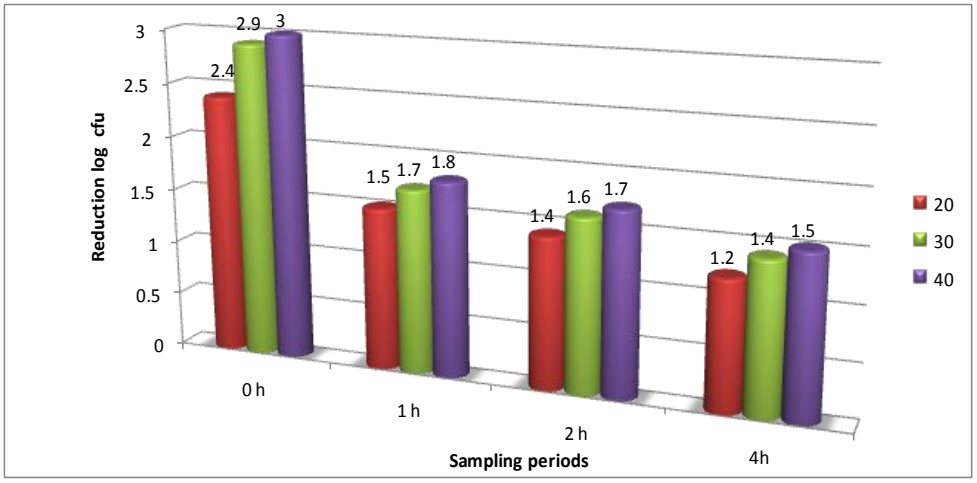

Fig. 3: Effect of different concentration of chlorine on *S.* Enteritidisin chicken fillet

DISCUSSION

1- Incidence of *Salmonella* spp. in the examined chicken fillet:

The isolation of invasive *Salmonella* serotypes in our study indicates the public health significance of these serovars as contaminated chicken meat may pose health hazards. The arised risk may further be higher if chicken meat is consumed undercooked or due cross contamination in the kitchen with *Salmonella* during meal preparation (FSIS/USDA, 2013).

Results achieved in Table (2) indicated that *Salmonella* spp. were isolated from7.5% of examined chicken fillet. *Salmonellae* could be identified serologically as *S.* Enteritidis (2.5%), *S.* Typhimurium (2.5%), *S.* Virginia (1.25%) and *S.* Finken worder (1.25%).

The incidence of *Salmonella* spp. in examined chicken fillet samples in the present study showed marked lower than that recorded by Arafat *et al.* (2011); Saad *et al*. (2011); Lidia *et al*. (2006) and Ibrahim *et al*. (2014) where their findings were,

(52%), (16%) and (15.39%) and (13%) respectively. Other studies of isolation of *Salmonella* spp. in chicken carcass which also were higher than the obtained result conducted by Paião *et al.* (2013) (45%), Alali *et al.* (2012) (27%), Chang (2000) (25.9%), Dhaher *et al*. (2011) (24.76%), Molla and Mesfin (2003) (15.4%), and Todd (1999) (13.3%). On the other hand, the incidence of this organism was nearly agreed with that results obtained by Straver *et al*. (2007) and Abdellah *et al*. (2009) who recorded 8.6 and 6.25% chicken fillets, respectively. Husnu and Walid (2015) cited 3.1% in chicken fillet which seem to be lower than this results. Moreover, Vaidya *et al.* (2005) and Bajaj *et al.* (2003) reported negligible prevalence as low as 5% of chicken carcasses.

The findings outlined in the same Table declared that the incidence of *S.* Enteritidisin chicken fillet samples was (2.5%). This trend was lower than that obtained by Ibrahim *et al.* (2014) who recorded 7% in chicken fillet, Hee *et al.*(2007) (21.9%), Paião *et al*. (2013) (12%) and Mohammad *et al.* (2014) (5.8%) in chicken carcases, respectively. While Saad *et al.* (2011) failed to recover *S.* Enteritidis from the examined samples of chicken fillet.

Salmonela Typhimurium as presented in Table (1) was recovered from chicken fillet in a percentage of 2.5% which was lower than reported by Saad *et al.* (2011) (8%) and Ibrahim *et al*. (2014) (7%) in chicken fillet, Mohammed (2013) (44%) and Hee *et al*. (2007) (23.4%) in chicken carcass. While, this study showed some what higher findings than that reported by Mohamed *et al.* (2014) who could isolate *S.* Typhimurium from 1.2% of chicken fillet. The prevalence of *S.* Typhimuriumin the examined chicken carcass recovered by Paião *et al.* (2013) (3%) was nearly agreement to the obtained.

Regarding *S.* Virginia, the findings illustrated in Table (1) revealed that examined chicken fillet samples proved to harbor 1.25% *S.* Virginia. Hee *et al*. (2007) and Mohamed *et al.* (2014) recorded that the incidence of *S.* Virginia was 6.3 and 3.5%, respectively in chicken carcass which was higher than that obtained in this study. Furthermore, Chang (2000) could isolate *S.*Virginia from raw chicken carcasses.

It is worth to mention that in this study, we have identified *S.* Finkenworder (1.25%) which is uncommon invasion non typhoidal *Salmonella*. Its presence in the examined chicken fillet may be due to the hazard exportation chicken or poultry products. Most cases of Salmonellosis which arecaused by non typhoidal *Salmonella* are mild (WHO, 2013).

The presence of *Salmonella* in chicken meat may be attributed to poor hygiene conditions, regarding the temperature of storage, the equipment and the employees' personal hygiene and the cutting tables were seldom washed, These benches could therefore be reservoirs from which Salmonellae could spread to other equipment through flies or direct contact (Stevens *et al.*, 2006).

The strains which identified biochemically and serologically as *Salmonella* spp. were subjected to PCR test. The result indicated that, two strains were *S.* Enteritidis, another two were *S.* Typhimurium, one strain was *S.* Virginia and the last strain was *S.* Finkenworder.

The virulence of any particular *Salmonella* organisms is determined by its invasiveness, which depend upon the attachment of the organism to the mucosal epithelium and production of enzymes and toxins that damage the epithelium and /or alter epithelial permeability and facilitate bacterial entry into the mucosal cells and infection of the lamina propria (Coburn *et al.*, 2007). One of the earliest steps in the pathogenic cycle of the facultative intracellular pathogen *Salmonella* spp is the invasion of cells of intestinal epithelium. Gene *Inv* A ne is amember of *Inv* genes those allow Salmonella organisms to enter the epithelial cells. In the present study *Inv* A gene was detected in 100% of the examined isolates (fig1) the used primer of the gene multiply a region of 284 bp. Many authors as Jacopsen and Holben (2007)**,** Abd Elfatah (2014) and Osman (2015) could detect the *Inv* A gene and explained that the gene was necessary for the invasion of the cell. During the course of this study, *Stn*, gene *Salmonella* enterotoxin recovered in all isolates was investigated by using PCR. The *Stn* gene was a heat labile *Salmonella* enterotoxin. All isolates (*S.* Typhimurium, *S.* Eenteritidis and *S.* Virginia) except *S.* Finkenworder were detected to harbor *Stn* gene that encoded on plasmid DNA and amplified region 260 bp (Fig 1).

This result agrees with ElEbeedy (2011) who reported that *Stn* gene is widely distributed among *Salmonella* irrespective to their Serovars and source of isolation. The *Stn* gene was encoded mainly on plasmid DNA and hence on total DNA. Several workers have corroborated this findings (Rahman, 1999; Rahman *et al.*, 2000 and El Ebeedy, 2011).

Characterization of the *fim* H gene encoding the fimbrial adhesions indicated two allelic variants. *fim* H is responsible for mediating binding to eukaryotic cells and mutants unable to produce *fim* H have been shown to lack adhesive ability (Hancox *et al*.,1998). Consequently, the fim H polypeptide is considered to be the adhesion molecule of type 1 fimbriae and confers the binding specificity upon the fimbriae. Jennifer *et al*. (2002) detected *fim* H gene is *S.* Typhimuriumserovars these results agree with our results. Other serovars didn't harbor *fim* Hgene (*S.*

Enteritidies, *S.* Virginia and *S.* Finkenworder) except one *S.* Enteritidis serovar harbor *fim* H gene.

2- Effect of acidified sodium chlorite (ASC) on *Salmonella* Enteritidis:

Acidified Sodium chlorite is activated when pH values in the range 2.5-2.9 Candian Food Inspection Agency, 2001). ASC is Known as Generally Recognized as Safe Acid (GRAS), (Shireen and Abdelmonem, 2014).

In present study the cubes of the chicken fillet witch inoculated with10^6 log cfu/g, of *S.* Enteritidis were dipped in different concentrations of ASC (400,600 and 900 ppm) and left to drain (leaving non treated sample as a control) then counted at the 0, 1, 2, and 4 hours of refrigeration storage for the viable cells of *S.* Enteritidis. Meanwhile, the initial count of control samples in zero time was 5.6 log cfu/g while the number differed in the different dipped samples with different ASC concentrations. The maximum reduction in the count of *Salmonella* to the inoculated chicken cubes (4.1 log cfu/g, with reduction value 1.5log cfu/g compared to control) was immediately occur after treatment (0hr) with concentration 900 ppm, as shown in (Fig2). As it was 4.5, and 4.3 in the concentration 400 and 600 ppm, whereas the corresponding values of reduction were 1.1 and 1.3 log cfu/g respectively. The cidal effect of acid decontamination of fresh meat surfaces depends principally on the immediate lethality (Dickson and Anderson, 1992).

The immediate and short-term bactericidal effect of ASC on *Salmonella* on chicken samples has been recorded by Sexton *et al.* (2006) who observed immediate reduction of 0.05 log cfu/g for *Salmonella* after 20 seconds dip in ASC (900-1000 ppm), *Salmonella* prevalence was reduced from 90% to 10%, which was initially very low.

During storage at 4ºC the counts of control samples after the first hour was 3.8 log cfu/g, while when examining samples dipped in 400,600 and 900 ppm ASC gave *S.* Enteritidis number as 3.2, 2.9 and 2.6 log cfu/g, with reduction values 0.6, 0.9 and1.2 log cfu/g respectively.

The control sample showed a slight decrease in the second hours of experiment 3.5 log cfu/g, while it was 3.1, 2.8 and 2.5 log cfu/g for samples dipped in ASC of the three concentrations, the corresponding values for reduction were 0.4, 0.7 and 1.0 log cfu/g respectively.

In the fourth hour of storage, *S.* Enteritidis in control samples, decreased to be 3.2 log cfu/g. while when examination of samples which dipped in 400,600 and 900 ppm ASC concentrations become 3, 2.7 and 2.3 log cfu/g with reduction values 0.2, 0.5 and 0.9 log cfu/g respectively.

From the mentioned results, the inoculated control samples with *S.* Enteritidis showed obvious decrease in its bacterial count during refrigeration storage, which approves that low temperature treatments can inactivate the living cell by the attribution of ice nucleation and dehydration (Brunnberg *et al.*, 2009).

Many investigators evaluated the effectiveness of ASC as a processing aid for dipping or spraying to control *Salmonella* increasing the safety of chicken carcasses. Kemp *et al.* (2000) cited that the using of ASC at concentrations (500ppm, 850 ppm) is an effective method for reducing microbial contamination on chicken carcasses, while the maximum antimicrobial activity of ASC when carcasses were dip in 1200 ppm for 5 seconds. While Kemp *et al.* (2001) applied an "ASC spray system" in reducing *Salmonella* levels on contaminated poultry carcasses, could report that the incidence of *Salmonella* was 10%. Moreover, Wang and Zhou (2014) evaluated the effects of ASC (concentrations ranging from 0 to 1 g/L) the results showed that bacterial numbers were significantly reduced with increasing concentrations of ASC reaching maximum reductions of 2.2 log cfu/g for *Salmonella*.

It is clearly evident from the previous results that the action of ASC on Salmonella occurred immediately after the cells were exposed to the treatment and minimal or delayed effects were noticed during storage. This is explained by Anderson and Marshall (1989) who recorded that ASC decontamination causes death and sub-lethal injury to microorganisms but in the favorable environment on meat surface after decontamination, injured cells on meat can repair and then grow normally.

The use of ASC can lead to substantial reductions in both the prevalence and concentration of important foodborne pathogens. The degree of reduction, if achieved commercially, is likely to substantially reduce consumer exposure while providing potential product quality benefits (FSANZ, 2003).

3- Effect of chlorine on *Salmonella* Enteritidis:

The decontaminating efficacy of chlorine depends on the concentration of chorine. Therefore, it is necessary to know its concentration in the treated solution, and a determination should be routinely done as quality control for decontamination (Fenner, 2005).

From the summarized results given in Table 3 it is evident that the cubes of the chicken fillet which inoculated with 10^6 cfu/g, of *S.* Enteritidis were dipped in different concentrations of chlorine (20, 30 and 40 ppm) and left to drain (leaving non treated

sample as a control) then counted in the 0, 1, 2 and 4 hours of refrigeration storage for the viable cells of *S.* Enteritidis. The count of control samples in zero time was 5.6 log cfu/g. The maximum reduction in the count of *Salmonella* to the inoculated chicken cubes (2.6 log cfu/g, with reduction values 3 log cfu/ gcompared to control) was immediately occurred after treatment (0h) with concentration 40ppm, as shown in (Fig3). However, the count was 3.2, and 2.7 log cfu/g at concentration 20 and 30 ppm, with reduction values 2.4 and 2.9log cfu/g respectively.

Nassar *et al.* (1997) recorded that the chicken carcasses when subjected to 20 ppm and 50 ppm, of chlorine was no reduction in the number of *Salmonella* but when Northcutt *et al.* (2005) used 50 ppm of chlorine, recorded 3.1 log10 reduction of Salmonella which similar to this result., On contrary, the presence of *Salmonella* reported by Whyte *et al.* (2001), Fabrizio *et al.* (2002) and Brunnberg *et al.* (2009) was lower than that obtained in our study, their reduction values were 1.04, 0.86and 0.75 log cfu/g respectively, treated by 20 and 25 ppm. While A higher reduction (4 log cuf/g) was recorded by Saad *et al*. (2015) when used 50 ppm.

The number of Salmonella in the control samples after the first hour of refrigeration storage was 3.8 log cfu/g, while when examining samples dipped in 20,30 and 40 ppm chlorine gave *S.* Enteritidis number as 2.3, 2.1 and 2 log cfu/g, with reduction values 1.5, 1.7 and1.8 % respectively. Chlorine is not available for a longer period; after one hour in this study we noticed that the effect of chlorine already was lowered because of the unstable chlorine.
In the second hour of experiment, the control sample was 3.5 log cfu/g, while it was 2.1, 1.9 and 1.8 log cfu/g for samples dipped in chlorine of the three concentrations respectively, the corresponding values for reduction were 1.4, 1.6 and 1.7 log cfu/ grespectively.

In addition, after 4 hours of storage, the control samples was 3.2 log cfu/g, where as the populations of *S.* Enteritidis for the samples treated with 20,30 and 40 ppm of chlorine was 2, 1.8 and 1.7 log cfu/g, with reduction values, 1.2 ,1.4 and 1.5 log cfu/g respectively. Our result was nearly agreement to that recovered by Saad *et al.* (2015) who recorded1.3 log cfu\g when used 30 ppm of chlorine.

From the qualitative observations, there was no detectable bleaching of the treated product with ASC and chlorine however, they were impossible to detect which carcasses had been treated. There was also no detectable odor difference between the control and treated samples either initially or throughout the shelf-life. Our results are in agreement to that recovered by FSANZ (2003) who reported that ASC treatment solution, after contact with food surfaces is either evaporated or reduced without any residual traces. While, Northcutt *et al.* (2005) observed that the use of chlorine did report no or only minor changes on poultry carcasses which nearly agreed with our obtained results.

However, when Izat *et al.* (1989) and Nassar *et al.* (1997) used high level of chlorine as100 ppm reduced the number of *Salmonella* organisms was reduced (70-75%) respectively, but had a yellowish appearance and a strong chlorine smell compared to the non-treated controls.

Levels of chlorine which used in the processing plants of Saudi Arabia are approximately 20 ppm to 50 ppm, since poultry processors believe that higher chlorine concentrations produce an undesirable tainting of and color in the carcass (Nassar *et al.,* 1997).

Therefore, these results clearly indicate that chlorine treatment is more efficient way of inactivating of *S.* Enteritidis than the use of ASC in the chicken fillet.

Although the use of antimicrobial agents is only one step in the process of pathogen reduction it would suggest that it is worth trialing a commercial system and following product through further processing stages (such as boning, portioning and marinating) to determine if the significant reduction in pathogens can be achieved on further processed products Sexton *et al.* (2006).

4- Measurement of the residual ASC and chlorine:
Measurement of the residual ASC:
Chlorite and chlorate residues in carcasses dipped in Acidified sodium chlorite (ASC) was assumed as a behaviour of the decontaminant chemicals similar to the decontamination process applying ASC (Alcide, 2002).

In present study, residual ASC were detected immediately after treatments of chicken fillet samples by 400, 600 and 900 ppm for 5 s and dip in rinse water. The residual were (0.03 µg/cm2 of meat surface) which below the estimated detection limit for chlorite and chlorate.

The residual concentrations of chlorite and chlorate as reported in the data submitted to the Joint Food and Agriculture Organization of the United Nations (FAO)/ World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (WHO, 2008) for raw meat and meat products, including poultry that had been treated with ASC solution were 0.1 mg/kg for both chlorite and chlorate.

Residues of chlorite and chlorate were reported by SCVPH (2003) for poultry carcasses immersed in a 150 mg/l ASC solution at pH 2.8 and 5 ^{o}C for 1 h, then drained for 5 min and rinsed for 5 min in clean water. The residue levels were lower than the detection limit (chlorate <19 µg/kg) or became so after 2 h (chlorite <16 µg/kg).

Measurement of the residual chlorine:
Hypochlorous acid and the hypochlorite ion together constitute aqueous chlorine. Aqueous chlorine is widely used throughout the food processing industry (McIntyre *et al.*, 2008).

In this trial, Residual chlorine was detected immediately after treatments of chicken fillet samples by 20, 30 and 40 ppm for 5s and dip in rinse water. The residue levels of chlorine could not be detected by using (HPLC) and suggested that residues were below measurability.

WHO Working Party has established a tolerable daily intake (TDI) for chlorine of 150 µg/kg body weight/day (International Programme on Chemical Safety, 2000). No information was found on chlorine residues in chicken flesh following treatment with aqueous chlorine. While there is some evidence for formation of chlorinated compounds in chicken treated with aqueous chlorine, oxidation reactions appear to predominate for chlorine dioxide and acidified sodium chlorite (Hoenicke *et al.*, 2004).

On the other hand, Robinson *et al.* (1981) could detect Chloroform in chicken treated with aqueous chlorine due to absorption of chloroform from chiller water, rather than direct formation in the chicken flesh. The highest average concentration of chloroform (177 µg/kg) was observed in muscle meat following dipping in a solution of 50 mg/L chlorine for 5 minutes at 15-16ºC. Thus, poultry (Scientific Committee on Veterinary Measures Relating to Public Health, 2003) chilled in chlorinated water contributes only 0.3 – 1% of the daily chloroform exposure, whereas water contributes most 99% of the exposure.

The residue levels for both ASC and chlorine were based on using Good Manufacturing Practice (assuming application and post-treatment recommendations are followed).

IN CONCLUSION

This study shown that *Salmonella* spp. was widespread among the chicken fillet. It may be due to insufficient hygienic condition. This, subjecting the consumer health to great hazards, so the need of antimicrobial agent to minimize and control such organisms is important. Treatment of chicken with ASC and chlorinecan improve the safety of chicken without any physical changes or sensory alterations, besides it minimizing tissue damage and maximizing antimicrobial effects. Additional studies are needed that consider the effect of chemical concentration, spray pressure, contact time, solution recycling, and point of application in commercial processing to ascertain the effectiveness of chemical applications against *Salmonella* spp. Further studies are needed to improve surveillance strategies to decrease the prevalence of *Salmonella* spp. in chicken population.

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تقليل مخاطر ميكروب السالمونيلا فى الدجاج الفيليه باستخدام انواع مختلفه من المثبطات البكتيريه

غادة محمد محمد ، هالة إسماعيل شعراوى
E-mail: ghada02468@yahoo.com Assiut University web-site: www.aun.edu.eg

أجريت هذه الدراسة على ٨٠ عينة من الدجاج الفيليه تم تجميعها من أماكن مختلفة من السوق المحلية بمحافظة أسيوط لمعرفة مدى تواجد ميكروبات السالمونيلا وتصنيفها والتأكيد على تواجد جينات الضراوة المختلفة باختبار انزيم البلمرة المتسلسل. كانت نتائج عزل ميكروبات السالمونيلا بنسبة ٧.٥% ونسبة العترات المعزوله بعد تصنيفها كالاتى: سالمونيلا تيفيميوريم وسالمونيلا إنيتريتيدس ٢.٥% لكل منها- وسالمونيلا فيرجينيا وسالمونيلا فينكينثيردير ١.٢٥% لكل منهما. أما بالنسبة لعزل جينات الضراوة (*Inv*A) فقد تم تواجدها في جميع العترات بنسبة ١٠٠%- أما جين (*Stn*) فقد تواجد في جميع العترات ما عدا عترة السالمونيلا فينكينفيردير. أما بالنسبة لجين (*fim*H) فقد تواجد في إحدى عترتي كل من سالمونيلا تيفيميوريم وسالمونيلا إنتيريتيديس فقط. ولقد تم إستبيان مدي تاثير Chlorine وAcidified Sodium chlorite (ASC) علي تواجد ميكروب السالمونيلا إنيتريتيدس المحملة إصطناعياً على الدجاج الفيليه وذلك بغمرها فى محلول يحتوى على^{٦}١٠ ملى من هذا الميكروب ثم غمرها فى تركيزات مختلفة من (ASC٤٠٠، ٦٠٠، ٩٠٠) جزء لكل مليون (تاركين عينات غير معالجة) ثم عد الميكروب بعد وقت (صفر، ١، ٢، ٤ ساعة) بعد وضعها في الثلاجة عند ٤ درجه مئويه. وقد كان أقصى اختزال لميكروب السالمونيلا ١.٥ وحده لوغاريتميه عند توقيت صفر وتركيز ٩٠٠ جزء لكل مليون- بينما كان الاختزال ١.١ ، ١.٣ وحده لوغاريتميه في التركيزات ٤٠٠، ٦٠٠ جزء لكل مليون على التوالي. بعد ساعة من وضع العينات في الثلاجة عند ٤ درجه مئويه كان الاختزال ٠.٦ ، ٠.٩ ، ١.٢ وحده لوغاريتميه بالترتيب على التوالي بينما الاختزال بعد الساعة الثانية كان ٠.٤ ، ٠.٧ ، ١ وحده لوغاريتميه على التوالي. أخيرا بعد ٤ ساعات كان الاختزال ٠.٢ ، ٠.٥ ، ٠.٩ وحده لوغاريتميه على التوالي. من ناحية أخرى عند استخدام (Chlorine) بتركيزات (٢٠، ٣٠، ٤٠) جزء لكل مليون فى عينات الدجاج الفيليه الملوثه اصطناعيا بالميكروب ثم العد بعد ٠ ، ١ ، ٢ ، ٤ ساعات كان أقصى اختزال لميكروب السالمونيلا ٣ وحده لوغاريتميه بعد المعاملة عند ساعة الصفر عند تركيز ٤٠ جزء لكل مليون. بينما كان ٢.٤ ، ٢.٦ وحده لوغاريتميه على التوالي عند تركيز ٢٠ ، ٣٠ جزء لكل مليون. بعد الساعة الأولى من التخزين كان اختزال الميكروب ١.٥ ، ١,٧ ، ١.٨ وحده لوغاريتميه بالترتيب على التوالي بينما بعد الساعة الثانية من التخزين كان الاختزال ١.٤ ، ١.٦ ، ١.٧ وحده لوغاريتميه بالترتيب على التوالي بينما بعد مرور ٤ ساعات كان الاختزال١.٢ ، ١.٤ ، ١.٥ وحده لوغاريتميه بالترتيب على التوالي. وقد اوضحت هذه الدراسه ان هذه المضادات لم تؤثر على رائحة أو ملمس الدجاج الفيليه واخيرا تم قياس المتبقيات لكل من ASC and chlorine كما تم مناقشة أهمية الميكروب للصحة العامة وطرق التحكم في هذا الميكروب.