PREVALENCE OF SALMONELLA AND STAPH. AUREUS MICROORGANISMS IN BROILER MEAT AT ZAGAZIG CITY AND THE EFFECTS OF SOME ORGANIC ACIDS ON THEIR VIABILITIES.

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Abstract

A total of 100 broiler chicken carcasses at the marketing age (approximately 45 day) were collected from slaughtering houses at Zagazig City during winter 2011- 2012s and spring 2012s for detection the prevalence of both Salmonella spp. and staphylococcus aureus and count of the latter mentioned microorganism in the examined broiler meat. The obtained results exhibited that the prevalence of the tested microorganisms were 2% and 24% for salmonella spp. and staphylococcus aureus respectively. Also, the mean bacterial count of staphylococcus aureus was 2.39 X103 ±6.68X102.

Sixty samples from the negative examined samples of both Salmonella spp. and S. aureus used for the organic acid treatments. Thirty samples were inoculated with salmonella enteritidis and another 30 samples were inoculated with staphylococcus aureus. The inoculation level for both microorganisms was about 105 cfu/gm. These inoculated samples exposed to citric and acetic acids in concentrations of 0.5% and 1%, respectively by immersion for of 1 and 2 minutes for each mentioned organic acids, and mixture of the both 2 organic acids 1% for 1 and 2 minutes. The obtained results showed that the reduction values in each of the two examined microorganisms after organic acid treatments comparing with the initial count varied from >102 to <104, which indicated relatively high effects of the organic acids on salmonella enteritidis and staphylococcus aureus. The statistical analysis showed that the treatment with acetic acid was more effective against the two examined bacterial types than citric acid. Moreover, the mixture solution of both citric acid plus acetic acid (1% for each) could not exhibit more antimicrobial effect than acetic acid (1%) alone.

INTRODUCTION

During the last few decades, broiler meat became the most public and widespread source of animal protein in Egypt. It contained all essential amino acids beside many vitamins and minerals which are necessary for maintaining life and promoting growth *(Pearson and Dutson,1997)*. Since few years ago, the selling of live birds was prohibited in Egypt for following the hygienic measures and saving the public health. These new circumstances lead to change of the traditional consumption behaviors of poultry meat consumer. Therefore, the Egyptian consumer begin depend

upon the slaughtered frozen or chilled broilers instead of the live birds. Subsequently, any defect of the hygienic measures in the slaughtering houses or during broiler meat transferring leads to microbiological contaminations, which cause serious diseases for the consumer. Thus, raw poultry products are reported to be responsible for a significant number of cases of human food poisoning *(Geornaras et al., 1995)*. Many previous studies recorded that *salmonella Spp.* and *staphylococcus aureus* were the most common bacterial types which can contaminate the broiler carcasses *(Cohen et* al., 2007 and Gody et al., 2012).

Salmonellosis is one of the widely distributed food borne diseases. It constitutes a major public health burden and represents a significant cost in many countries. Millions of human cases are reported worldwide every year and the disease results in thousands of deaths. Salmonellosis caused by the bacteria *salmonella*. Today, there are over 2500 known types, or serotypes, of Salmonella (WHO, 2012). Salmonellosis in humans is generally contracted through the consumption of contaminated food of animal origin (mainly meat, poultry, eggs and milk). Horizontal transmission of salmonella to broiler carcasses in the slaughterhouse was shown to be the main determinative factor (Heyndrickx et al., 2002). The causative organisms pass through the food chain from primary production to households or food-service establishments and institutions (WHO, 2012).

Staphylococcus is a group of bacteria that can cause a multitude of diseases as a result of infection of various tissues of the body. Staphylococcal food poisoning is a gastrointestinal illness. It is caused by eating foods contaminated with enterotoxin produced by *staphylococcus aureus*. The most common way for food to be contaminated with *staphylococcus* microorganism is through contact with food workers which carry the bacteria or through contaminated food. Staphylococcal toxins are fast acting, sometimes causing illness in as little as 30 minutes. Symptoms usually develop within one to six hours after eating contaminated food. Patients typically exhibit several of the following: nausea, vomiting, stomach cramps, and diarrhea. The illness is usually mild and most patients recover after one to three days. In a small minority of patients the illness may be more severe (CDC, 2006).

Therefore, the objective of the current investigation in to determine the prevalence of both salmonella spp. and *staphylococcus* aureus in count of *staphylococcus* aureus in the broiler meat samples collected from slaughtering houses at Zagazig City, and also; study the effects of the treatments by some organic acids on the viability of the mentioned microorganisms.

MATERIALS AND METHODS

Sampling

A total of 100 broiler carcasses at the marketing age (approximately 45 day) were collected from slaughtering houses at Zagazig City during winter 2011- 2012s and spring 2012s for detection the prevalence of both salmonella spp. and *staphylococcus* aureus and count of the latter mentioned microorganisms. Also, 60 of broiler carcasses free from bacteria were use in this study.

Isolation and identification of Salmonella spp. and S. aureus

Preparation of samples

Ten grams of each sample (broiler muscles) were aseptically taken and mixed with 90 ml of 0.1% sterile peptone water in a sterile homogenizer to get a dilution of 1:10. One ml was transferred to a separate sterile test tube containing 9 ml of sterile saline from which serial dilution up to 108 were prepared. One ml of each dilution was transferred to sterile petri dish, then the media (tryptone dextrose agar) was poured after solidification, plates were incubated in inverted position at 37°C for 48-72 hours as well as 0.1 ml of each dilution was transferred and spread over specific media then using surface plate technique after 24 and 48 hours incubation at 37°C. The *staphylococcus* aureus colonies appear on the surface, there colonies were counted. (Cheesbrough Monica, 1993)

Identification of Salmonella

After incubation of original sample at 37°C for 20 hours, one tenth of each dilution was transferred to tetrathionate broth and incubated at 43°C for 24 hours and 48 hours. These cultures were streaked on plates of brilliant green phenol red agar after incubation. The plates were incubated at 37°C for 24 hours. Then suspected colonies were examined biochemically. (Mulder, 1977 and, Cheesbrough Monica, 1993).

Staphylococcus aureus count

Over a dry surface Baird- Parker (B-P) agar plates, 0.1 ml amount from each of prepared dilutions (ten fold dilutions) of samples under investigation was transferred and evenly spread using surface plating technique (Thatcher and Clark, 1975).

Preparation of bacterium inoculum

Salmonella enteritidis culture (inoculum):

Bacteria were maintained on brain–heart infusion agar slants held at 4°C. Prior to use, each culture was subjected to two successive transfers by loop inoculate to 5ml brain–heart infusion broth. A final transfer of 0.2 ml was made into 20 ml BHIB with

incubation at 36 °C for 18 h under static conditions. Bacterial cells were harvested by centrifugation at 4 °C, and the cell pellets were washed in salt–peptone [0.85% NaCl, 0.05% Bacto-peptone (BBL/Difco)]. The cell pellets were used to prepare the inoculum consisting of the individual bacterial strains at approximately 1.3x105 cfu/ml in 3 liter of 0.1% (w/v) peptone-water (Ukuku and Sapers, 2007).

Staphylococcus aureus culture (inoculum)

Stock cultures were maintained at -20 °C in brain heart infusion broth with 10% (wt/vol) added glycerol. Working cultures maintained at 4°C on brain heart infusion agar were prepared monthly from frozen stock cultures. To obtain a working culture, a strain was cultured twice successively at 35 °C for 18 to 24 h in BHIB, streaked onto a BHIA plate, incubated at 35 °C for 18 to 24 h, examined for uniform colony morphology, and then stored at 4 °C. Inoculation cultures were prepared for each strain by transferring a loopful of growth from the working culture plate to 9 ml of BHIB and incubating the broth at 35 °C for 20 to 24 h. To prepare a five-strain inoculum cocktail of each organism, the BHIB cultures of each organism were combined into one sterile 50-ml centrifuge tube and centrifuged for 12 min at 5,000 g. The supernatant in each tube was decanted, and the pellets were re-suspended with approximately 20 ml of Butterfield's phosphate diluent (Ingham et al., 2006).

Sample inoculation

Sixty samples of both salmonella enteritidis and *staphylococcus* aureus resulted from the examined broiler meat samples. These samples were grinded; 30 of the ground sample were mixed with salmonella spp. and another 30 samples were mixed with *staphylococcus* aureus at a ratio of 1 ml of culture per 100 gm of broiler meat sample. The inoculation level for each 2 microorganisms were about 105 CFU/gm. Inoculated broiler meat samples were kept at 4°C for 30 min to allow bacterial cells attachment to meat.

Treatment

Both citric and acetic acids were used for sample treatments in concentrations 0.5% and 1% for each acid at 1 and 2 minutes for each concentration. Furthermore, mixed acids (citric + acetic acid) 1% of each used for treatment for one and 2 minutes (3 samples used for each treatment). The mentioned organic acids treatment

used by immersion of both inoculated samples by salmonella enteritidis and *staphylococcus* aureus. After organic acid treatments, the bacterial cell count was estimated.

Statistical analysis

Statistical analysis of data was conducted using "Statistic for animal and veterinary science" (Petric and Watson, 1999).

RESULTS AND DISCUSSION

Table 1. The prevalence of salmonella spp. and *staphylococcus aureus* and bacterial count per gm. of *staphylococcus. aureus* in the examined broiler meat (n=100).

Bacterial Types	The incidence of positive samples		Bacterial count per gm.		
			Max.	Min.	Mean ±S.E.*
	No.	%			
Salmonella spp.	2	2	-	-	-
Staphylococcus aureus	24	24	1.1 X104	1.5 X102	2.39 X103 ± 6.68X102

*: In the mean ±S.E. calculation, only the positive samples were estimated.

The prevalence of the salmonella spp. in the examined broiler carcasses showed in Table 1. It coincided with those reported by Cohen et al., (2007) which estimated 1.6% salmonella spp. in poultry meat in Morocco. Moreover, the mentioned microorganism was not detected in the examined broiler meat in Iran (Javadi and Safarmashaei, 2011). Contrarily, Godoy et al., (2012) recorded higher incidence of salmonella spp. (27%) than our estimations in broiler meat in Colombia.

Concerning *staphylococcus aureus*, the obtained results (Table 1) revealed that the incidence of the mentioned microorganism was higher than those estimated by Cohen et al., (2007) which recorded 10.4% of *staphylococcus aureus* in the poultry meat. Meanwhile, higher incidences of *staphylococcus aureus* than our findings were recorded by Bystron et al., (2005) in Poland, Kozacinski et al., (2006) in Croatia and Javadi and Safarmashaei, (2011) in Iran, they estimated 48%, 46.15% and 65% of the mentioned microorganism in the examined poultry meat respectively. On the other hand, the mean value of *staphylococcus aureus* count in the present study was higher than those detected by Kozacinski et al., (2006) and Cohen et al., (2007) which estimated 2.7 and 2.1- 2.2 log cfu/ gm. respectively in the examined poultry meat. On contrast, Javadi and Safarmashaei, (2011) recorded 4.7 log cfu/gm of *staphylococcus*

aureus in the examined poultry meat which was higher than those in the current investigation.

All the positive samples for salmonella spp. or *staphylococcus aureus* in the current study (24 sample) were unfit for the human consumption according to the Egyptian standard (E.O.S.Q.C., 2000), which reported that the poultry meat must be free from salmonella spp. in 25 gm. and free from *staphylococcus aureus* and its toxins.

Table 2. The effect of organic acids treatment on the mean count ±S.E. (cfu/ gm.) of the inoculated microorganism in the broiler meat samples (n = 3 for each treatment).

Treatment	Citric acid				Acetic acid				Citric acid + Acetic acid	
	0.5%		1%		0.5%		1%		1%	
Micro-organism*	After 1 minutes	After 2 minutes	After 1 minutes	After 2 minutes	After 1 minutes	After 2 minutes	After 1 minutes	After 2 minutes	After 1 minutes	After 2 minutes
salmonella enteritidis	203.3 ±23.3^{a}	102 ±4.35^{c}	151.6 ±4.4^{b}	104.3 ±2.96^{c}	103 ±2.08^{c}	95 ±7.63^{c}	42 ±2.08^{d}	20.33 ±2.02^{d}	37.33 ±0.33^{d}	20.0 ±1.15^{d}
staph. Aureus	8 X10^{2} ±1 X10^{2a}	2.6 X10^{2} ±65.0^{b}	94 ±5.5^{c}	55 ±6.08^{c}	1.2X10^{2} ±20.8^{c}	75 ±1.73^{c}	44.66 ±2.60 3^{c}	20.0 ±4.04^{c}	41.33 ±1.76^{c}	18.33 ±0.88^{c}

*: Initial count= 10^{5} (for each)

N.B.: Different letters within the same category (*salmonella enteritidis and staph. aureus*) mean significant variations between the values of the bacterial counts ($P \leq 0.01$).

Fig. 1: The different effects of organic acids treatment on the mean count ±S.E. (cfu/ gm.) of the inoculated *salmonella enteritidis* in broiler meat samples (Initial count= 10^5).

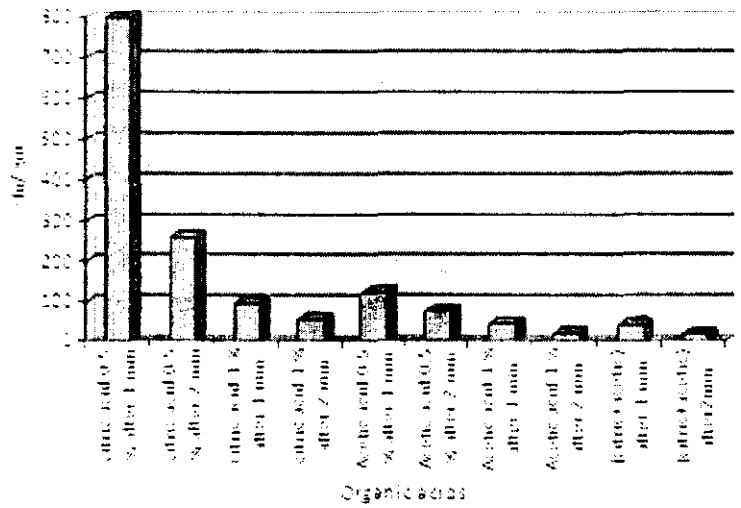

Fig. 2: The different effects of organic acids treatment on the mean count ±S.E. (cfu/ gm.) of the inoculated *staphylococcus aureus* in broiler meat samples (Initial count= 105).

The obtained results in Table 2 and Figures (1,2) exhibited obvious significant reductions of both salmonella enteritidis and *staphylococcus aureus* count in the treated samples with different concentrations of organic acids for different durations as shown, comparing with the initial bacterial count for the each microorganisms (105). The statistical analysis revealed that the salmonella enteritidis count in the samples treated with citric acid 0.5% for 1 minute recorded significant higher levels

than the others followed by samples treated with citric acid 1% for 1 minute. Also, significant lower bacterial levels than the mentioned samples were estimated in samples treated with citric acid 0.5% and 1% for 2 minutes, and in samples treated with acetic acid 0.5% for 1 and 2 minutes. The most significant lower bacterial count than the others were recorded in samples treated by acetic acid 1% for 1 and 2 minutes and samples treated with citric + acetic acid 1% (for each) for 1 and 2 minutes also. Concerning the statistical analysis of the organic acid treatments of the *staphylococcus aureus* inoculated samples, it revealed that the samples treated with citric acid 0.5% for 1 minute recorded significant higher *staphylococcus aureus* levels than others followed by another samples treated with citric acid 0.5% for 2 minutes. All the other treated samples recorded significant lower *staphylococcus aureus* levels comparing with the 2 mentioned samples without significant variations within all of them.

The obtained results recorded that the reduction values in each of the two examined microorganisms in the comparing with the initial count varied from >102 to <104. These relatively recorded high effects of organic acids on salmonella enteritidis and *staphylococcus aureus*. The findings recorded by Mikolajczky (2010) detected lower organic acid effects on salmonella spp. than our figures, he recorded that the contaminated turkey meat with the mentioned microorganism reduced 2 log from the bacterial count comparing with the control after dipping for 15 minutes in 0.05% citric acid solution. Moreover, lower effects of acetic acid than our findings were estimated by Cutter and Betancourt, (2000), they recorded 2 log reduction of Salmonella typhemurium in beef meat after treatment by 2% acetic acid. Also, Raftari et al., (2009) revealed that acetic acid in concentrations 1%, 1.5% and 2% reduced bacterial count by 1, 1.14 and 1.28 log respectively in meat samples inoculated with 103 cfu/ gm *staphylococcus aureus* microorganism. Furthermore, Cosansu and Ayhan (2012) recorded that 1% and 2% acetic acid solutions reduced Salmonella enteritidis count in chicken leg meat by 0.85 and 0.95 log respectively from 4- 5 log initial number; while, the same concentrations of the citric acid reduced the same initial Salmonella enteritidis count in the breast chicken meat by 0.95 and 1.58 log respectively. On the other hand, the statistical analysis showed that the treatment with acetic acid was more effective against the two examined bacterial types than citric acid. Moreover, the mixture solution of both citric acid plus acetic acid (1% for each) could not exhibit more antimicrobial effect than acetic acid (1%) alone.

Generally, we could be concluded that the treatment of broiler meat samples with both citric acid and acetic acid in low concentrations at short durations as mentioned in the present study declared obvious antibacterial effects against salmonella enteritidis and *staphylococcus aureus* without considerable changes in taste or odor of these poultry meat.

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مدى تواجد ميكروبي السالمونيلا و العنقود الذهبي في لحوم دواجن التسمين بمدينة الزقازيق و تأثير المعالجات ببعض الأحماض العضوية على حيويتها

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تم إجراء هذه الدراسة لاستبيان مدى تواجد ميكروبي السالمونيلا و العنقود الذهبي في عدد ١٠٠ من عينات لحوم دجاج التسمين المجمعة من عدد من المجازر بمدينة الزقازيق، خلال شتاء ٢٠١١- ٢٠١٢ و ربيع ٢٠١٢، بالإضافة لدراسة أثر بعض الأحماض العضوية على حيوية نوعي البكتيريا محل الدراسة.

و قد أسفرت الدراسة عن تواجد ميكروب السالمونيلا في العينات محل الفحص بنسبة ٢% فقط من إجمالي عدد العينات، في حين تواجد ميكروب العنقود الذهبي بنسبة ٢٤% مع متوسط عدد بلغ ٢.٣٩ × ٣١٠ خلية/ جرام.

تم حقن عدد ٦٠ من العينات السلبية ببكتيريا سالمونيلا انترتيدس و العنقود الذهبي، ٣٠ لكل نوع من النوعين محل البحث و بتركيز قدره ٥١٠ خلية/ جرام و تمت المعالجة بكل من حمض الستريك (الليمونيك) و الأسيتك (الخليك) بتركيزات قدرها ٠.٥% ، ١% لكل من نوعي الحمض المذكورين و ذلك بالغمر في الأحماض المذكورة لمدة دقيقة و دقيقتان لكل تركيز و لكل حمض، بالإضافة لمخلوط من الحمضين بتركيز ١% لكل منهما و الغمر فيها لمدة دقيقة و دقيقتان. و قد أسفرت النتائج عن نقص معنوي كبير في أعداد البكتيريا بعد المعاملة بالأحماض العضوية المذكورة، حيث تراوح التناقص في أعداد البكتيريا عن العدد الأولي الذي تم حقنه ما بين > ٢١٠ إلى < ٤١٠ ، كما أوضحت النتائج الإحصائية أن حمض الأسيتك أكر فاعلية على كلا الميكروبين محل البحث مقارنة بحمض الستريك، كما بينت النتائج أن المعاملة بمخلوط الحمضين المذكورين بنسبة ١% و لمدة دقيقة او دقيقتان لم تسفر عن تحسن معنوي في تقليل أعداد نوعي البكتيريا محل البحث مقارنة بحمض الأسيتك منفردا بتركيز ١%، مما يدل على عدم جدوى عملية الخلط.