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Prevalence of *Staphylococcus aureus* in chicken meat products and their contact surfaces with a reduction trial by using electrolyzed water

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Abstract

Chicken meat products are essential sources of protein, vitamins, and minerals. Chicken meat products can be regarded as potential sources of foodborne pathogens such as *Staphylococcus aureus (S. aureus)*. Such organisms can find their way to the chicken meat products via their contaminated contact surfaces such as knives, cutting boards, and packaging bags. This study aimed to investigate the prevalence of *S. aureus* in three chicken meat products including chicken mince, burger, and luncheon, 10 each, sold in Eldakahlya local marketplaces, Egypt, and their contact surfaces including knives, cutting boards, and packaging bags, 10 each. Second, PCR was applied to detect the coding genes of *S. aureus*-enterotoxins including *SEa*, *SEb*, *SEc*, and *SEd*. An experimental trial was conducted to evaluate the effect of the electrolyzed water either acidic, alkaline, or both on reduction of *S. aureus* load on the cutting boards. The obtained results revealed that chicken mince had the highest contamination rate among the examined chicken meat products, followed by burger, and luncheon, respectively. Cutting boards had the highest contamination rate among the examined contact surfaces. PCR revealed that *SEa* was the highest enterotoxin detected among the recovered *S. aureus* isolates, followed by *SEc*, *SEb*, and *SEd*, respectively. Electrolyzed water could significantly reduce *S. aureus* load with treatment by both alkaline, and acidic water achieved the highest reduction. Therefore, it is highly recommended to treat the contact surfaces of chicken meat products with electrolyzed water to reduce *S. aureus* contamination.

Keywords: Chicken meat products, *S. aureus*, virulence genes, electrolyzed water.

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Introduction

Chicken meat products contribute significantly to human nutrition since they are a competitively priced alternative to red meat, which is woefully undersupplied in Egypt. High-quality animal protein, necessary amino acids, and trace elements are abundant in chicken meat. The rapid improvements in food processing and technology have led to the production and distribution of a number of chicken meat products into the chicken meat markets, such as chicken mince, chicken luncheon, and chicken burger. These significant products stand out for their unique flavor and aroma, which attract consumers, particularly children **(El Bayomi et al., 2018; Morshdy et al., 2024)**.

The key factor contributing to the microbial contamination of chicken carcass surfaces is the presence of diverse microorganisms in the atmosphere of poultry slaughterhouses and butcher shops. Cross-contamination can occur at many stages of processing, such as slaughtering, de-feathering, blucking, venting, carcass transportation, and distribution. The birds itself as well as objects that come into contact with the carcass, such as butcher knives, cutting boards, walls, floors, air, and water are sources of microbial contamination **(Darwish et al., 2018; 2022)**. As a result, chicken meat becomes contaminated with a wide range of microorganisms, including food-poisoning organisms such as *Staphylococcus aureus (S. aureus)* which could have serious effects on public health.

S. aureus produce a range of heat-resistant enterotoxins, including staphylococcal enterotoxin (SE), which can result in severe foodborne intoxication if consumed. Staphylococci are typically found on skin, in

nasal and oral cavities of humans and animals, and in the environment. These are the main routes by which they can spread to food **(Crago et al., 2012)**. Because of its dangerous side effects for the medical and veterinary fields, *S. aureus* is thought to have a significant influence on the chicken meat industry globally **(Ruban and Fairoze 2011)**.

Electrolyzed water (EW) has been identified as a new, potent, and versatile sanitizer due to its primary active components, known as free available chlorine compounds (FAC), particularly hypochlorous acid (HOCL). HOCL is highly effective in rapidly eliminating a wide variety of disease-causing microorganisms. Consequently, EW is being considered as a safe alternative to chemical sanitizers **(Hsu et al., 2019)**. Development in the food sector is the ability to regulate and reduce germs without altering the sensory properties of the food product **(Hsu, 2003)**. Electrolyzed water (EW) is extensively employed for food sterilization, encompassing various types of foods such as chicken, shrimp, beef, fish, eggs, and others **(Hao, 2013)**.

A primary responsibility of the food safety industry is to guarantee the implementation of sanitary protocols throughout every stage of meat processing. Therefore, this study was undertaken to investigate the prevalence of *S. aureus* in three chicken meat products including chicken mince, luncheon, and burger. Besides, the prevalence of *S. aureus* in the chicken meat products' contact surfaces including cutting boards, packaging materials, and knives was also examined. Detection of enterotoxins on *S. aureus* isolates recovered from chicken meat products was also done using PCR. In addition, the effects of the EW on the contamination rate of *S. aureus* to the cutting boards were also screened.

Material and Methods:

1- *Collection of chicken meat samples:*

This study was done according to the guidelines of the Animal Health research Institute (AHRI), and no living animals were used in the present study. Thirty randomized samples of chicken meat products including chicken mince, burger, and luncheon (10 of each type). Samples (100 g/ each) were collected from market places in Dakahlya governorate. Every sample was separately stored in a sterile plastic bag, kept cold in an ice-box, and then brought straight into Food Hygiene Department, Animal Health Research Institute (AHRI), Mansoura Branch, where it was kept in perfect aseptic conditions. Besides, swabs were taken from the chicken meat contact surfaces including knives, cutting boards, and packaging bags (10 of each). The collected samples underwent a bacteriological analysis as soon as it arrived to identify *S. aureus* and characterize the genes related to enterotoxin production.

2- *Bacteriological examination of chicken meat samples:*

Preparation of samples (ISO (4833-1: 2013).

After 225 ml of 0.1 % sterile peptone water was precisely added to 25 g of the chicken meat product samples and carefully blended for 1.5 minutes using a sterile blender, ten-fold serial dilutions were also made from the chicken meat product samples. Each swab from meat contact surfaces was mixed in 10 ml of 0.1 % sterile peptone water.

Determination of total Staphylococcus aureus count (FDA, 2001).

With a sterile curved glass spreader, 0.1 ml from each of the prepared serial dilutions was evenly distributed on the Baired Parker agar plate. Following their inversion, the control and inoculated plates were incubated for duration of 48 hours at a temperature of 37°C. The colonies of *S. aureus*, which were black, shiny, spherical, smooth, convex, and had a small white margin, were observed and counted. The number of *S. aureus* colonies was determined by multiplying the count in each dilution factor.

Identification of Staphylococcus aureus:

The process involved morphological examination **(Cruickshank et al., 1975)**, followed by biochemical identification **(MacFaddin, 2000)** and testing for the presence of hemolysis, coagulase, thermostable nuclease test "D-Nase activity," mannitol, growth at 10% NaCl, bile esculent test, catalase activity, oxidase, and fermentation of sugars **(Lachia et al., 1971)**.

Polymerase Chain Reaction for the coding genes of Staphylococcus aureus enterotoxins

DNA Extraction by QIA amp kit:

Utilizing the DNA extraction kit (Qiagen, GmbH, Germany) per the manufacturer's instructions, genomic DNA was extracted from 24-hour cultures of *S. aureus* isolates in BHI broth **(Shah et al., 2009)**. The quantity and purity of DNA was measured by NanodropTM 1000 spectrophotometer (Thermofisher scientific, USA). The coding genes for *S. aureus enterotoxins* (SE) types a, b, c, and d were identified by PCR using primers (Pharmacia Biotech) described before **(Rall et al., 2008)**.

Multiplex polymerase chain reaction for the coding genes of Staphylococcus aureus enterotoxins:

Using a total volume of 25 µL for the multiplex PCR. This volume includes 1 µL of bacterial suspension obtained using the rapid DNA extraction method, 80 mM MgCl2, PCR buffer, 3.5 mM DNTP mix (Fermentas), 10 picomoles µL-1 of each primer, and 1 unit of Taq polymerase (BioSyntech Technologies). The thermal cycling profile used for the amplifications consisted of a first denaturation step at 94°C for 5 minutes, followed by 10 cycles of amplification with denaturation at 94°C for 30 seconds, annealing at 64°C for 30 seconds, and extension at 72°C for 45 seconds. This was followed by 25 cycles of amplification with denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 1 minute. Finally, an extension step was performed at 72°C for 10 minutes. After amplification, the reaction mixture was loaded onto a 2% agarose gel. Electrophoresis was

performed to assess the sizes of the amplified products. A 100-bp molecular size reference ladder (MBI Fermentas) was used for comparison. Following the application of ethidium bromide to the gel, the gel was captured using a UV laser **(Mehrotra et al., 2000)**.

The use of EW in reducing Staphylococcus aureus contamination on the cutting boards

Plastic cutting boards underwent sterilization using autoclaving at a temperature of 121°C for duration of 20 minutes prior to their utilization in an experimental trial. The boards were submerged in a *S. aureus* bacterial suspension obtained from the current study for duration of 5 minutes. The initial count of *S. aureus* on the surface was determined by swabbing a single face (50 cm^2) of a surface that had been contaminated and allowed to dry in the air, using a sterile cotton swab. The swab was rinsed in 5 ml of sterile peptone water solution, and 1 ml of suitable dilutions of this solution was spread onto duplicate Baired Parker agar plates as previously described. The surfaces that were contaminated with *S. aureus* were washed for 5 minutes in separate sterile bags containing 250 ml of recently made electrolyzed water (either alkaline alone, acidic alone, or first exposed to the alkaline followed by the acidic which was named as a mixture of both), sterile electrolyzed water was used as a control. The alkaline EW contained NaOH, while the acidic EW contained hypochlorous acid and both are kindly gifted from the Animal Health Research Institute, Dokki, Giza, Egypt. Following immersion, surfaces were cleaned using sterile tongues, and the count of *S. aureus* bacteria was assessed on one side of the surface by swabbing, as previously described **(Deza et al., 2007)**.

Statistical Analysis:

S. aureus count was expressed as means ± SE log 10 cfu/g or /cm. The Analysis of Variance (ANOVA) followed by Tukey's Kramer HSD test was used to statistically evaluate the obtained results **(Feldman et al., 2003)**.

Results and Discussion

Products made from chicken meat may become contaminated at any stage of the processing, packing, and shipping process with various pathogens. The chicken products become hazardous to customers and inappropriate for human consumption due to these pathogens. The obtained results of the current study revealed isolation of *S. aureus* from the examined chicken meat product samples at 50%, 60%, and 90% from the examined chicken luncheon, burger, and mince, respectively **(Fig. 1A)**. Chicken mince had the highest *S. aureus* counts (4.17±0.10 log 10 cfu/g) followed by burger (3.59±0.18 log 10 cfu/g), and luncheon (3.09±0.15 log 10 cfu/g), respectively **(Fig. 1B)**. Mincing machine plays a major role in the contamination of the mince with foodborne pathogens; this could explain the high *S. aureus* count in the mince **(Darwish et al., 2022**). In agreement with our findings, **Ibrahim et al. (2018)** recorded an incidence rates of *S. aureus* in samples of chicken luncheon, nuggets, and shawerma retailed in Benha city , Egypt at 7 (23.3%), 9 (30%) and14 (46.6%). In Saudi Arabia, *S. aureus* mean count ranged from less than 10^2 cfu/g in raw chicken thigh, and breast to 10^4 and 10^6 cfu/g for mince and frankfurter samples, respectively **(Al-Dughaym, and Altabari, 2010)**.

Food contact surfaces have been consistently identified as sources of bacterial cross-contamination. Fluid residues from raw meat or poultry left on a cutting surface can transmit disease-causing agents to other meals that will not undergo further cooking before consumption. A previous study has demonstrated that when cutting boards get contaminated, harmful microorganisms can persist and proliferate on the surface. These pathogens can easily spread to adjacent surfaces, posing a significant risk of infection **(Cools et al., 2005)**. Foodborne pathogens such as *Salmonella spp., Campylobacter spp., Bacillus cereus, Staphylococcus aureus*, **and** *Listeria monocytogenes* have been found on surfaces and equipment used for food preparation in home kitchens **(Deza et al., 2007)**.

Contrary to commonly held beliefs, epidemiological evidence shows that the majority of foodborne disease outbreaks originate from infections that occur within our kitchens **(Tirado and Schmidt, 2001)**. Additionally, inadequate hygiene practices, particularly involving the hands and other surfaces are identified as a contributing factor in up to 39% of domestic food poisoning outbreaks **(Ryan et al., 1996)**. Multiple surveys indicate that 20% of customers do not consistently wash their hands or cutting boards after handling raw meat or chicken **(Altekruse et al., 1999)**.

The obtained results of the present investigation revealed isolation of *S. aureus* from the examined chicken meat product contact surfaces at 40%, 30%, and 100% from the examined knives, packaging bags, and cutting boards, respectively **(Fig. 2A)**. Cutting boards had the highest *S. aureus* counts (4.52±0.09 log 10 cfu/g) followed by knives (2.98±0.28 log 10 cfu/g), and bags (2.49±0.06 log 10 cfu/g), respectively **(Fig. 2B)**. This variation is attributed to the differences in the frequency of cleaning, use of packaging bags as a single time use, and the material from which the cutting boards are made. In agreement with our findings, **Darwish et al. (2018)** isolated *S. aureus* from washing water of chicken carcasses. Besides, **Mahyudin et al. (2019)** isolated multiple drug resistant S. aureus strains from cutting boards of commercial food premises that represented a threat to food and public health.

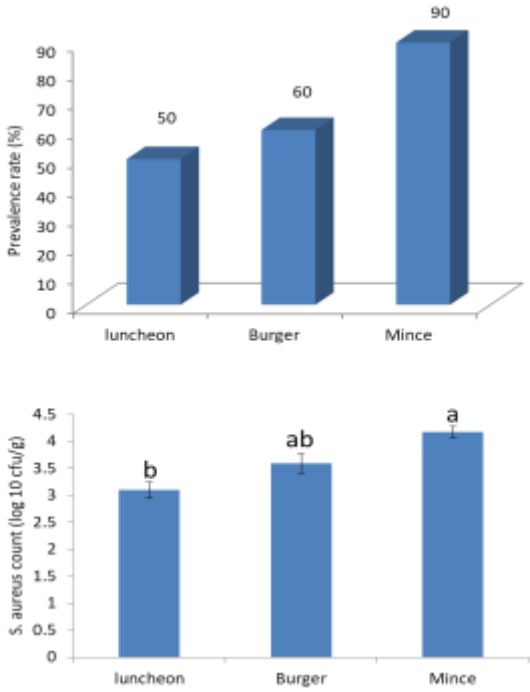

Fig. 1: A) Prevalence rate (%) of S. aureus in the examined chicken meat products.

B) Mean ± SE count (log 10 cfu/g) of S. aureus in the examined chicken meat products.

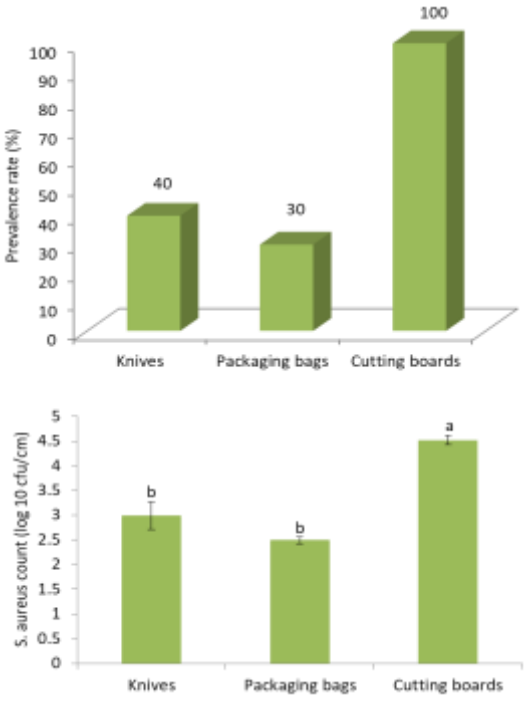

Fig. 2: A) Prevalence rate (%) of S. aureus in the examined chicken meat products contact surfaces.

B) Mean ± SE count (log 10 cfu/cm) of S. aureus in the examined chicken meat products contact surfaces.

Hospitalizations due to *S. aureus* are commonplace worldwide. Diarrhea, stomach pain, vomiting and dehydration are signs of *S. aureus* intoxication **(Darwish et al., 2022)**. The obtained results in **Fig. 3** revealed detection of SEs in selected isolates of *S. aureus* recovered from the examined chicken meat products with the *Staphylococcus aureus* enterotoxins type a *(SEa)* was the most prevalent enterotoxin. Likely, **Elomossalamy et al. (2020)** detected *SEa*, and *SEc* in the recovered *S. aureus* isolated from chicken burger. Besides, an analysis of the enterotoxin genes in S. aureus isolates obtained from chicken meat products sold in India identified the presence of 9 genes (*SEa- SEj*). Out of the 80 isolates examined, 52.50% (42/80) were found to be enterotoxigenic, containing one or more of these genes. The data clearly indicated that most of the isolates included the *SEb* gene, followed by *SEg*, *SEi*, *SEc*, *SEd*, and *SEj*, either individually or in combination **(Savariraj et al., 2021)**.

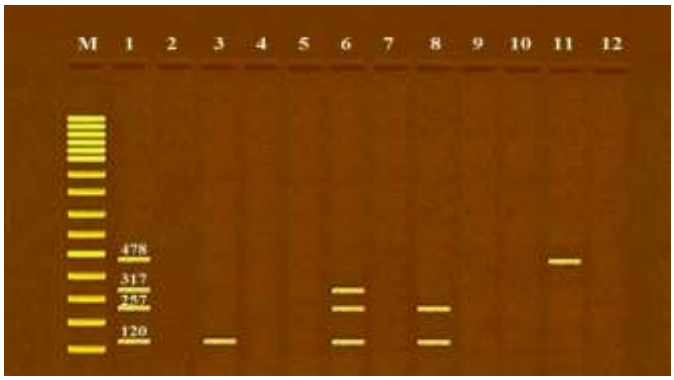

Fig. (3): DNA expression of *Staphylococcus aureus* enterotoxins genes by using multiplex PCR.
Agarose gel electrophoresis of multiplex PCR of sea (120 bp), seb (478 bp), sec (257 bp), and sed (317 bp) enterotoxin genes.

Regularly washing and disinfecting surfaces used for food preparation is an efficient method to decrease the spread of bacteria between different foods and to prevent outbreaks of foodborne illnesses. Nevertheless, the act of rinsing cutting boards with water and home chemical cleansers does not guarantee complete elimination of bacteria **(Cogan et al., 2002)**. Therefore, the use of antimicrobial agents becomes essential in order to obtain thorough cleanliness of the surfaces. The range of disinfectants utilized in kitchens and food sectors is extensive, encompassing quaternary ammonium compounds, amphoteric products, biguanides, iodophors, and peroxo acids **(Taylor et al., 1999)**. In recent years, alkaline and acidic electrolyzed water have been introduced for application as sanitizers. In the current investigation, the application of either alkaline, acidic or both of the alkaline and acidic electrolyzed water on the cutting boards used during cutting of chicken carcasses had caused marked reduction in *S. aureus* counts by by 24.14% in case of alkaline electrolyzed water, 39.66% in case of acidic electrolyzed water, and by 56.90% in case of using both of the alkaline and acidic electrolyzed water, respectively **(Fig. 4)**. In agreement with the obtained results of the present study, **Deza et al. (2007)** recorded that electrolyzed water was very effective in reducing the contamination of cutting boards with several foodborne pathogens such as *S. aureus*, *E. coli*, and *Listeria monocytogens*. In addition, **İplikcioğlu et al.**

(2020) reported that electrolyzed water was very effective in controlling *S. aureus* growth on the cutting boards which were regarded as ideal sources for cross-contamination of different food stuffs with various foodborne pathogens.

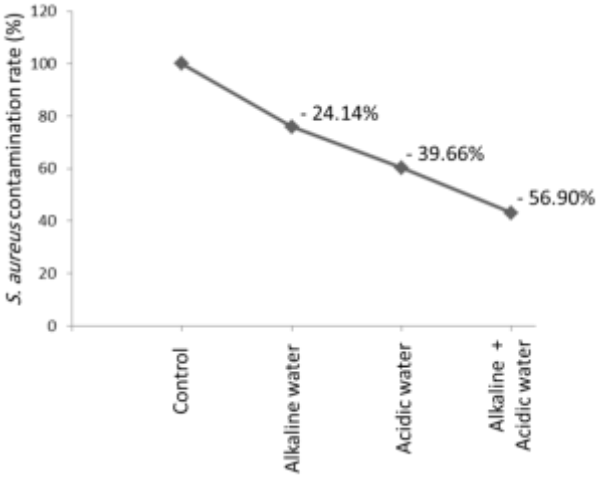

Fig. 4: Anti-S. aureus activities of electrolyzed water. The figure shows the reduction rates of the EW on S. aureus contamination rates.

Conclusion:

The findings of this study demonstrated that improper hygienic measures and contamination of the contact surfaces might result in contamination of chicken meat products with enterotoxigenic *S. aureus*. The use of EW either acidic, alkaline, or both is considered as a reliable method to inhibit *S. aureus* contamination of the chicken meat products' contact surfaces.

Conflict of interest: The authors have no conflicts of interest.

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