Prevalence and decontamination of *Staphylococcus aureus* in ready to eat chicken meat.

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Abstract

Two hundred and fifty ready to eat chicken products were collected randomly from different restaurants at Zagazig city, Sharkia governorate, Egypt, at different sanitation levels. The collected samples represented by (50 chicken ready to eat products each of fajitas, shawarma, burger, pane and luncheon). The *S. aureus* was detected in 56, 48, 68, 42 and 36% with mean counts 3.24 ± 0.38, 3.62 ± 0.41, 4.12 ± 0.42, 3.12 ± 0.34 and 2.45 ± 0.14 log10 CFU/g of examined RTE chicken fajitas, chicken shawarma, chicken burger, chicken pane and chicken luncheon, respectively. Ready to eat chicken products classified into good 125 (50%), acceptable 36 (14.4%), unsatis-factory 54 (21.6%) and potentially hazard 35 (14%) according to the count of S.aureus. Addition of 1% rosemary oil, 1% thyme oil and 1% garlic oil during formulation of chicken burger artificially inoculated with *S.aureus* achieved reduction percentages 86.53%, 88.94% and 92.51 % for rosemary1%, thyme 1% and garlic 1% treated groups, respectively on the 6th day of storage at 4° C. Generally essential oils decrease *S.aureus* population in artificially inoculated chicken burger and considered as a promising in controlling of food pathogens contaminate chicken products.

Keywords: Staphylococcus aureus, Chicken shawarma, Chicken burger, Garlic oil, Thyme oil.

1. Introduction:

Ready to eat chicken products may be contaminated with microorganisms from meat handlers carrying of pathogenic bacteria during the processes of manufacturing, packaging and marketing. Improper cooking, refrigeration or storage may lead to meat borne illness. Foodborne pathogens are the leading causes of illness ranging from an upset stomach to more serious symptoms such as diarrhea, fever, vomiting, abdominal cramps and dehydration and death depending on the etiological agents **(Van et al., 2007)**.

Foodborne illnesses not only affects the health of individuals, but it can also has dramatic economic impact. The economic losses from various factors, such as medical treatment, lost wages and productivity, loss of business, recall and destruction of products.

Delicatessen foods such as salads and sandwiches have also been implicated in foodborne illness outbreaks. These foods are often prepared by hand and this direct contact may lead to an increased incidence of contamination with potential foodborne pathogens, such as *Staphylococcus aureus* **(Colombari et al., 2007)**. *Staphylococcus aureus* is a non-motile,

non-spore producer, Gram positive coccus. It is one of the normal inhabitant's microorganisms of skin and nares (man and animals) which may also act as pathogen under certain circumstances **(Licitra, 2013).** *Staphylococcus aureus* produces Staphylococcal enterotoxin that is responsible for almost all Staphylococcal food poisoning. Symptoms that generally have a rapid onset, appearing around (1–6 hours) after ingestion. Common symptoms include nausea, vomiting, abdominal cramps, diarrhea, headache, muscle cramping, transient changes in blood pressure may occur in severe cases **(FDA, 2012).** The rapid evolution of antibiotic resistance in *S. aureus* is a global concern. *S. aureus* has multiple resistances to different antimicrobial agents. Antibiotic resistance depends on the presence of genes, which encode the antibiotic resistance **(Momtaz *et al.*, 2013)**. The present study was conducted to evaluate the prevalence of S. aureus in RTE chicken products in addition to decontamination trial using essential oils during manufacturing of chicken burger.

2.Materials and Methods:

2.1. Collection of samples:

Two hundred and fifty RTE chicken products were collected randomly from different restaurants at Zagazig city, Sharkia governorate, Egypt, at different sanitation levels. The collected samples represented by (50 chicken ready to eat products each of fajitas, shawarma, burger, pane and luncheon).

All samples were directly transferred to the laboratory of Food Control Department in an ice box under hygienic conditions without undue delay then the core content were microbiologically examined.

2.2. Microbial examination:

The samples were prepared according to **APHA (1992).**Twenty five grams of each sandwiches core were homogenized aseptically for 1 min with 225 ml of 0.1 % peptone water in a stomacher (Colworth, 400) then serially dilutedto 10-fold in the same diluent.

2.3. Enumeration and identification of Staphylococcus aureus:

The technique of *S. aureus* count was applied according to **AOAC (2003).** 0.1 ml from each of the prepared dilutions was spread onto duplicate plates of Baird Parker (BP) agar (Oxoid CM 275), supplemented with egg yolk tellurite emulsion (50 ml/L, Oxoid SR54) and incubated at 37ºC for 24-48 hrs. Typical colonies of *S. aureus* were circular, black, shiny colonies surrounded by clear halo zone extending into opaque medium, were count and recorded.

2.3.1.Biochemical identification:

The top parts of five suspected colonies from plate containing 20-200 colonies were inoculated into test tubes containing 5 ml of Brain Heart Infusion broth (BHI) (**FAO, 1992).** The inoculated tubes are then incubated at 37ºC for 18 hours and the growth cultures were identified biochemically as follow:

2.3.2 Coagulase activity (Rayman et al., 1975):

Seven hundred microlite from thoroughly mixed BHI broth culture was added to tubes containing 0.3 ml of sterile citrated rabbit plasma in 10×75 mm tubes and incubated at 35-37ºC.

The tube was examined for clotting after 4 hrs and if not positive they were incubated at a room temperature and re-examine after 24 hours for clot formation. This clot indicated a coagulase positive activity.

2.4. Decontamination trial using of Essential oils:

About 6 Kg of chicken burger (Minced broiler breast 70 %, fat 15 %, starch 3.5 %, cold water 10 % and salt 1.5 %) are directly transported to the laboratory in an ice box. The mixture was artificially inoculated with *Staphylococcus aureus* then divided into four groups.First control group , second group treated with addition of 15 ml of rosemary oil, thired group treated with addition of 15 ml of thyme oil and fouth group treated with 15 ml garlic oil (each group one and half kilograms). The groups were sampled immediately after preparation (zero time) then sampled at 1st day, 2nd day, 4th day and 6th day of storage at 4^{O} C.

Twenty five grams of each sample were homogenized 25 g with 225ml of sterile pepton water 0.1% then serial dilutions (10^{-1} to 10^{-6}) were prepared following the recommendation of **APHA (1992).** One hundred microliter from each prepared serial dilution of the samples under investigation was evenly spread over a dry surface of the Paired parker Agar. The inoculated plates were incubated at 35°C for 48 hrs.

2.5. Statistical analyses:

The experimental data were evaluated using mixed model's procedure, *post hoc* comparisons were applied, whenever appropriate, using Duncan's test. All statistical procedures were performed using PASW statistics 18 (SPSS Inc., USA). Statisticalsignificance was considered at $P \leq 0.05$.

3. Results and discussion:

Staphylococcal food poisoning usually occurs in foods that require human handling during preparation and left at room temperature for long periods before consumption. The data illustrated in table (1)) declared that *S. aureus* was detected in 56, 48, 68, 42 and 36% with mean counts 3.24 ± 0.38, 3.62 ± 0.41, 4.12 ± 0.42, 3.12 ± 0.34 and 2.45 ± 0.14 log10 CFU/g of examined RTE chicken fajitas, chicken shawarma, chicken burger, chicken pane and chicken luncheon, respectively.

The obtained *S.aureus* counts nearly similar to **Sharaf and Sabra (2012)** they found S.aureus $4.1x10^2$ and $5.6x10^3$CFU/g. for luncheon and shawarma. Higher counts in chicken luncheon obtained by **Essa et al (2004)** they found 1.37×10^4 CFU /g of thirty chicken luncheon in Assiut

Table1. Prevalence and count log_{10} CFU/g of *S. aureus* in examined ready to eat chicken products in comparison to standard of ready to eat meat (n= 250).

Chicken products	prevalence	Mean ± SE (Min – Max)	Categories according Food Standards Australia New Zealand (2001).			
			Good $>10^2$	Acceptable 10^2 to $<10^3$	Unsatisfactory 10^3 to $<10^4$	Potentially Hazardous $\geq 10^4$
Chicken fajitas	56%	3.24 ± 0.38^{ab} 2.56 - 4.32	22	8	15	5
Chicken shawarma	48%	3.62 ± 0.41^{ab} 2.84 - 4.11	26	3	17	4
Chicken burger	68%	4.12 ± 0.42^{a} 2.95 - 4.78	16	4	9	21
Chicken pane	42%	3.12 ± 0.34^{b} 2.18 - 4.19	29	9	7	5
Chicken luncheon	36%	2.45 ± 0.14^{c} 2 - 3.45	32	12	6	0
Total			125 (50%)	36(14.4%)	54 (21.6%)	35(14%)

(a,b and c) Means within the same column bearing different small superscript letters are significantly different ($p < 0.05$).

Min= Minimum Max= Maximum SE= Standard Error

City.The high prevalence of *S. aureus* attributed to the poor personal hygiene during processing of sandwiches, which resulted in higher contamination **(Tambekar et al., 2008)**. There were significant differences between RTE chicken produts ($p<0.05$). The lowest mean value detected in chicken luncheon, which attributed to preparation of chicken luncheon under commercial sterilization and packaging. Moreover, the variation could be related to the difference of the cooking system (frying, roasting, grilling, etc.), the microbiological quality of raw ingredient, the shape and size of the meat piece(s), the utensils used for cooking (oven, crock pot, stew pot, grill, etc.), the presence of seasoning ingredients (sauces, spices, vegetables, etc.) **(Daelman et al., 2013)**. *S. aureus* previously isolated from 17.9 % of RTE and count ranged from 2.30 to 3.60 log10 CFU/g in Taiwan **(Fang et al., 2002)**. Enterotoxigenic *S. aureus* detected in 60% of coriander sauce, 58% of coconut slices and in 86% samples of RTE salads collected from New Delhi and Patiala City, India **(Ghosh et al., 2007)**. There is no available regulation for *S. aureus* count in Egyptian RTE food, so the examined sandwiches were categorized as shown in table (2) and figure (2.B) according to the Compendium of Microbiological Criteria for Food **(Food Standards Australia New Zealand , 2001)** into good 125 (50%), acceptable 36 (14.4%), unsatis-factory 54 (21.6%) and potentially hazard 35 (14%). The unsatisfactory plus potentially hazard samples carry a significant risk if applied under certain conditions as excessive handling, bad hygienic conditions, time and temperature abuse that help *S. aureus* to proliferate and produce different staphylo-coccal enterotoxin (SEs) that may cause food poisoning when ingested **(Bennett et al.,2013)**. *S.aureus* produces a number (SEs) that responsible for inflammation and hyperemia of the gastro-intestinal mucosa. Moreover, symptoms as nausea, vomiting, and abdominal cramps, and diarrhea rarely occur. These usually start within one to eight hours after ingestion of food contaminated with *S. aureus* and symptoms last one or two days. The illness seldom is fatal **(Kerouanton et al., 2007)**.

Decontamination of *Staphylococcus aureus* using essential oils:

An increase in bacterial resistance to antibiotics and the lack of new antibiotics introduced into the market resulted in a need to find alternative strategies so as to cope with infections resulting from drug-resistant bacteria **(Bajera et al., 2017).** Development of alternatives for antibiotics and the discovery or development of adjuvants are amongst the potential strategies proposed (**Bush et al., 2011)**.The results in table (2)) revealed that addition of 1% rosemary oil, 1% thyme oil and 1% garlic oil during formulation of chicken burger artificially inoculated with *S.aureus*. The *S. aureus* count could be reduced from $2.4\times10^5\pm0.79\times10^5$ in control group to $5.8\times10^4 \pm 0.92\times10^4$, $3.23\times10^4 \pm 0.94\times10^4$ and $2.98\times10^4 \pm 0.51\times10^4$ CFU/g with reduction percentages of 75.83% , 86.54% and 87.58% in rosemary1%, thyme 1% and garlic 1%, respectively at zero time table (10) and figure (10).

On the 1st day the counts were$13.6\times10^5\pm0.9\times10^5$, $17.25\times10^4\pm2.65\times10^4$, $15.14\times10^4\pm1.95\times10^4$ and $12.52\times10^4\pm1.82\times10^4$ CFU/g in control, rosemary1%, thyme 1% and garlic 1%. The

reduction percentages were 87.31%, 88.86% and 90.79% for rosemary1%, thyme 1% and garlic 1% treated groups, respectively.

Garlic oil significantly reduced *S. aureus* count ($p<.05$) at zero time and 1st day of application. While there were no significant differences presented with rosemary or thyme oil 1%. The finding coincides with **Prabaand Kumaresan (2014)** whom found garlic extract showed better activity.

Table (2) Effect of essential oils on *Staphylococcus aureus* artificially inoculated in chicken burger stored at 4^{O} C (N=5).

	Control	Rosemary 1%	Thyme 1%	Garlic 1%
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Zero time	$2.4\times10^{5}\pm0.79\times10^{5a}$	$5.8\times10^{4}\pm0.92\times10^{4a}$	$3.23\times10^{4}\pm0.94\times10^{4a}$	$2.98\times10^{4}\pm0.51\times10^{4b}$
1st day	$13.6\times10^{5}\pm0.9\times10^{5a}$	$17.25\times10^{4}\pm2.65\times10^{4a}$	$15.14\times10^{4}\pm1.95\times10^{4ab}$	$12.52\times10^{4}\pm1.82\times10^{4b}$
2nd day	$19.8\times10^{5}\pm1.2\times10^{5a}$	$20.8\times10^{4}\pm2.32\times10^{4b}$	$18.32\times10^{4}\pm2.15\times10^{4b}$	$14.56\times10^{4}\pm1.74\times10^{4b}$
4th day	$30.6\times10^{5}\pm3.1\times10^{5a}$	$25.7\times10^{4}\pm3.9\times10^{4b}$	$21.87\times10^{4}\pm3.21\times10^{4b}$	$17.62\times10^{4}\pm2.35\times10^{4c}$
6th day	$5.68\times10^{6}\pm0.94\times10^{6a}$	$7.65\times10^{5}\pm1.14\times10^{5a}$	$6.28\times10^{5}\pm1.09\times10^{4a}$	$4.25\times10^{5}\pm1.23\times10^{5b}$

(a, b,c) Means on the same rows carrying different superscript letters are significantly different ($P< 0.05$).

Table (3) Effect of essential oils on reduction percentages on *Staphylococcus aureus* artificially inoculated in chicken burger stored at 4^{O} C.

	Reduction % of rosemary 1%	Reduction % of thyme 1%	Reduction % of garlic 1%
Zero time	75.83%	86.54%	87.58%
1st day	87.31%	88.86%	90.79%
2nd day	89.49%	90.74%	92.64%
4th day	91.6%	92.85%	94.24%
6th day	86.53%	88.94%	92.51%

Reduction Percentage = $\frac{\text{Control}-\text{treated}}{\text{Control}} \times 100$

%= percentage

On the 2nd day the counts were $19.8\times10^5\pm1.2\times10^5$, $20.8\times10^4\pm2.32\times10^4$, $18.32\times10^4\pm2.15\times10^4$, and $14.56\times10^4\pm1.74\times10^4$ CFU/g in control, rosemary1%, thyme 1% and garlic 1%. The reduction percentages were 89.49%, 90.74% and 92.64 % for rosemary1%, thyme 1% and garlic 1% treated groups, respectively.

On the 4th day the counts were $25.7\times10^4\pm3.9\times10^4$, $30.6\times10^5\pm3.1\times10^5$, $21.87\times10^4\pm3.21\times10^4$ and $17.62\times10^4\pm2.35\times10^4$ CFU/g in control, rosemary1%, thyme 1% and garlic 1%. The reduction percentages were 91.6%, 92.85% and 94.24 % for rosemary1%, thyme 1% and garlic 1% treated groups, respectively.

The *S. aureus* on 2nd and 4th day significantly reduced in all treated groups ($P< 0.05$) which may attributed to the effect of phenolic compounds that present in essential oils.

On the 6th day $5.68\times10^6 \pm 0.94 \times 10^6$, $7.65 \times 10^5 \pm1.14\times10^5$, $6.28\times10^5 \pm1.09\times10^4$ and $4.25\times10^5\pm1.23\times10^5$ CFU/g in control, rosemary1%, thyme 1% and garlic 1%. The reduction percentages were 86.53%, 88.94% and 92.51 % for rosemary1%, thyme 1% and garlic 1% treated groups,respectively.

The antimicrobial activity exhibited by plant extracts against food poisoning bacteria has been demonstrated by several studies as, **Alzoreky and Nakahara (2003), Verma *et al.*(2012) and Akinpelu *et al* (2015)** they found that the antimicrobial activity of the *thyme* essential oil is more effective on Gram-positive than Gram-negative bacteria by interfering with the cell wall. Plant extracts components as phenolic compounds possess antimicrobial activity by interfere with the phospholipids bilayer of cell membranes causing an increase in permeability and leakage of vital intracellular components and affect bacterial enzymes systems.

The effect of rosemary oil decalred in previous studies by **Barbosa *et al.* (2009) Kwiatkowski et al.(2015)** and **Rezaei and Shamloofar (2016)** they found antibacterial properties of commercial essential oil rosemary, significantly reduce the number of *S. aureus*. α- pinene is reported as the major component of rosemary essential oil, followed by 1, 8 – cineole, camphene, β- myrcene, camphor and borneole. It was determined that rosemary essential oil exhibits antimicrobial activity by passing through the cell wall and cytoplasm membranes and disrupting their structure as a typical lipophilic substance **(Stojanović-Radić et al., 2010)**.

Regarding to the effect of thyme similar results were recorded by **Stojković *et al.* (2013**) and **Maksimov (2017)** they demonstrated that *Thymus. Vulgaris* essential oil was active against *S. aureus* at MIC of (1 µL/ml) and (0.6 –1 mg/mL), respectively. Higher protection were recorded by **Gonçalves *et al.* (2017)** who noted that *thyme* essential oil inhibited antimicrobial growth with the strongest activity against *S. aureus (*MIC varied between 0.125 and 0.6 mg/ml), furthermore, **Mostafa *et al.* (2018)** reported that *thymus* vulgaris was effective against *S. aureus*, as it is the most susceptible strains to the extracted plants. Thymol which isfound in thyme oil act on bacterial cell membrane is considered to be the main mode of antibacterial action effect on morphology, structure, function, modification in the transport of nutrients, membrane disruption and extensive leakages from the bacterial cells leading to cell death (**Bajpai et al., 2012).**

The effect of garlic on S.aureus declared by **Shokrzadeh and Ebadi, (2006)** they found concentration-dependent antibacterial activity against *S. aureus* and **Fakoor and Rasooli (2008)** who revealed also inhibitory effect of cumin oil against *S. aureus* in meat products. Allicin and

other sulfur compounds are thought to be the major compounds responsible for the antimicrobial effect of garlic. Garlic is effective against a number of gram-negative, gram-positive, and acid-fast bacteria, including *Staphylococcus*, *Salmonella*, *Vibrio*, *Mycobacteria*, and *Proteus* species (**Abdullah et al., 1988)**. The gram positive *S. aureus* was more susceptible to the toxic effects of garlic than its gram negative counterparts (**Abubakar, 2009**).

Generally essential oils decrease *S.aureus* population in artificially inoculated chicken burger considered as a promising in controlling of food pathogens contaminate chicken products.

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المخلص العربى

مدى تواجد المكورات العنقوديةالذهبية فى مصنعات الدجاج الجاهزة للأكل مع محاولة للحد منها

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تعتبر الدواجن ومنتجاتها من الاغذية المفضلة لدى كثير من المستهلكين لسهولة تحضيرها و طعمها المستساغ بالأضافة الى قيمتها الغذائية العالية لاحتوائها على نسب عالية من البروتين الحيوانى، بالاضافة الا انها رخيصة الثمن مقارنة بباقى مصادر البروتين الحيواني لذلك تم تجميع250 عينة عشوائية من مصنعات الدجاج الجاهزة للأكل 50 عينة من كل من فاهيتا الدجاج ، شاورما الدجاج ، برجر الدجاج، بانية الدجاج ولانشون الدجاج من المطاعم ذات المستويات الصحية المختلفة بمحافظة الشرقية لفحصها بكتيرلوجيا لعزل وتصنيف المكور العنقودى الذهبى. تواجدت المكورات العنقودية الذهبية في 56 و 48 و 68 و 42 و 36 ٪ مع متوسط حسابى3.24 ± 0.38 ، 3.62 ± 0.41 ، 4.12± 0.42 ، 3.12 ± 0.34 و 2.45 ± 0.14 مستعمرة بكتيرية لوغاريتم $_{10}$/ جرام لكل من فاهيتا الدجاج ، شاورما الدجاج ، برجر الدجاج، بانية الدجاج ولانشون الدجاج على الترتيب . بمقارنة أعداد ميكروبات المكور العنقودى الذهبى مع الأعداد المسموح بها فى المواصفات الدولية وجد أن 125 (50%) من مجموع العينات المختبرة جيدة، 36 (14.4%) من مجموع العينات المختبرة مقبولة.اما العينات الغير مقبوله فكانت54 (21.6%) كما بلغ عدد العينات التى تؤدى إلى خطر صحى على المستهلكين الي 35(14%). فى هذة الدراسة تم عمل 6 كجم من برجر الدجاج وتم تلويث الخليط باستخدام المكور العنقودى الذهبى ثم تم تقسيمه إلى أربع مجموعات. المجموعة الأولى الضابطة ، المجموعة الثانية عولجت بإضافة 15 مل من زيت إكليل الجبل ، مجموعة ثالثة بإضافة 15 مل من زيت الزعتر ومجموعة رابعة بإضافة 15 مل زيت الثوم. تم أخذ عينات من المجموعات فور تحضيرها ، ثم أخذ عينات منها في اليوم الأول واليوم الثاني واليوم الرابع و السادس بعد التخزين درجة حرارة 4 سيليزية وفى كل مرة تم عد المكور العنقودى الذهبى فى المجموعات المختلفة. وجد أن جميع الزيوت المتسخدمة لها القدرة على خفض أعداد المكور العنقودى الذهبى فى أيام التخزين المختلفة عند درجة حرارة 4 درجة سيليزية إلا أن أكثر المجموعات خفضا كانت المجموعى التى تم معالجتها بزيت الثوم.