PREVALENCE OF *Staphylococcus aureus* AND *Salmonella* SPECIES IN RETAILED CHICKEN MEAT WITH A REDUCTION TRIAL USING *Nigella sativa* AND ROSEMARY ESSENTIAL OILS

Amina H.A. Habashy¹, Waiel M. Salah El-Dien¹, Mohamed A. M. Hussein², Wageh Sobhy Darwish²

¹Food Control Department, Animal Health Research Institute, Zagazig branch, ²Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Sharkia, 44511, Egypt

*Corresponding author, Email: katkoot_moftaris2008@yahoo.com

Abstract: Chicken meat represents an important source of animal derived proteins, vitamins, and minerals. However, chicken meat might act as a potential source of human exposure to foodborne pathogens such as *Staphylococcus aureus (S. aureus)* and *Salmonella* species. The objectives of the present study were first to investigate the prevalence rates of *S. aureus* and *Salmonella* species in the retailed chicken breast and thigh muscles at Zagazig city, Egypt. Second, serological identification of the isolated bacteria was followed. Third, screening of *S. aureus* enterotoxin coding genes (*sea, seb, sec, sed, and see*) and methicillin resistance (*mecA*) gene, as well as *Salmonella* virulence associated genes including *Salmonella* hyper-invasive locus (*hilA*), and *Salmonella* enterotoxin (*stn*) was done using PCR. Thereafter, the inhibitory effects of *Nigella sativa*, and rosemary essential oils were investigated against *S. aureus*, and *Salmonella* Typhimurium. The obtained results revealed isolation of *S. aureus* from the examined breast and thigh muscles at percentages of 23.3%, and 26.6%, respectively, whereas percentages of *Salmonella* spp. isolation from both samples were 33.3%, and 16.6%, respectively. Four *Salmonella* serotypes namely, *S. Typhimurium, S. Enteritidis, S. Kentucky, and S. Anatum* were further identified. The recovery rates of *S. Typhimurium* from breast and thigh muscles were 23.33% and 13.33%, respectively followed by *S. Enteritidis* (3.33% each). *Staphylococcus* enterotoxin genes (*sea, and see*), and *mecA* gene were detected in *S. aureus* isolates. Besides, *Salmonella* *hilA* and *stn* genes were also detected in *Salmonella* Typhimurium isolates. *Nigella sativa* and rosemary essential oils at 0.1%, and 0.5% could significantly reduce *S. aureus*, and *Salmonella* Typhimurium in chicken breast meat.

Key words: chicken muscles; *Staphylococcus aureus; Salmonella* species; *Nigella sativa*; rosemary essential oils

Introduction

Chicken meat is regarded as an important source of animal-derived protein, essential amino acids, polyunsaturated fatty acids, vitamins, and minerals. Economically, chicken meat plays an important role in solving food security issues related to the shortage in red meat industry because of its wide availability, and relatively low price compared with the red meat (1, 2).

Microbial contamination of chicken meat might take place during any step of the manufacture process starting from the slaughter, defeathering, deplopping, evisceration, distribution, and storage. Therefore, chicken meat is regarded as a potential source for spreading of food poisoning pathogens such as *Staphylococcus aureus (S. aureus)* and *Salmonella* spp. (3, 4).

Ingestion of foods contaminated with *S. aureus* enterotoxins is the major cause of food poisoning cases that characterized by their rapid onset (1-6 hours post ingestion of contaminated foods), nausea, vomiting, abdominal cramps, and diarrhea (5).
S. aureus and staphylococcal enterotoxins (SEs) were isolated and detected in retailed chicken giblets in Egypt (1, 6), chicken meat and giblets retailed in US markets (7), and in chicken breast and thigh retailed in Cambodian markets (8).

Non-typhoidal salmonellosis is a foodborne disease caused by ingestion of foods contaminated with Salmonella spp. The incubation period of this disease is 12 to 36 h post-ingestion of contaminated foods. The major symptoms of this foodborne infection include nausea, vomiting, fever, severe diarrhea, abdominal cramps, and malaise (9). Salmonella spp. was isolated from chicken meat in Vietnam (10), Japan (11), and chicken giblets in Egypt (3).

There is an increasing demand for natural antimicrobials as food preservatives to avoid the undesirable effects of the chemical preservatives. In this regard, plant essential oils offer friendly alternatives for chemical preservatives. Black seed (Nigella sativa) essential oil has a documented antimicrobial effect, particularly against Listeria monocytogenes (12). Besides, rosemary (Rosmarinus officinalis L.) essential oil has recorded antimicrobial activities against several food poisoning microorganisms (13).

Insight of the previous facts, this study was undertaken to investigate the prevalence rates of two food poisoning organisms, including S. aureus, and Salmonella spp., in the retailed chicken meat (breast, and thigh) in Egypt. Furthermore, detection of enterotoxin coding genes and virulence-associated genes were screened using PCR. In addition, the inhibitory effects of Nigella sativa, and rosemary essential oils against S. aureus, and Salmonella spp. were examined.

**Materials and methods**

**Sampling and samples preparation**

Sixty random chicken muscle samples, including breast and thigh muscles (30 each) were collected directly after slaughter from chicken butchery shops with different sanitation levels at Zagazig city, Egypt. The samples were cooled and transferred without delay to Animal Health Research Institute, Zagazig, Egypt for bacteriological examination.

Samples were prepared according to the recommended guidelines (14). In brief, ten grams from each sample were mixed with 90 mL of 1% sterile peptone water (Oxoid CM9, UK) and blended for 2.5 min at 2000 rpm. The mixture was then allowed to stand for 15 min at room temperature.

**Isolation and identification of S. aureus**

S. aureus isolation and identification were done according to Animal and Plant Health Agency (APHA) (14) procedures. In short, 0.1 mL of each prepared homogenate was spread over a Baird Parker agar (Oxoid, UK) plate supplemented with egg yolk emulsion using a sterile glass spreader. Plates were incubated on inverted positions at 37°C for 48 h. S. aureus colonies appear as black, shiny, circular, smooth, and convex with narrow white margin and surrounded by a clear zone extending into the opaque medium. Five suspected S. aureus colonies were picked up and purified on nutrient agar (Oxoid, UK) slopes. S. aureus colonies were subjected to morphological, biochemical (catalase, coagulase, and mannitol sugar fermentation tests), and serological identification (14).

**Isolation and identification of Salmonella species**

The procedures of ISO 6579 (15) for Salmonella isolation and identification were followed. In brief, ten mL of the previously prepared meat homogenate from each sample were incubated at 37°C for 18 ± 2 h as pre-enrichment on non-selective liquid medium. Selective enrichment was done on Rappaport Vassiliadis soya broth (Oxoid, UK) and incubation at 41.5°C for 24 ± 2 h. A loopful from the previously enriched culture was streaked on the surface of xylose lysine deoxycholate (XLD) agar (Oxoid, UK) plate and incubated 37°C for 24 ± 2 h. Suspected colonies (non-lactose fermenters) were red with or without black centers. Such colonies were purified and sub-cultured onto nutrient agar slopes and incubated at 37°C for 24 h. The purified colonies were subjected to morphological, biochemical (indole, methyl red, Voges Proskauer, citrate utilization, hydrogen sulfide production, and urease tests), and serological identification (15).
Prevalence of *Staphylococcus aureus* and *Salmonella* species in retailed chicken meat with a reduction... 301

Table 1: Oligonucleotide primer sequences used in the study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5'-3')</th>
<th>Product size (bp)</th>
<th>Annealing temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA</td>
<td>GTAGAAATGACTGAACGTCCGATAA CCAAATCACCATTGTTTCCGGTCTAA</td>
<td>310</td>
<td>50</td>
<td>(16)</td>
</tr>
<tr>
<td>sea</td>
<td>GGTTATCAATGTGCCGGGTG  CGGCACTTTTTTCTTTCGG</td>
<td>102</td>
<td>50</td>
<td>(17)</td>
</tr>
<tr>
<td>seb</td>
<td>GTATGGTGGTGAATCAGGC  CCAAATGTCAGGATTGAG</td>
<td>164</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>sec</td>
<td>AGATGAAATGATGTGTGGTATGG</td>
<td>451</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sed</td>
<td>CCAAATAGGAAAAAATAAAG  ATTTGATTTTTTTTGTTTC</td>
<td>278</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>see</td>
<td>AGGTTTTTCCACAGGTATCC  CTTTTTTTCTTGGGTCAATC</td>
<td>209</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>hilA</td>
<td>CGGAAGCTTATTTGCGCCATGCTGAGGTAG  GCA TGG ATC CCC GCC GGC GAG ATTGTG</td>
<td>854</td>
<td>60</td>
<td>(18)</td>
</tr>
<tr>
<td>stn</td>
<td>TTGGTGCGATCAC TGG CAACC  ATTCGTAACCCGCATCGTTC</td>
<td>617</td>
<td>60</td>
<td>(19)</td>
</tr>
</tbody>
</table>

Molecular identification of *Staphylococcal* enterotoxins and *Salmonella*-associated virulence genes

DNA isolation from the cultured and identified bacteria was done using Genomic DNA extraction kit according to the instructions of the manufacturer (Alliance Global, Dubai, UAE). Primer pairs for *S. aureus* enterotoxin genes (sea, seb, sec, sed and see), methicillin resistance (mecA) gene for detecting methicillin resistant *S. aureus* (MRSA), and *Salmonella* hilA, and stn virulence associated genes were purchased from Metabion International, Gmbh, Germany, and displayed in Table 1. PCR amplification reactions and cycling conditions were performed (16-19) on a Thermal Cycler (Master cycler, Eppendorf, Germany) using uniplex PCR approaches. Amplified PCR products were run on 1.5% agarose gel electrophoresis (AppliChem, Gmbh, Germany) in 1x Tris Borate EDTA (TBE) buffer stained with ethidium bromide then visualized on a UV transilluminator. DNA Ladder (100 bp, Qiagen, Gmbh) was used to determine the fragment sizes.

An experimental trial to investigate the inhibitory effects of *Nigella sativa* and rosemary essential oils against *S. aureus* and *Salmonella* species

The antibacterial effects of *Nigella sativa*, and rosemary essential oils extracted, prepared, and purchased from National Research Center, Dokki, Giza, Egypt were tested at two concentrations for each (0.1 and 0.5% prepared in pure corn oil (100%)) based on pre-experimental trials (Data are not shown). Two parallel experimental trials were conducted to test the inhibitory effects of these oils, one against *S. aureus*, and the other against *Salmonella Typhimurium*. In the two experiments, microbiologically negative breast muscles were prepared as slices (20). Each slice was four cm width, six cm length, 1.0 cm thickness and 25 g in weight. Five groups for each experiment were planned (n = 3/group). Group 1 was employed as a control, where breast muscles were artificially inoculated with the tested organism (10⁷ CFU/mL) (21), group 2 treated with *Nigella sativa* oil 0.1%, group 3 treated with *Nigella sativa* oil 0.5%, group 4 treated with rosemary oil 0.1%, and group 5 treated with rosemary oil 0.5%. Treatment groups were soaked in the tested oils for 30 min at room temperature and examined for bacterial counts (22).

Statistical analysis:

All microbial counts were transferred into base-10 logarithms of CFU/g. Data were analyzed using one-way ANOVA procedure of SPSS v.23 (SPSS Inc., Chicago, Illinois, The USA). Tukey’s multiple comparison tests were used to detect significant variations. Data were expressed as means ± SD, with a P-value of 0.05 is considered significant.
Results

The results revealed that isolation percentages of *S. aureus* from the examined breast and thigh muscles were 23.3 and 26.6%, respectively, whereas *Salmonella* spp. was isolated from these samples with percentages of 33.3, and 16.6%, respectively (Figure 1).

![Figure 1](image)

**Figure 1:** Prevalence rates (%) of *S. aureus* and *Salmonella* spp. in the examined chicken breast and thigh muscles retailed in Zagazig city, Egypt

Four *Salmonella* species were recovered namely, *S. Typhimurium*, *S. Enteritidis*, *S. Kentucky*, and *S. Anatum*. *S. Typhimurium* and *S. Enteritidis* were isolated at 23.33% and 3.33% from breast muscles; and at 13.33% and 3.33% from thigh muscles. *S. Kentucky* and *S. Anatum* were isolated only from breast muscles at 3.33% (Table 2).

PCR testing of three randomly selected *S. aureus* isolates for harboring meca and *Staphylococcal* enterotoxins revealed detection of meca in two of the tested isolates and only sea and see in one *S. aureus* isolate. Similarly, PCR testing of four selected *S. Typhimurium* isolates for harboring bilA, and stn virulence associated genes revealed detection of bilA in two of the isolates; while stn was detected in only one *S. Typhimurium* isolate (Data are not shown). These PCR screened isolates were subjected to the two essential oils under study.

*Nigella sativa* and rosemary essential oils were used to reduce *S. aureus*, and *Salmonella* spp. in an experimental trial using chicken breast meat. The obtained results revealed significant inhibitory effects for the two used oils against *S. aureus* in a concentration-dependent manner and against *Salmonella* spp. without clear effect for the dose (Table 3). At the same time, the used oils did not change the sensory characteristics (blue-whitish color, firm in consistency, and fresh odor) of the breast meat at the two tested oil concentrations (0.1%, and 0.5%) (Data are not shown).

Discussion

*Staphylococcus aureus* is a major foodborne pathogen (23-25). It is capable of producing heat-stable enterotoxins such as sea, seb, sec, sed, and see. Moreover, MRSA is responsible for many nosocomial infections worldwide. Herein, *S. aureus* was isolated from retailed chicken breast and thigh at variable rates. In addition, some of the identified *S. aureus* isolates harbored meca, sea, and see-coding genes, indicating that chicken breast and thigh muscles might be considered potential sources for enterotoxigenic MRSA. In agreement with the obtained results, toxin producing MRSA were isolated from retailed chicken meat and meat products in Egypt (1, 6), United states (7, 26), and Canada (27).

| Table 2: Serological identification of *Salmonella* spp. isolated from breast and thigh muscles |
|--------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| **Salmonella serotypes** | **Breast muscles** | **Thigh muscles** | **Antigenic structure** |
| | No. | % | No. | % | O | H |
| *S. Typhimurium* | 7 | 23.33 | 4 | 13.33 | B | 1,4,5,12 | i : 1,2 |
| *S. Enteritidis* | 1 | 3.33 | 1 | 3.33 | D | 1,9,12 | g,m : - |
| *S. Kentucky* | 1 | 3.33 | - | - | E1 | 8,20 | iZ6 |
| *S. Anatum* | 1 | 3.33 | - | - | E1 | 3,10, | e,h : 1,6 |
| **Total** | 10 | 33.33 | 5 | 16.66 | | | |
Table 3: Inhibitory effects of Nigella sativa and rosemary essential oils against S. aureus, and S. Typhimurium growth in chicken breast

<table>
<thead>
<tr>
<th>Experimental trials</th>
<th>S. aureus</th>
<th>S. Typhimurium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (log 10 cfu/g)</td>
<td>Reduction (%)</td>
</tr>
<tr>
<td>Control</td>
<td>8.39 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Nigella sativa 0.1%</td>
<td>6.58 ± 0.09&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>21.57</td>
</tr>
<tr>
<td>Nigella sativa 0.5%</td>
<td>5.69 ± 0.60&lt;sup&gt;d&lt;/sup&gt;</td>
<td>32.10</td>
</tr>
<tr>
<td>Rosemary 0.1%</td>
<td>6.81 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.81</td>
</tr>
<tr>
<td>Rosemary 0.5%</td>
<td>6.22 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.82</td>
</tr>
</tbody>
</table>

Values within the same column carrying different letter are significantly different at *P* < 0.05

Staphylococci can be found on the skin, hair, and nails of food handlers. Additionally, washing water used in the cleaning of chicken carcasses is also considered as a source for MRSA (1). Such sources might explain the isolation of S. aureus from retailed chicken products in the present study. Detection of enterotoxins in the identified S. aureus isolates agrees with Shawish and Al-Humam (28) who detected at least one of the S. aureus enterotoxins (sea, seb, sec, and sed) in the examined meat products sold in Egypt and Saudi Arabia. S. aureus is responsible for many cases of food poisoning worldwide. For instances, Center for Disease prevention and Control (CDC) reported a food poisoning outbreak in a military unit, US, 2012. This outbreak was due to S. aureus (29). Furthermore, in Europe, European Food Safety Association reported that 293 food poisoning outbreaks in 2011 were due to Staphylococcus spp (30).

Salmonella spp. was isolated from the examined chicken breast and thigh muscles at different rates. The Egyptian legislation prohibits the presence of Salmonella spp. in meat, and this reflects the poor hygienic practices adopted during processing of chicken carcasses in chicken retail markets in Egypt (31). In agreement with the recorded results in the present study, high prevalence rates for Salmonella spp. in retailed chicken meat were recently reported in Malaysia (32), and Bangladesh (33). Four Salmonella serotypes were identified in the present study. Of these serotypes, S. Typhimurium and S. Enteritidis are major food poisoning agents. These serotypes are responsible for several food poisoning outbreaks worldwide. For instances, Salmonella enterica serovar Typhimurium -associated outbreak was reported in Switzerland during May-June 2008 (34). In addition, seven S. Typhimurium outbreaks were reported in three Australian states and territories (35). Two virulence factors namely Salmonella enterotoxin (Stn) and hyper-invasive locus (hilA) coding were detected in the isolated S. Typhimurium. Salmonella enterotoxin (Stn), which is contributed to the pathogenicity process of Salmonella, primarily diarrhea had been identified in Salmonella serovars Typhi, Typhimurium and Enteritidis (36). The hilA gene encodes a regulator that activates the expression of invasion genes in response to both environmental and genetic regulatory factors (37).

There are continuous efforts to find alternatives to the chemical preservatives with antimicrobial activities (38-41). In this regard, a trial to investigate the inhibitory effects of Nigella sativa, and rosemary essential oils against S. aureus and Salmonella spp was performed. Interestingly, the two used essential oils had significant inhibitory effects against the examined microorganisms. In agreement with these findings, rosemary oil significantly retarded growth of Listeria monocytogenes in mozzarella cheese (42). Besides, Nigella sativa oil had significant antibacterial activities against Gram-positive bacteria (43).

**Conclusion**

The obtained results in the current investigation revealed isolation of S. aureus, and Salmonella spp. from retailed chicken breast and thigh muscles at variable rates. This indicates unsatisfactory hygienic measures adopted during slaughtering, defeathering, and processing of chicken carcasses. Therefore, strict hygienic procedures should be followed in chicken processing plants. In addition, using *Nigella sativa* and rosemary ess-
essential oils at 0.1%, and 0.5% is of value in reducing the microbial load of chicken carcasses, particularly S. aureus, and Salmonella spp.

Acknowledgment

Authors are grateful to staff members at Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Egypt for their kind support. The authors guarantee that there is no conflict of interest.

References

Class 1 integron and associated gene cassettes mediating multiple-drug resistance in some food borne pathogens. Int Food Res J; 23: 332-339.


