Prevalence and distribution of staphylococcal enterotoxin genes among Staphylococcus aureus isolates from chicken and turkey carcasses in Algeria

F. Mebkhout1, L. Mezali1, T. M. Hamdi1, Z. Cantekin2, Y. Ergun3, N. Ramdani-Bouguessa4, P. Butaye5,6

1Food Hygiene and Quality Insurance System Laboratory, High National Veterinary School of Algiers, Algeria.
2Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Microbiology, Hatay, Turkey.
3Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Hatay, Turkey.
4Laboratory of medical biology, Algiers, Algeria.
5Ross University, School of Veterinary Medicine, Department of Biomedical Sciences, West Farm, St Kitts and Nevis
6Ghent University, Faculty of Veterinary Medicine, Department of Pathology, Bacteriology and Poultry diseases, Merelbeke, Belgium

ABSTRACT. This study is aimed to determine the prevalence of staphylococcus aureus (S.aureus) by biochemical tests in poultry carcasses. It is also intend to detect the distribution of genes for classical staphylococcal enterotoxins A, B, C, D and E (sea, seb, sec, sed and see) and for gene femA, specific for S.aureus species, using multiplex PCR. A total of 385 samples of neck skins from fresh poultry carcasses were collected during the period 2012-2013 from 16 different slaughterhouses located in the region of Algiers, Algeria. The overall prevalence of S.aureus in freshly slaughtered poultry carcasses was 41.56%, with an individual prevalence of 40.63% and 45.71% for chicken and turkey respectively. From the 95 strains of S.aureus identified by biochemical tests, 82 (86.32%) isolates were femA positive using multiplex PCR. The investigation has also revealed the presence of both enterotoxins B and D, with a predominance of seb (13.33%) followed by sed (1.67%), in the chicken carcasses while in turkey only sed was detected (4.55%). It has been found that strains of S.aureus of poultry origin can be enterotoxigenic with the predominance of genes encoding for enterotoxins seb in chicken and sed in turkey. As enterotoxins can be produced in adequate amounts to induce foodborne illnesses, these potential dangers must be considered in terms of a real risk to public health.

Keywords: Staphylococcus aureus, Poultry carcasses, Staphylococcal entérotoxine, Algiers.
INTRODUCTION
Recent studies have revealed that high risk of foodborne diseases is often related to the contamination of poultry carcasses by pathogens micro-organisms (Escudero-Gilete et al., 2007). Among the pathogen bacteria that maybe present in poultry carcasses is *Staphylococcus aureus*. Its presence in food indicates poor hygiene and improper storage conditions (Gundogan et al., 2005).

*S. aureus* is an opportunistic pathogen that can colonize the skin and mucous membranes particularly in the nose of both healthy humans and animals (Nader et al., 2016). In addition to colonizing various hosts, it can also be the origin of a wide range of different infections in poultry ranging from septicemia, pneumonia, endocarditis, and arthritis (Smyth and McNamee, 2008).

Amongst the foodborne pathogens *S. aureus* is a major one contaminating meat products. It is considered the third largest cause of food related illnesses throughout the world (Achi and Madubuik, 2007; Aydin et al., 2011; Sasidharan et al., 2011).

*S. aureus* is characterized by its ability to produce a large variety of Staphylococcal enterotoxins (SEs) (A, B, C, D, E, G, H, I, J, K, L, M, N, O, P, Q, R and U), but 95% of food poisoning are caused by the enterotoxins A, B, C, D and E (Letertre et al., 2003). It is notable that SEs are heat stable toxins. Indeed, the heat used in cooking and pasteurization is insufficient to destroy them. Moreover, they are difficult to be perceived in food due to the lack of taste and food appearance (Aycicek et al., 2005). For the case of food ingestion contaminated with SEs, food poisoning occurs shortly after, 30 min to 8 hrs while infected individuals usually recover from the toxicity within 24-48 hours (Argudín et al., 2010).

Based on recent published studies, just a little is known about enterotoxigenic *S. aureus* strains from poultry. Particularly in Algeria, the number of studies discussing the prevalence of enterotoxins genes of *S. aureus* in poultry meats is really limited. Therefore, the present study is aimed to determine the prevalence of *staphylococcus aureus* (*S. aureus*) by biochemical tests in poultry carcasses. It is also intend to detect the distribution of genes for classical staphylococcal enterotoxins A, B, C, D and E (*sea, seb, sec, sed* and *see*) and for gene *femA*, specific for *S. aureus* species, using multiplex PCR.

MATERIALS AND METHODS
Sample collection and microbiological analysis
A total of 385 samples of neck skins from fresh poultry carcasses were collected from 16 different slaughterhouses located in the region of Algiers, Algeria. These samples have been examined during 2012-2013. They consist of chicken carcasses (n = 315), and turkey carcasses (n = 70). All samples were placed in sterile plastic bags and brought to the laboratory in cold chain and analyzed within the following 3 h. Microbiological analysis in this study has been carried out according to the international standard NF EN ISO 6888-1/A1 (ISO, 2004). Twenty-five portions of the samples were weighed into sterile stomacher bags diluted with 225 ml sterile buffered peptone water (BPW; Oxoid CM 509) and homogenized in a stomacher (Seward 400) for 2 min. The samples were diluted with BPW, and 0.1 ml portions of dilution levels were streaked on Baird-Parker (BP) agar (Oxoid CM 275) supplemented with egg yolk-tellurite emulsion (Oxoid SR 54) and incubated at 37°C for 24-48 h.

The colonies suspected for Staphylococci positive coagulase were cultured on 5% blood agar and identified by catalase, coagulase tests. *S. aureus* was identified by Staphaurex (Bio-Rad). The reference strain used for microbiological analysis was *S. aureus* ATCC 25923. The pure isolates were then stored at −20°C in TSB-broth and 10% glycerol for future studies.

Statistical Analysis
Data collected from the detection of *S. aureus* on turkey and chicken were analyzed by SPSS Statistics 20 software, to test the significance of differences among the biochemical tests and the Polymerase-Chain Reaction (PCR), and the toxigenic potency of *S. aureus* between the two species (turkey and chicken).

Genomic DNA Extraction
The methodology of DNA extraction is taken from Sambrook and Russel, (2001). For nucleic acid isolation, 95 strains of Staphylococcus species isolated from poultry carcasses were activated on trypticase soya agar (TSA) (BioMérieux, France). After overnight incubation at 37°C, one to two colonies for each strain was re-suspended on 300 μl of TNE buffer (Tris- Nacl-EDTA- SDS) and then vortexed, 10 μl of lysozyme was added plus 200 μl of SETS (Sodium- EDTA- Tris- SDS). The mix was incubated at 37°C for 1 h. After incubation
Each tube was vortexed once every 15 min. Then, 10 μl of proteinase-K (Vivantis Technologies, Malaysia) and 100 μl of TNE were added. The final solution was vortexed and incubated at 50°C for 1 h. After that each microcentrifuge tube was vortexed once every 15 min. A volume of 150 μl of NaCl (5M) was added and vortexed. The obtained DNA was harvested by centrifugation at 10,000 rpm for 10 min at room temperature. The supernatant was transferred to a new microcentrifuge tube for washing by 99% cold ethanol and then stored at -20°C overnight. A second washing of DNA was done by 70% cold ethanol. The sample was dried for 10-30 min and re-suspend in 100 µl sterile distilled water and stored at -20°C until PCR analysis.

Multiplex PCR Conditions
The multiplex PCR has been performed as described by Mehrotra et al. (2000). The primers used in the multiplex PCR are presented in Table 2. Multiplex PCR mix contained 400 μM deoxynucleoside triphosphates (dNTPs); 5 μl of 10X reaction buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl); 4 mM MgCl₂; 20 pmol of each sea, seb, sec, see, and femA primers; 40 pmol of sed primers; 2.5 U of Taq DNA polymerase (AmpliTaq DNA polymerase, Perkin-Elmer), and 5μl of template DNA. The final volume was adjusted by adding 50μl with sterile ultra-pure water. DNA thermocycling (CFX 96 thermal cycler, Bio-Rad) was carried out with the following thermal cycling profile: an initial denaturation at 94°C for 2 min, annealing at 57°C for 2 min, and extension at 72°C for 1 min, ending with a final extension at 72°C for 7 min. Ten microliters of the PCR products were then analyzed by electrophoresis on 2% agarose (BIOMAX) gel, stained with ethidium bromide. Note that the DNA fragments were visualized using a UV transiluminator (EC3, UVP Biomaging systems, Inc (BioMax) while immigration was made by Electrophoresis Power Supply (Model EC 1000 XL Thermo Scientific, Inc.) in TBE buffer (0.09 M Tris–HCl, 0.09 M boric acid, 2 mM EDTA, pH 8.3) for 45 min at 120. Primers are shown in Table 1.

## RESULTS
Table 2 illustrates the overall prevalence of *S. aureus* in freshly slaughtered poultry carcasses that are collected from 16 slaughterhouses. The prevalence was 160 of

<table>
<thead>
<tr>
<th>Type of meat sample</th>
<th>No. of samples</th>
<th><em>S. aureus</em> positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken carcasses</td>
<td>315</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40.63%</td>
</tr>
<tr>
<td>Turkey carcasses</td>
<td>70</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45.71%</td>
</tr>
<tr>
<td>Total</td>
<td>385</td>
<td>160</td>
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<td></td>
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<td>41.56%</td>
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385 samples (41.56%), with an individual prevalence of (40.63%) and (45.71%) for chicken and turkey respectively.

Table 3 shows the confirmation of the isolated strains as *S.aureus* by multiplex PCR. The multiplex PCR has revealed that 82 (86.32%) from these isolates were *femA* positive, i.e., 60 (85.71%) in chicken and 22 (88%) for turkey. Statistical analysis showed no significant difference between the two methods (Classical method and PCR) (p< 0.05).

It has also been observed that 12.19% of *S.aureus* isolates encoded classical staphylococcal enterotoxins, i.e., nine (15%) from chicken and 1 (4%) from turkey carcasses. Statistical analysis showed no significant difference for the prevalence of enterotoxigenic isolates from chicken carcasses and turkey for p< 0.05.Moreover, detected the presence of both enterotoxins B and D in poultry carcasses with a prevalence of 9.76% and 2.44% respectively.

As indicates in Figure 1 and Table 4, isolates from chicken present both enterotoxins B and D, with a predominance of *seb* (13.33%) followed by *sed* (1.67%), while in turkey only *sed* was detected (4.55 %). It is notable that none of the isolates was positive for *sea*, *sec* or *see* genes.

**DISCUSSION**

The isolation of *S.aureus* from chicken and turkey carcasses (41.56%) confirms the contamination of the poultry carcasses by *Staphylococcus aureus*. Indeed, the present study is mainly focused on the determination of the prevalence of *S.aureus* in freshly slaughtered chicken and turkey, in 16 slaughterhouses in the Wilaya of Algiers. Moreover, it also aims to the molecular characterization of the isolated strains by highlighting the presence of enterotoxins genes.

The prevalence of *S.aureus* recorded for chicken (40.63%) was in good agreement with those advocated
by Citak and Duman (2011) and Nader et al. (2016). In Algeria a study conducted in the region of Biskra, carried out on 60 chicken carcasses revealed a prevalence similar to that obtained in the present study (Alloui et al., 2013). However, lower prevalence rates of Staphylococcus aureus were found by others studies (Khallaf et al., 2014; Sarrafzadeh Zargar et al., 2014). In our study, the recorded prevalence of S. aureus present in turkey carcasses (45.71%) was higher than that obtained by Sarrafzadeh Zargar et al. (2014) (16.6%) and El Allouï (2013) (41.6%).

Two other studies that have been carried out in Turkey recorded a prevalence of 9.61% (Kiliç et al., 2009) and 48% (Bystron et al., 2005) of coagulase-positive Staphylococcus from meat and hash meat respectively.

The high prevalence recorded in both species often indicates a lack of good hygiene practices (Lindblad et al., 2006; Pacholewicz et al., 2016) which are linked to the multiple handling of carcasses during slaughter processing. The staff represents a source of contamination spreading the germs by inadequate clothing hygiene, or by hosting (sores, angina, sinusitis and nasopharyngitis).

The lack of control of good manufacturing practices can also be at the origin of these contaminations - multiple contacts with the equipment: tables, bags, knives, towels, scalding bath and feather (Azelmad et al., 2017; Kotula and Pandya, 1995; Mead et al., 1993).

It could also be related to the sanitary status of poultry intended for slaughter (carried by the skin and feathers, or various pathologies as osteomyelitis, arthritis and synovitis).

The variations in the prevalence between our results and other studies, that have been carried-out in other countries, can be attributed to several factors, including the size of the samples tested, the sampling mode (Whole chicken or parts of carcasses), the seasons in which the sampling was done, the methods of isolation used and the hygiene conditions of the slaughterhouse (Teramoto et al., 2016; Wang et al., 2013).

The use of PCR and biochemical identification to confirm S. aureus strains revealed similar results (average 86.32%), which confirms the concordance between the two techniques, already reported by the study of Benhamed (2014).

The present study shows that the poultry isolates can be enterotoxigenic (12.19%). These results are in good agreement with the previous published studies which revealed that the percentage of S. aureus strains producing enterotoxin A to E, bovine and avian, can vary from 0 to 15% (Bergdoll, 1991; Genigeorgis, 1989; Rosec et al., 1997). More recent studies showed that the genes encoding the classical enterotoxins, were absent or occur in less than 3% in S. aureus strains isolated from poultry (Hazarivala et al., 2002; Normanno et al., 2007; Smyth et al., 2005).

As for the chicken some authors note relatively higher percentages. For instance, Nader et al. (2016) show that 7 strains out of 12 (58.3%) of S. aureus isolated from chicken meat were enterotoxigenic. They also reported the presence of sea, seb and sed genes in chicken meat isolates, with percentages of 33.3%; 8.3% and 16.7% respectively.

Kitai et al. (2005) revealed that 21.7% of S. aureus strains, isolated from chicken carcasses were enterotoxigenic. Moreover, Nemati (2013) observed that 71% of S. aureus strains isolated from chicken nose and cloaca were enterotoxigenic. As for the turkey isolates, Koluman et al. (2011) showed that the production of enterotoxins in turkey meat was 36%.

However, the present study demonstrated that the prevalence of enterotoxigenic isolates from chicken carcasses (15%) and turkey (4.54%) is relatively lower.

Although, there was a variability in the frequency of different enterotoxins types production, in chicken we observed the dominance of seb gene with a rate of 13.33% compared to the sed gene (1.67%). Similar results were stated by Kitai et al. (2005). Nevertheless, only type D enterotoxins (4.54%) were detected in turkey isolates. This variability between the two species had not yet been defined.

Madahi et al. (2014) showed that S. aureus strains isolated from chicken nuggets present staphylococcus enterotoxins genes, with values of 33.33%; 4.16%; 12.50%; 8.33%; 12.50%; 12.50% for sea, seb, sec, sed, sea + sec and sea + sed respectively. No see gene was detected. Another study by Nemati (2013) also reported that 33.3% of S. aureus strains isolated from the nose and cloaca of healthy chicken were positive for sea and 5% had a mixed sea + egc enterotoxins production, whereas the genes encoding seb, sec, sed and see were absent.

A study of the incidence of enterotoxigenic S. aureus strains in turkey meat, was carried out by Bystron et
CONCLUSION

We have experimentally determined the prevalence and enterotoxins genes of *S. aureus* in fresh poultry carcasses that are collected from 16 different slaughterhouses located in the region of Algiers, Algeria. The investigation has revealed that the microbial risk by *S. aureus* in poultry carcasses is not negligible. It has been found that the prevalence of *S. aureus* in 385 carcasses was 41.56% with an individual prevalence of 40.63% and 45.71% for chicken and turkey respectively. The strains of *S. aureus* of avian origin can be enterotoxigenic with the predominance of the genes encoding for enterotoxin *seb* in chicken and *sed* in turkey.

When meat is preserved under favorable conditions for germs multiplication and toxinogenese, enterotoxins can be produced in sufficient amount to trigger foodborne illnesses. These potential dangers must be considered in terms of real risk to public health. To ensure the safety and hygienic quality of meat, the application of good hygiene practice (GHP) and the implementation of HACCP in poultry slaughterhouses has become an absolute necessity to protect consumer’s health.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.
REFERENCES


