Evaluation of growth and transfer of *Staphylococcus aureus* from poultry meat to surfaces of stainless steel and polyethylene and their disinfection

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**A B S T R A C T**

The growth of *Staphylococcus aureus* inoculated onto poultry meat was investigated under different incubation periods and temperature patterns. Transfer of this microorganism to surface materials and their disinfection was also evaluated. The evaluation of transfer was carried out by placing the contaminated meat cubes on stainless steel and polyethylene surfaces for 10 s and 10 min each, and the surfaces were disinfected with 0.5% chlorhexidine digluconate (CHXdG) for 1 and 10 min each. After 24 h, there was a significant increase of the bacteria count at 20 °C, but not at temperatures between 7 and 15 °C. Significant counts of *S. aureus* were transferred after a few seconds of contact of the cubes with both materials, and significant differences of transferred cell counts were not detected among the surface materials or durations of contact. The CHXdG solution was able to inactivate all the transferred cells after 10 min of exposure; however, the same result was not observed with 1-min exposure. The time of contact and the type of surface material did not influence the quantity of the transferred cells. The 0.5% CHXdG solution was effective for the disinfection of the contaminated surfaces without previous cleaning.

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**1. Introduction**

*Staphylococcus aureus* is one of the most important agents of food poisoning around the world (Aarestrup, Wegener, & Rosdahl, 1995; Balaban & Rasooly, 2000), and in many countries, it is the main bacterial agent causing foodborne diseases. The primary habitat of this microorganism is the mucosa of the nasopharynx and the skin of humans and animals (Casey, Lambert, & Elliot, 2007; Genigeorgis, 1989); however, it has been found in water, dust, and air also (Hamann, 1986). The presence of *S. aureus* in food is often attributed to inadequate hygiene during handling by the individuals involved in the production of food (Hatakka, Björkroth, Asplud, Maki-Petays, & Korkeala, 2000), and the products of chicken have been involved with many outbreaks caused by this microorganism (Khakhria, Woodward, Johnson, & Poppe, 1997).

The production of chicken meat has significantly increased in Brazil lately, classifying this country as the biggest exporter in the world and extending the importance of the poultry industry to the country’s economy. In 1990, 2267 tons of poultry meat was produced, whereas the production in 2005 was 9297 tons. In 2007, Brazil exported 3203 tons of poultry meat, followed by the USA and European Union, which exported 2508 and 685 tons, respectively (Associação Brasileira dos Produtores e Exportadores de Frango, 2007). The low cost, low fat content, and short time needed for preparation have contributed to poultry meat and other poultry products being widely consumed (Álvarez-Astorga, Capita, Alonso-Calleja, Moreno, & García-Fernández, 2002). In spite of the increasing automation and certification of the companies, the control of pathogenic microorganisms in poultry meat is still a major concern for suppliers, consumers, and public health organizations. Although undesirable, the bacterial contamination of poultry products occurs due to its improper control that depends on various factors, such as initial level of contamination of carcasses, the duration and temperature of storage, and hygienic practices during handling (El-Leithy & Rashad, 1989).

In Brazil, according to Regulation N° 210/1998 (Brasil, 1998), meat poultry handled in the cutting section must be stored at temperatures below 7 °C. According to the same Regulation, if this temperature is exceeded, the meat must be discarded, thereby avoiding possible public health problems, although this act generates large economic losses for the companies. Similarly, the Regulation N° 46/1998 of the Ministry of Agriculture (Brasil, 2003) declares that one of the Critical Control Points in the production of poultry meat for exportation must be the temperature of the meat at the exit of the cooling tunnel. This temperature must be controlled to avoid proliferation of the microorganisms possibly present in poultry meat, specifically *S. aureus* and its enterotoxin production.

In addition to temperature control, the prevention of cross-contamination in poultry industries has been widely emphasized. The
transfer of microorganisms from food to equipments and utensils is receiving increased attention, especially in studies about microbial adhesion to materials used in food industries (Marques et al., 2007).

The materials most commonly used in poultry industries are stainless steel and polyethylene. Stainless steel is more easily disinfected compared to polyethylene (Rossoni & Gaylarde, 2000); however, studies conducted with different types of stainless steel that have undergone a corrosion process indicate an increase in bacterial adhesion (Kusumaningrum, Riboldi, Hazeleger, & Beumer, 2003; Kusumaningrum, Van Putten, Rombouts, & Beumer, 2002). Both materials have irregular surfaces when observed microscopically, which facilitate the deposition of organic material, facilitating the proliferation of microorganisms and hindering the action of the disinfectants (Sinde & Carballo, 2000).

The aim of this work was to evaluate the growth of S. aureus in poultry meat exposed to different durations and temperatures possibly found in slaughterhouses. Furthermore, the transfer of S. aureus from the contaminated poultry meat to stainless steel and polyethylene was also evaluated, in addition to the disinfection of these surfaces by using chlorhexidine digluconate (CHXdG), which is a disinfectant commonly used in poultry industries.

2. Materials and methods

2.1. Samples of poultry meat

Samples of refrigerated chest poultry meat were supplied by a poultry meat producer, the industrial plant of which was localized in Rio Grande do Sul, Southern Brazil. The industrial plant undergoes federal inspection and has permission to export meat products to many countries. The samples were sent to the Food Microbiology Laboratory of the Food Science and Technology Institute of Federal University of Rio Grande do Sul (ICTA/UFRGS), where they were cut in cubes of approximately 10 g under aseptic conditions. The chest poultry meat samples were previously analyzed to prove the absence of initial contamination with coagulase-positive Staphylococci.

2.2. Bacterial strain

The strain used in the experiments was S. aureus ATCC 25923. Before each experiment, the microorganism was cultivated in Brain–Heart Infusion agar (BHI) (Biobras, Belo Horizonte, Brazil) at 37 °C for 24 h.

2.3. Evaluation of the growth of S. aureus in poultry meat

Approximately 4 log colony-forming units (CFU)/mL of S. aureus ATCC 25923 were inoculated onto the surface of the chest poultry meat cubes. The cubes were maintained aseptically for 2 h at the following temperatures to stabilize the temperature before every experiment: 7 °C, 10 °C, 12 °C, 15 °C, and 20 °C. The temperatures were chosen on the basis of extreme failure conditions recorded in poultry meat industries before implementation of the Hazard Analysis and Critical Control Points approach. The inoculated cubes were separately set aside at these temperatures for 24 h, and counts of S. aureus ATCC 25923 were estimated at 0, 1, 2, 3, 4, 6, 8, and 24 h. Every count was carried out in duplicate on Baird-Parker Agar (Merck, Darmstadt, Germany) plates, according to the procedure of Milles and Misra (1938).

2.4. Transfer of S. aureus to stainless steel and polyethylene

2.4.1. Coupon preparation

Stainless steel AISI 316 (1 mm thick) (Metalbras, Porto Alegre, Brazil) and polyethylene (7 mm thick) (Sanremo, Esteio, Brazil) coupons, of size 2 × 2 cm, were prepared. Before testing for bacterial transfer, the coupons were washed in potable water and neutral detergent. Subsequently, the coupons were rinsed with distilled water and disinfected with 70% (v/v) ethyl alcohol. The coupons were dried at 60 °C for 2 h and autoclaved at 121 °C, for 15 min (Rossoni & Gaylarde, 2000).

2.4.2. Evaluation of S. aureus transfer

To evaluate the transfer of S. aureus, the poultry meat cubes were artificially contaminated with approximately 7 log10 colony-forming units (log CFU)/mL of S. aureus ATCC 25923. The contaminated coupons were placed in contact with the stainless steel and polyethylene coupons for 10 s and 10 min each at room temperature. Subsequently, the coupons were immersed in 25 mL of 0.1% sterile peptone water (Merck, Darmstadt, Germany) and immediately submitted to sonication for 10 min using a bath sonicator Unique USC 700 (Unique Group, Indaiatuba, Brazil), with an ultrasonic frequency of 40 kHz. Each coupon was sonicated for two stages of 10 min for achieving the release of the cells adhered on the surfaces of the couponssurfaces.

Decimal dilutions of the peptone water containing each sonicated coupon were conducted, and 20 μL of each dilution were placed on Baird-Parker and BHI agar for assessment of microbial counts, according to the procedure described by Milles and Misra (1938).

2.5. Evaluation of disinfection of artificially contaminated stainless steel and polyethylene coupons using 0.5% CHXdG

The stainless steel and polyethylene coupons were kept in contact for 10 min with the poultry meat cubes that were previously contaminated with 7 log CFU/mL of S. aureus ATCC 25923. Two experiments were carried out in the next step. In the first experiment, the disinfection of the coupons was done with 0.5% CHXdG immediately after contact with the contaminated poultry meat cubes. In the second experiment, the contaminated coupons were incubated at 35 °C for 3 h before the disinfection, to facilitate the formation of a S. aureus biofilm. In both experiments, the coupons were transferred to a container containing 15 mL of 0.5% CHXdG and placed in the solution for 1 min and 10 min each. Subsequently, the coupons were immersed in a neutralizing solution (Tween 80, 0.5%) for 30 s, transferred to 25 mL of 0.1% peptone water, and immediately sonicated for 10 min. The samples obtained were decimally diluted, and 20 μL were plated on Baird-Parker and BHI Agar for determination of viable cell counts, according to the procedure of Milles and Misra (1938). Both media (BHI and Baird-Parker) were used because non-selective agars allow the growth of both non-injured and sublethally injured cells, but cannot differentiate target pathogens from a mixed population (Wu, 2008).

The control coupons were treated with distilled water instead of disinfectant.

2.6. Statistical analysis

The counts of S. aureus were converted to log CFU, with the growth of S. aureus in the chest poultry meat expressed in log CFU/g of poultry meat. The quantity of S. aureus transferred from chest poultry meat to the stainless steel and polyethylene surfaces and the number of cells removed by the disinfectant were expressed in log CFU/cm² of surface. In all cases, samples were tested...
in triplicate, and the experiment was repeated at least three times. The counts obtained were compared using Tukey’s T test.

Data analyses were carried out using the software Statistica, v 7.1, and a level of $P < 0.05$ was considered statistically significant.

3. Results and discussion

The first part of the present study was done to evaluate whether possible variations in the temperature of the poultry meat might cause significant multiplication of *S. aureus*. Incubation for a few hours was done at the temperatures of 7°C, 10°C, and 12°C to simulate the actual environmental variations in poultry industries. The parameters considered as extreme abuse were also tested, at temperatures of 15°C and 20°C for 8 h and 24 h, which are uncommon in poultry meat industries, especially those firms that export the poultry products.

Before the experiments, all the meat samples were negative to coagulase-positive *Staphylococci*. The counts of *S. aureus* ATCC 25923 artificially inoculated into cubes of poultry meat were approximately 3 log CFU/g (Table 1). The results indicated that there was no significant increase ($P < 0.05$) in the microbial counts for at least 6 h under any of the evaluated temperatures (7°C, 10°C, 12°C, 15°C, and 20°C). Under these conditions, quantities of *S. aureus* considered necessary to cause outbreaks, i.e., approximately 5–6 log CFU/mL or g (Bremer, Fletcher, & Osborne, 2004; Su & Wong, 1997) were not reached. Counts potentially able to cause foodborne outbreaks in healthy persons (8.06 ± 0.088 log CFU/g) were observed only after 24 h of cultivation at 20°C. For individuals to show symptoms of food poisoning by *S. aureus*, the bacterial cells should produce enterotoxins (Troller, 1971). It was reported that *S. aureus* cells can grow at 7°C, but enterotoxin production occurs only above 10°C (Bremer et al., 2004). Furthermore, the microbe must have a density of at least 5 log CFU/g or mL of food to produce sufficient quantities of enterotoxins to cause food poisoning. Considering these data and the results of the present study, the exposure of poultry meat to a temperature of 7°C for short periods of time (operational limit), with tolerance up to 10°C (critical limit), does not appear to present the risk of causing *S. aureus* intoxication. These parameters can be easily reached and controlled in an industrial environment; nevertheless, it is important to emphasize that this control must be implemented in parallel with other factors, such as the monitoring of the quality of raw material and appropriate hygienic handling by food handlers, to restrict the amount of *S. aureus*, possibly existing in the sample, at low levels.

Poultry meat cubes, artificially contaminated with about 7 log CFU/g of *S. aureus*, were able to transfer approximately 4 log CFU/cm² when maintained in contact with stainless steel and polyethylene coupons (Table 2). In spite of polyethylene displaying a more irregular surface than stainless steel (Oliveira et al., 2006), no significant difference was observed in the quantities of cells adhering to the coupons. Furthermore, there was no difference ($P < 0.05$) in the transfer levels between the two contact periods (10 s and 10 min), indicating that the exposure periods tested did not influence the quantity of transferred cells.

The results presented in this study showed that stainless steel and polyethylene were able to be colonized by expressive counts of *S. aureus* transferred from poultry meat, even with short periods of contact. According to Beresford, Andrews, and Shama (2001), during bacterial adherence, both weak and strong interactions are found, reversible or irreversible, independent of nutrient availability. When the contact time is short (less than 2 h), the microorganisms are bound in a reversible manner to both the surface and between themselves through weak interactions. However, according to the authors of the present study, as time passes, these interactions become stronger and irreversible. Oulahal, Brice, Martial, and Degraeve (2008) tested the survival of *S. aureus* CNRZ3 on stainless steel AISI 304 and polypropylene placed in contact with three dairy products (pasteurized skim milk, raw milk, and cheese curd) at 12°C and 25°C. Both the tested materials were used for 5 yr in a dairy industry and were contaminated with the microorganism (7.6 ± 0.2 log CFU/mL) in a BHI broth for 5 h, before being placed in contact with the dairy products. The results indicated that *S. aureus* adhered on the surfaces and remained viable for 8 days, the microbial biofilm formation being the main explanation for the bacterial survival in both materials.

Surface contamination is an important factor to be considered in the transmission of pathogens to foods during processing. Although the food particles are removed from the surfaces after a thorough cleaning, the bacteria adhering to these surfaces might not be removed (Kusumamangrum et al., 2003).

Table 3 shows the results of the disinfection, using 0.5% CHXdG, of surfaces that were contaminated with *S. aureus* ATCC 25923, recovered in BHI agar. There were no differences between the counts obtained from selective agar (Baird-Parker) and non-selective agar (BHI) (data not shown), probably due to neutralization with Tween 80. Both stainless steel and polyethylene coupons were completely disinfected after immersion for 10 min (approximate reduction by 4 log CFU/cm²). However, when the coupons were immersed in disinfectant for only 1 min, approximately 2 log CFU/cm² of the microorganisms survived in both materials. These results are important mainly because the contact time of the disinfectant can be shorter than 1 min in many sanitization routines of the food industries, due to the frequent necessity of

<table>
<thead>
<tr>
<th>Contact time</th>
<th>Stainless steel</th>
<th>Polyethylene</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 s</td>
<td>4.25 ± 0.07</td>
<td>4.46 ± 0.22</td>
</tr>
<tr>
<td>10 min</td>
<td>4.24 ± 0.17</td>
<td>4.36 ± 0.29</td>
</tr>
</tbody>
</table>

*SD: standard deviation.

### Table 1

Counts of *Staphylococcus aureus* (log CFU/g ± SD) in poultry meat after incubation under different temperatures.

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>Temperatures (°C)</th>
<th>7</th>
<th>10</th>
<th>12</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.65 ± 0.048</td>
<td>3.59 ± 0.043</td>
<td>3.18 ± 0.160</td>
<td>3.65 ± 0.224</td>
<td>3.25 ± 0.135</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.63 ± 0.043</td>
<td>3.46 ± 0.097</td>
<td>3.15 ± 0.046</td>
<td>3.56 ± 0.146</td>
<td>3.19 ± 0.103</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.45 ± 0.187</td>
<td>3.32 ± 0.115</td>
<td>3.31 ± 0.117</td>
<td>3.37 ± 0.266</td>
<td>3.19 ± 0.199</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.26 ± 0.152</td>
<td>3.38 ± 0.090</td>
<td>3.13 ± 0.153</td>
<td>3.28 ± 0.210</td>
<td>3.12 ± 0.046</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3.26 ± 0.230</td>
<td>3.29 ± 0.102</td>
<td>3.30 ± 0.055</td>
<td>3.43 ± 0.383</td>
<td>3.17 ± 0.073</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3.31 ± 0.063</td>
<td>3.20 ± 0.039</td>
<td>3.17 ± 0.026</td>
<td>3.57 ± 0.131</td>
<td>3.36 ± 0.184</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.28 ± 0.162</td>
<td>3.07 ± 0.247</td>
<td>2.96 ± 0.241</td>
<td>3.50 ± 0.164</td>
<td>4.19 ± 0.103</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>3.15 ± 0.174</td>
<td>3.05 ± 0.156</td>
<td>3.02 ± 0.151</td>
<td>4.55 ± 0.120</td>
<td>8.06 ± 0.088</td>
<td></td>
</tr>
</tbody>
</table>

*SD: standard deviation.
time-reduction procedures. However, in the present study, the coupon washing was not done before disinfection, which certainly is not recommended in the food industries.

The above results also showed that no differences were found between the disinfection levels of the coupons that remained with S. aureus for 3 h compared with those disinfected just after contamination. These results can be explained by the fact that there was no expressive increase in the S. aureus counts after 3 h of incubation at 35 °C, and the population that adhered did not indicate more resistance to disinfection after this period. According to the results presented in this work, contaminated poultry meat can transfer expressive amounts of S. aureus to stainless steel and polyethylene surfaces; however, 0.5% CHX·DG could completely inactivate this microbial population after 10 min of contact.

References


Table 3
Survival of Staphylococcus aureus (log CFU/cm² ± SD) after treatment with 0.5% chlorhexidine digluconate.

<table>
<thead>
<tr>
<th>Contact time with disinfectant</th>
<th>Coupons immediately disinfected</th>
<th>Coupons incubated at 35 °C for 3 h before disinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stainless steel</td>
<td>Polyethylene</td>
</tr>
<tr>
<td>Controlb</td>
<td>4.68 ± 0.08</td>
<td>4.62 ± 0.07</td>
</tr>
<tr>
<td>1 min</td>
<td>2.42 ± 0.21</td>
<td>2.83 ± 0.05</td>
</tr>
<tr>
<td>10 min</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a SD: standard deviation.

b Surfaces treated with distilled water instead of disinfectant.