QUANTIFICATION OF ENTEROBACTERIA AND CLOSTRIDIUM SPP. IN POULTRY SLAUGHTERHOUSES SANITARY CONVEYORS BELTS

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RESUMO

O aumento da produção e do consumo per capita de carne de frango ocorreu devido a modernização neste setor. Tal aumento gerou preocupação com a transmissão de patógenos para o ser humano, porém com uma higienização adequada essa transmissão pode ser controlada. Assim, o objetivo deste estudo foi avaliar a higiene pré-operacional e operacional das esteiras condutoras de cortes de frangos através da quantificação de Clostridium spp. e Enterobactérias. As análises estatísticas da contagem de Clostridium spp. mostraram uma diferença entre os frigoríficos visitados e entre os tipos de limpeza realizados nas esteiras (p<0,0001). Já as análises estatísticas para a contagem de Enterobactérias mostraram diferenças significativas somente entre os frigoríficos visitados (p<0,0001), não havendo diferença entre os tipos de limpeza das esteiras (p=0,4057). Os resultados demonstraram que houve uma variação na contagem bacteriana entre os frigoríficos e que a higienização das esteiras foram deficientes pois apresentaram contagens superiores aos valores recomendado pelas organizações internacionais.


SUMMARY

The increase in production and consumption of chicken meat has occurred due to modernization in this area. Such increase caused the concern about the transmission of pathogens to humans; however, with proper hygiene this transmission can be controlled. Thus, this study aimed to evaluate the pre-operational and operational hygiene in sanitary conveyors belts of chicken cuts in slaughterhouses through Clostridium spp. and Enterobacteria quantification. Statistical data analysis for Clostridium spp. colony count showed a difference between the studied slaughterhouses and the types of cleaning performed on sanitary conveyors belts (p<0,0001). Already statistical analysis for Enterobacteriaceae colony count showed significant differences only between the visited slaughterhouses (p<0,0001), with no difference between the types of conveyors belts cleaning (p=0,4057). The results showed that there was a variation in bacterial count among the slaughterhouses and the hygiene process in sanitary conveyors belts were deficient because they presented counts higher than the values recommended by the international organizations.


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INTRODUCTION

The modernization and industrialization of Brazilian poultry production chain started in the 1950s, through a series of changes in this chain, which resulted in the production of chicken on a large scale (TAVARES; RIBEIRO, 2007; VASCONCELOS et al., 2015). According to ABPA (2017), Brazil occupies second position in a world ranking, behind only the USA, with 12.90 million tons produced, and the top exporter, with 4.38 million tons.

The most important concern around the poultry production chain is to obtain products and byproducts such as meat and chicken cuts with low contamination rate, in order to avoid economic losses and risks to the public health (SOUZA et al., 2014). To prevent contamination by pathogenic microorganisms in animal products, it is necessary to sanitize the environment and equipment and it must be carried out in a judicious manner, according to norms established by MAPA (SOUZA et al., 2014; FLORES; MELO, 2015).

According to MAPA, the conveyors belts hygiene is performed in two stages, preoperational and operational cleaning. The preoperational cleaning is made after the end of each work shift, using detergents, organic acids, and potable water under pressure at 45°C. The rinse with water is necessary to remove the chemical substances that might come into contact with meat. For the operational cleaning, it is used only the potable water under pressure at 45°C on the sanitary conveyors belts for carcasses waste removal. The potable water is the one with microbiological safety and with 0.5mg.L⁻¹ to 2.0mg.L⁻¹ of chlorine (BRASIL, 1998).

Based on these matters, the present study aimed to evaluate the hygiene in sanitary conveyors belts in chicken-cutting area of slaughterhouses located in Southeastern Brazil, before and after the preoperational and operational hygiene. For this, it was carried out Enterobacteriaceae and Clostridium spp. quantification in sanitary conveyors belts of poultry slaughterhouses.

MATERIALS AND METHODS

Samples of sanitary conveyors in slaughterhouses

For this experiment, five samplings were carried out in two poultry slaughterhouses located in south of Minas Gerais State, in Passos region (SH1) and countryside of São Paulo State, in Campinas region (SH2). These samplings were collected at the surface of sanitary conveyors belts, which were made with polyurethane plastic, before and after the preoperational and operational hygiene with water spray. Both plants are focused on exporting chicken meat and the cutting areas kept the temperature controlled at around 12°C.

Three samplings were made in slaughterhouse SH1, located in Passos Region, in the first one (S1) were collected 48 samples, the second (S2) and third (S3) were collected 60 samples each. In slaughterhouse SH2, located in Campinas Region, were carried out two samplings, the first one (S1) were collected 52 samples and the second (S2) were collected 55 samples, for a total of 275 samples.

The samples were collected using sterile swabs, in a predetermined area of 20cm² with a metal template, previously sterilized. The samples were taken successively before and after the preoperational and operational hygiene. The swab was placed in a test tube containing 10 mL of 0.1% peptone water. All the samples were refrigerated, approximately, at 4°C during the transportation to the laboratory for subsequent analysis.

Quantification of Enterobacteria and Clostridium spp. in sanitary conveyors belts of chicken cuts

The tubes containing peptone water 0.1% and the swab were homogenized with Vortex. Serial dilutions were performed until 10⁻² and 10⁻³ for Clostridium spp. and Enterobacterial colony counts, respectively. Each diluted sample for Clostridium spp. was submitted to heat-shocked at 80°C for 10 minutes to allow the spores to germinate and to remove contaminants and then cooled in frozen water (CASAGRANDE et al., 2013).

An aliquot of 1 mL of each dilution was transferred to a Petri dish and were added, by the pour plate method, Reinforced Clostridial Agar (RCA, Himedia, France) for Clostridium spp. and MacConkey agar (Himedia, France) for Enterobacteriaceae. The plates for Clostridium spp. were incubated in anaerobic jars using the GasPak® System (Probac, Brazil) at 37°C for 48h, and Enterobacteriaceae plates were incubated in aerobic conditions at 37°C for 24h (APHA, 2001).

After the bacterial growth, Gram method was performed in typical colonies of Clostridium spp. and Enterobacteriaceae, and the colony forming units per mL (CFU.mL⁻¹) were counted. Typical colonies of Clostridium spp. in RCA agar are opaque with light yellow color, and they are Gram-positive, rod-shaped and sporulated. The colonies of Enterobacteriaceae in MacConkey agar are pink with a bile precipitate, they are Gram-negative and rod-shaped. The quantification data were transformed into colony forming units per cm² (CFU.cm⁻²) as performed on international standards.

Statistical analysis

The data from Clostridium spp. and Enterobacteria quantification were statistically analyzed using analysis of variance. The means were grouped by completely randomized design (CRD) and a 6x4 factorial design was performed, through the F-test, at 5% significance level. Before proceeding with statistical analysis, the results were converted into log CFU.mL⁻¹. Analyses of variance were carried using the CAR package (JOHN; SANFORD, 2011) and means were estimated by the method of least squares using LSMEANS package (LENTH, 2013).
RESULTS AND DISCUSSION

Quantification of Enterobacteria and Clostridium spp. in sanitary conveyors belts of chicken cuts

The results of Clostridium spp. quantification showed a variation among the studied slaughterhouses. Only at first sampling, there was none bacterial multiplication in RCA. The highest score, 6.79x10^3 CFU.cm^{-2}, was found before preoperational cleaning in the third sampling performed in the slaughterhouse SH1.

Enterobacteria quantification also showed a variation among the visited slaughterhouses and the highest score, 9.76x10^9 CFU.cm^{-2}, occurred before preoperational cleaning in the third sampling performed in the slaughterhouse SH1, same as the Clostridium spp. results. There was no bacterial count in preoperational cleaning for the second sampling at SH2.

The mean of Clostridium spp. colony count at SH1.S2 and at SH2.S1, in preoperational cleaning, decreased after the hygiene process, whereas in other samplings, it was noted an increase this mean. For the operational cleaning, there was a decrease in bacterial count after the hygiene process on establishment SH2.S1.

For Enterobacteria, the preoperational and operational cleaning did not result in a drastic population decrease, indicating that these cleaning processes were insufficient to eliminate this bacterial group.

In this way, it is possible to say that there is a deficiency of the cleaning processes among slaughterhouses samples for both bacterial groups, which may result in a contamination of chicken cuts. Thus, it is necessary the improvement of the hygienic process in order to prevent contamination. According to Russell et al. (1997) cited by Potter et al. (2012), the insufficient cleaning process can lead to cross-contamination of the carcasses, resulting in damage to human health.

In Brazilian legislation for food industries, there are no standards for bacteria quantification in sampling carried out on equipment and utensils. According to Massagué (2006), ideal standards considered by the Foods and Drugs Administration (FDA) and the American Public Health Association (APHA) for equipment, are 2.0 CFU.cm^{-2}, as for the slaughterhouses utensils are less than 100 CFU/utensil. In this study, higher counts were found than the ones recommended by these organizations, for both Clostridium spp. and Enterobacteriaceae, thus not meeting international standards.

According to European agencies, the Enterobacteria colony count may not exceed 1.0 CFU.cm^{-2} in slaughterhouses after preoperational conveyors cleaning, demonstrating that Brazilian slaughterhouses need more care about hygiene when performing these processes, since as it was shown in this study, the quantifications means were higher than European Union requirement (EC, 2010).

Statistical data analysis for Clostridium spp. colony count showed a statistical difference between the studied slaughterhouses and the types of cleaning performed on sanitary conveyors belts (p<0.0001). The interaction between slaughterhouses versus conveyors belts cleaning differed statistically at a significance level of 5%, demonstrating that there was a correlation between these two factors. The statistical ANOVA showed a mean of 1.132 log CFU.m^{-1}, a SD of 0.675 and a CV of 59.578%.

Already statistical analysis for Enterobacteriaceae colony count showed statistically significant differences only between the visited slaughterhouses (p<0.0001), with no difference between the types of conveyors belts cleaning (p = 0.4057). The interaction between slaughterhouses and conveyors belts cleaning was also statistically different at the level of significance of 5%. Analysis of variance showed a mean of 1.640 log CFU.m^{-1}, a SD of 0.939 and a CV of 57.229%.

The results of statistical means for Clostridium spp. colony count were 0.71 log CFU.m^{-1} for the first sampling in SH1, 0.77 log CFU.m^{-1} for the second sampling and 2.22 log CFU.m^{-1} for the third sampling at the same establishment. In the SH2, those averages were 0.95 log CFU.m^{-1} for the first sampling and 0.88 log CFU.m^{-1} for the second. Only third sampling in SH1 was statistically different from the others.

The analysis of statistical means, according to the types of cleaning performed on sanitary conveyors belts, showed a significant difference between the preoperational and operational cleaning, but there was no difference about the period that the samples was collected if it was performed before or after each hygiene process. The quantification mean before the preoperational cleaning was 0.83 log CFU.m^{-1} and after such this procedure, increased to 0.93 log CFU.m^{-1}. On the other hand, higher values were observed before and after cleaning process, with means for Clostridium spp. were 1.40 log CFU.m^{-1} and 1.26 log CFU.m^{-1}, respectively (Table 1).

The statistical average for Enterobacteriaceae quantification, in the SH1, were 2.24 log CFU.m^{-1} for the first sampling, 1.51 log CFU.m^{-1} for the second and 0.93 log CFU.m^{-1} for the third. In SH2, the means were 2.92 log CFU.m^{-1} for the first sampling and 0.83 log CFU.m^{-1} for the second. Only the average colony count for the third sampling in SH1 and the second in SH2 were statistically similar, differing from the others.

In both conveyors belts cleaning processes for Enterobacteriaceae, the averages do not differ from each other, which were 1.72 log CFU.m^{-1} in sampling made before the preoperational cleaning and 1.66 log CFU.m^{-1} after this procedure. The mean of samples taken before and after operational cleaning were 1.55 log CFU.m^{-1} and 1.82 log CFU.m^{-1}, respectively (Table 1).

The interaction between the slaughterhouses and type of conveyors belts cleaning performed were analyzed statistically for both bacteria, Clostridium spp. and Enterobacteria, in order to verify that these
The analysis of *Clostridium* spp. showed that there were a significant difference between the preoperational and operational cleaning, only in slaughterhouse SH1.S3, but there was no difference for the time that the samples was collected. The analysis of this bacterium in operational cleaning showed a difference between the period that the samples were taken, before and after cleaning, and the highest averages were found in the same slaughterhouse (SH1.S3) (Table 2).

For *Enterobacteriaceae* statistical analysis, there was a higher variation between the means. Among the slaughterhouses, only in SH1.S1, was observed differences between the cleaning processes, but there were no significant difference between the samples taken before the preoperational cleaning from the others, in the same establishment. The lowest average in *Enterobacteriaceae* counts were observed in the samples collected before the operational cleaning and the highest was found after this procedure (Table 2).

The cleaning procedure analysis showed a significant difference between the type of processing and the period of which sampling was collected. In SH1.S1 and SH2.S1, it was observed similar means for hygiene performed before the preoperational and after

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**Table 1** - The comparison between the statistical means of bacteria quantification in slaughterhouses chicken-cutting area and comparison between different conveyors cleaning hygiene in relation of all sampling.

<table>
<thead>
<tr>
<th>Slaughterhouses</th>
<th><em>Clostridium</em> spp.</th>
<th><em>Enterobacteria</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Means (log CFU.mL⁻¹)</td>
<td>Means (log CFU.mL⁻¹)</td>
</tr>
<tr>
<td>SH1.S1</td>
<td>0.71ᵃ</td>
<td>2.24ᵇ</td>
</tr>
<tr>
<td>SH1.S2</td>
<td>0.77ᵇ</td>
<td>1.51ᵇ</td>
</tr>
<tr>
<td>SH1.S3</td>
<td>2.22ᵇ</td>
<td>0.93ᵃ</td>
</tr>
<tr>
<td>SH2.S1</td>
<td>0.95ᵇ</td>
<td>2.92ᵈ</td>
</tr>
<tr>
<td>SH2.S2</td>
<td>0.88ᵇ</td>
<td>0.83ᵃ</td>
</tr>
</tbody>
</table>

**F test** 51,177 (p<0.0001) 48,005 (p<0.0001)

<table>
<thead>
<tr>
<th>Conveyors Cleaning¹</th>
<th>Means (log CFU.mL⁻¹)</th>
<th>Means (log CFU.mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPO</td>
<td>0.83ᵃ</td>
<td>1.72ᵃ</td>
</tr>
<tr>
<td>APO</td>
<td>0.93ᵇ</td>
<td>1.66ᵇ</td>
</tr>
<tr>
<td>BO</td>
<td>1.40ᵇ</td>
<td>1.55ᵇ</td>
</tr>
<tr>
<td>AO</td>
<td>1.26ᵇ</td>
<td>1.82ᵇ</td>
</tr>
</tbody>
</table>

**F test** 10,903 (p<0.0001) 0.9737 (p=0.4057)³

¹ SH – Slaughterhouse, S – Sampling, BPO – Before Preoperational Cleaning, APO – After Preoperational Cleaning, AO – After Operational Cleaning. ³ Means within a column with unlike superscripts differ significantly (F-test with α = 5%).

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**Table 2** - Comparisons means of *Clostridium* spp. and Enterobacteria that showed a significant between slaughterhouses and conveyors cleaning type.

<table>
<thead>
<tr>
<th>Slaughterhouses (SH)</th>
<th><em>Clostridium</em> spp.</th>
<th>Enterobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH1.S1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.71ᵃ</td>
<td>2.24ᵇ</td>
</tr>
<tr>
<td>SH1.S2</td>
<td>0.94ᵇ</td>
<td>1.51ᵇ</td>
</tr>
<tr>
<td>SH1.S3</td>
<td>1.09ᵇ</td>
<td>0.93ᵃ</td>
</tr>
<tr>
<td>SH2.S1</td>
<td>0.71ᵇ</td>
<td>2.92ᵈ</td>
</tr>
<tr>
<td>SH2.S2</td>
<td>0.71ᵃ</td>
<td>0.83ᵃ</td>
</tr>
</tbody>
</table>

**F test** 61.42 (p<0.0001) 2.31 (p=0.08) 0.71 (p=0.55)

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</thead>
<tbody>
<tr>
<td>BPO</td>
<td>0.71ᵃ</td>
<td>0.94ᵇ</td>
<td>1.09ᵇ</td>
<td>0.71ᵇ</td>
<td>0.71ᵇ</td>
</tr>
<tr>
<td>APO</td>
<td>0.71ᵃ</td>
<td>0.97ᵇ</td>
<td>1.01ᵃ</td>
<td>1.18ᵃ</td>
<td>1.07ᵃ</td>
</tr>
<tr>
<td>BO</td>
<td>0.71ᵃ</td>
<td>0.71ᵃ</td>
<td>3.55ᵇ</td>
<td>1.22ᵃ</td>
<td>0.83ᵃ</td>
</tr>
<tr>
<td>AO</td>
<td>0.71ᵃ</td>
<td>0.71ᵃ</td>
<td>3.25ᵇ</td>
<td>0.71ᵃ</td>
<td>0.93ᵃ</td>
</tr>
</tbody>
</table>

**F test** 0 (p=1.00) 0.45 (p=0.72) 0.71 (p=0.55)

<table>
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<tbody>
<tr>
<td>BPO</td>
<td>2.56ᵇ</td>
<td>1.54ᵇ</td>
<td>0.71ᵇ</td>
<td>3.08ᵇ</td>
<td>0.71ᵇ</td>
</tr>
<tr>
<td>APO</td>
<td>1.90ᵇ</td>
<td>1.48ᵇ</td>
<td>0.97ᵇ</td>
<td>3.23ᵇ</td>
<td>0.71ᵇ</td>
</tr>
<tr>
<td>BO</td>
<td>1.60ᵇ</td>
<td>1.92ᵇ</td>
<td>0.98ᵇ</td>
<td>2.35ᵇ</td>
<td>0.89ᵇ</td>
</tr>
<tr>
<td>AO</td>
<td>2.90ᵇ</td>
<td>1.10ᵃ</td>
<td>1.06ᵇ</td>
<td>3.00ᵇ</td>
<td>1.01ᵃ</td>
</tr>
</tbody>
</table>

**F test** 4.89 (p=0.003) 1.92 (p=0.13) 0.41 (p=0.75) 2.20 (p=0.09) 0.34 (p=0.79)

³ F-test = 5%; ¹ SH – Slaughterhouse, S – Sampling, BPO – Before Preoperational Cleaning, APO – After Preoperational Cleaning, BO – Before Operational Cleaning, AO – After Operational Cleaning. Åh, åb Means marked by the same letter (capital letters in the column and lowercase letters in the row) are not significantly different from each other (F-test with α = 5%).

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The factors were independent. These interactions were significant at 5%, p<0.0001 for *Clostridium* spp. and p=0.009 for *Enterobacteriaceae* counts, demonstrating that these factors are dependent upon each other in both cases, thus the statistical analysis were performed to examine better the data (Table 2).
operational cleaning, but there was different from the others. In regard to the samples collected after operational cleaning, the SH1.S1 had the lowest mean of Enterobacteriaceae colony count and SH2.S1 had the highest. For the sampling before operational cleaning, SH2.S1 had the highest average differing from the others slaughterhouses, the SH2.S2 had the lowest average, and the SH1.S1 was statistically similar to the others (Table 2).

The evaluation of Clostridium spp. interaction, for all sampling in SH1, showed a statistical difference between cleanings only in the third sample, and the mean was higher than others, more precisely in operational cleaning. In the case of SH2, all cleanings procedure had a statistical similarity.

In Enterobacteriaceae interaction, was observed in SH1 that the cleaning procedures, after the operational and before the preoperational cleaning were statistically similar, but was statistically different from the others. For the SH2 samples, there were a higher difference between the first and second samples, wherein the second sampling there was no difference among the hygiene types.

The study conducted by Soares et al. (2014), which aimed to evaluate the Enterobacteriaceae and Aerobic mesophilic bacterial counts in conveyors belts of chicken cuts in Brazil, that were submitted or not to the cleaning system with water under pressure at 45°C in different times, obtained statistically similar results between the population counts of these microorganisms independently of the evaluated period. At the present study, it was found statistical differences between the preoperational and operational cleaning for Clostridium spp. and Enterobacteriaceae colony count, being the results similar to the ones found by the researchers.

In developing countries, animal products can be the most important sources of pathogen transmission, such as E. coli O157: H7, as the cleaning process at the slaughterhouse are inadequate. Therefore, it is extremely important that proper hygiene should be performed from poultry farms, slaughterhouses up to commercialization of animal products for human consumption, in order to limit such transmission (FEGAN et al., 2004; ATEBA; MBEWE, 2014).

Thus, the lower the bacterial count on sanitary conveyors, for Clostridium spp. and Enterobacteriaceae, the lower is the chance of pathogens transmission to chicken carcasses, as it come into contact with the sanitary conveyors before packaging for commercialization.

**CONCLUSION**

The hygiene process were insufficient in most chicken-cutting conveyors that were sampled in this study, since Clostridium spp. and Enterobacteria quantification were higher than those recommended by international organizations. In this way, the slaughterhouses must review the cleaning process on their equipments, especially in chicken-cutting area, with effective improvement of programs.

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**REFERÊNCIAS**


