Quantificação de Enterobactérias e *Clostridium* spp. em esteiras condutoras de cortes de frango em frigoríficos.

Quantification of Enterobacteria and *Clostridium* spp. in poultry slaughterhouses sanitary conveyors.

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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 verificar a higienização das esteiras condutoras de cortes de frango em frigoríficos através da quantificação de *Clostridium* spp. e Enterobactérias. 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.

Palavras-chaves: avicultura, bactérias patogênicas, higienização em frigoríficos.

ABSTRACT

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 verify the hygiene in sanitary conveyors of chicken cuts in slaughterhouses through *Clostridium* spp. and Enterobacteria quantification. The results showed that there was a variation in bacterial count among the slaughterhouses and the hygiene process in sanitary conveyors were deficient because they presented counts higher than the values recommended by the international organizations.

Key words: pathogenic bacteria, poultry production, slaughterhouses hygiene.

INTRODUCTION

The modernization and industrialization of Brazilian poultry chain started in the 1950s, through a series of changes in poultry production 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 exported.

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).

Based on these matters, the present study aimed to evaluate the hygiene in sanitary conveyors 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. counting in sanitary conveyors 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 (SH3). These samplings were collected at the surface of sanitary conveyors, 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 20cm^2 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.

According to MAPA Normative n°210, 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 for carcasses waste removal. According to Agriculture Ministry, potable water is the one with microbiological safety and with 0,5mg.L^{-1} to 2,0mg.L^{-1} of chlorine (BRASIL, 1998).

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The tubes containing peptone water 0,1% and the swab were homogenized with the Vortex. Serial dilutions were performed until 10^{-2} and 10^{-3} for *Clostridium* spp. and Enterobacterial 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 ice 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) for *Clostridium* spp. and MacConkey agar (Himedia) for *Enterobacteriaceae*. The plates for *Clostridium* spp. were incubated in anaerobic jars using the GasPak® System 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 data counts were transformed into colony forming units per cm^2 ($CFU.cm^{-2}$) 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 out 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

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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, $9{,}76x10^3$ 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. quantification at the second sampling on SH1 and at the first sampling on SH2, in preoperational cleaning, decreased after the hygiene process, whereas in other samplings, it was noted an increase of the 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 hygiene 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 counting for sampling carried out on equipment and utensils. According to Massaguer (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 utensil 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 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. count showed a statistical difference between the studied slaughterhouses and the types of cleaning performed on sanitary conveyors ($p<0,0001$). The interaction between slaughterhouses versus conveyors 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 $CFU.mL^{-1}$, a SD of 0,675 and a CV of 59,578%.

Already statistical analysis for *Enterobacteriaceae* count showed statistically significant differences only between the visited slaughterhouses ($p<0,0001$), with no difference between the types of conveyors cleaning ($p = 0,4057$). The interaction between slaughterhouses and conveyors cleaning was also statistically different at the level of significance of 5%. Analysis of variance showed a mean of 1,640 log $CFU.mL^{-1}$, a SD of 0,939 and a CV of 57,229%.

The results of statistical means for *Clostridium* spp. count were 0,71 log $CFU.mL^{-1}$ for the first sampling in SH1, 0,77 log $CFU.mL^{-1}$ for the second sampling and 2,22 log $CFU.mL^{-1}$ for the third sampling at the same establishment. In the SH2, those averages were 0,95 log $CFU.mL^{-1}$ for the first sampling and 0,88 log $CFU.mL^{-1}$ 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, 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 mean count before the preoperational cleaning was 0,83 log CFU.mL^{-1} and after such this procedure, increased to 0,93 log CFU.mL^{-1}. On the other hand, higher values were observed before and after cleaning process, with means for *C. perfringens* were 1,40 log CFU.mL^{-1} and 1,26 log CFU.mL^{-1}, respectively (Table 1).

The statistical average for *Enterobacteriaceae* quantification, in the SH1, were 2,24 log CFU.mL^{-1} for the first sampling, 1,51 log CFU.mL^{-1} for the second and 0.93 log CFU.mL^{-1} for the third. In SH2, the means were 2,92 log CFU.mL^{-1} for the first sampling and 0,83 log CFU.mL^{-1} for the second. Only the average count for the third sampling in SH1 and the second in SH2 were statistically similar, differing from the others.

In both conveyors cleaning processes for *Enterobacteriaceae*, the averages do not differ from each other, which were 1,72 log CFU.mL^{-1} in sampling made before the preoperational cleaning and 1,66 log CFU.mL^{-1} after this procedure. The mean of samples taken before and after operational cleaning were 1,55 log CFU.mL^{-1} and 1,82 log CFU.mL^{-1}, respectively (Table 1).

The interaction between the slaughterhouses and type of conveyors cleaning performed were analyzed statistically for both bacteria, *Clostridium* spp. and Enterobacteria, in order to verify that these factors were independent. These interactions were significant at 5%, $p<0,0001$ for *Clostridium* spp. and $p=0,009$ for Enterobacteria, 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).

196 The analysis of *Clostridium* spp. means showed that there were a significant
197 difference between the preoperational and operational cleaning, only in slaughterhouse
198 SH1.S3, but there was no difference for the time that the samples was collected. The
199 analysis of this bacterium in operational cleaning showed a difference between the period
200 that the samples were taken, before and after cleaning, and the highest averages were
201 found in the same slaughterhouse (SH1.S3) (Table 2).

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

209 The cleaning procedure analysis showed a significant difference between the type
210 of processing and the period of which sampling was collected. In SH1.S1 and SH2.S1, it
211 was observed similar means for hygiene performed before the preoperational and after
212 operational cleaning, but there was different from the others. In regard to the samples
213 collected after operational cleaning, the SH1.S1 had the lowest mean of
214 *Enterobacteriaceae* count and SH2.S1 had the highest. For the sampling before
215 operational cleaning, SH2.S1 had the highest average differing from the others
216 slaughterhouses, the SH2.S2 had the lowest average, and the SH1.S1 was statistically
217 similar to the others (Table 2).

218 The evaluation of *Clostridium* spp. interaction, for all sampling in SH1, showed a
219 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* 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 counts 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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TABLES

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

Slaughterhouses	***Clostridium* spp.**	**Enterobacteria**
	Means (log CFU.mL^{-1})	**Means (log CFU.mL^{-1})**
SH1.S1	0,71^{a}	2,24^{c}
SH1.S2	0,77^{a}	1,51^{b}
SH1.S3	2,22^{b}	0,93^{a}
SH2.S1	0,95^{a}	2,92^{d}
SH2.S2	0,88^{a}	0,83^{a}
F test	51,177 (p<0,0001)	48,005 (p< 0,0001)
Conveyors Cleaning[1]	**Means (log CFU.mL^{-1})**	**Means (log CFU.mL^{-1})**
BPO	0,83^{a}	1,72^{a}
APO	0,93^{a}	1,66^{a}
BO	1,40^{b}	1,55^{a}
AO	1,26^{b}	1,82^{a}
F test	10,903 (p<0,0001)	0,9737 (p=0,4057)^{NS}

[1] SH – Slaughterhouse, S – Samples, BPO – Before Preoperational Cleaning, APO – After Preoperational Cleaning, BO – Before Operational Cleaning, AO – After Operational Cleaning. $^{a-b}$ Means within a column with unlike superscripts differ significantly (*F*-test with $\alpha = 5\%$).

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

***Clostridium* spp.**						
Conveyors Cleaning[1]	Slaughterhouses (SH)					**F test**
	SH1.S1	**SH1.S2**	**SH1.S3**	**SH2.S1**	**SH2.S2**	
BPO	$0,71^{Aa}$	$0,94^{Aa}$	$1,09^{Aa}$	$0,71^{Aa}$	$0,71^{Aa}$	0,96 (p=0,43)
APO	$0,71^{Aa}$	$0,71^{Aa}$	$1,01^{Aa}$	$1,18^{Aa}$	$1,07^{Aa}$	1,37 (p=0,24)
BO	$0,71^{Aa}$	$0,71^{Aa}$	$3,55^{Bb}$	$1,22^{Aa}$	$0,83^{Aa}$	47,97 (p<0,0001)*
AO	$0,71^{Aa}$	$0,71^{Aa}$	$3,25^{Bb}$	$0,71^{Aa}$	$0,93^{Aa}$	39,90 (p<0,0001)*
F test	0,00 (p=1,00)	0,45 (p=0,72)	61,42 (p<0,0001)*	2,31 (p=0,08)	0,71 (p=0,55)	
Enterobacteria						
Conveyors Cleaning[1]	Slaughterhouses (SH)					**F test**
	SH1.S1	**SH1.S2**	**SH1.S3**	**SH2.S1**	**SH2.S2**	
BPO	$2,56^{ABb}$	$1,54^{Aa}$	$0,71^{Aa}$	$3,08^{Ab}$	$0,71^{Aa}$	17,77 (p<0,0001)*
APO	$1,90^{Ab}$	$1,48^{Aab}$	$0,97^{Aab}$	$3,23^{Ac}$	$0,71^{Aa}$	14,96 (p<0,0001)*
BO	$1,60^{Aabc}$	$1,92^{Abc}$	$0,98^{Aab}$	$2,35^{Ac}$	$0,89^{Aa}$	6,05 (p=0,0001)*
AO	$2,90^{Bb}$	$1,10^{Aa}$	$1,06^{Aa}$	$3,00^{Ab}$	$1,01^{Aa}$	16,12 (p<0,0001)*
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)	

343 * *F*-test = 5%; [1]SH – Slaughterhouse, S – Samples, BPO – Before Preoperational
344 Cleaning, APO – After Preoperational Cleaning, BO – Before Operational Cleaning,
345 AO – After Operational Cleaning. $^{AB;\ ab}$ Means marked by the same letter (capital letters
346 in the column and lowercase letters in the row) are not significantly different from each
347 other (*F*-test with $\alpha = 5\%$).
348