Reflexes and microbiological interrelationships in pork processing and base

Reflexos e inter-relações microbiológicas no processamento e base de carne suína

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ABSTRACT
Brazilian pork production is expanding, due to the evolution of product characteristics, the international inclusion of technological systems and operating procedures. The objective of the work is to evaluate the microbiological quality at different stages of the slaughter of pigs in a slaughterhouse in the Northwest Region of Rio Grande do Sul, to ensure compliance with the sanitary requirements of the importing markets. The research was carried out in a pig slaughterhouse located in the northwest region of the State of Rio Grande do Sul, from October 2017 to January 2018, whose daily slaughter is greater than 2,000 animals. Through the results presented, it can be seen that the bleeding stage and before scalding, form the sampling points with the highest incidence of total coliforms. The points with the lowest results identified were the points of the shower passing into a clean area with 0% in all samples followed by the final shower with 33.33% in the average of the three days sampled. The contamination index found during the pig slaughter meets the microbiological limits in force. A high proportion of microorganisms occurred in bleeding in relation to groups of microorganisms, being aerobic mesophiles, *E. coli*, total coliforms and thermotolerants.

Key-words: Slaughter Process, Meat Health Quality, Microbiological Monitoring.

RESUMO
A produção brasileira de suínos está se expandindo, devido à evolução das características do produto, à inserção internacional de sistemas tecnológicos e procedimentos operacionais. O objetivo do trabalho é avaliar a qualidade microbiológica em diferentes etapas do abate de suínos em um abatedouro da Região Noroeste do Rio Grande do Sul, para garantir o atendimento às exigências sanitárias dos mercados importadores. A pesquisa foi realizada em um frigorífico de suínos localizado na região noroeste do Estado do Rio Grande do Sul, no período de outubro de 2017 a janeiro de 2018, cujo abate diário é superior a 2.000 animais. Pelos resultados apresentados, pode-se perceber que o estágio de sangramento e antes da escaldagem, formam os pontos de amostragem com maior incidência de coliformes totais. Os pontos com os menores resultados identificados foram os pontos de passagem do chuveiro para uma área limpa com 0% em todas as amostras seguidos do banho final com 33,33% na média dos três dias amostrados. O índice de contaminação verificado durante o abate de suínos atende aos limites microbiológicos

vigentes. Alta proporção de microrganismos ocorreu no sangramento em relação aos grupos de microrganismos, sendo mesófilos aeróbios, E. coli, coliformes totais e termotolerantes.

Palavras-chave: Processo de Abate, Qualidade Sanitária da Carne, Monitoramento Microbiológico.

1 INTRODUCTION

Brazilian pork production is expanding, due to the evolution of product characteristics, the international inclusion of technological systems and operating procedures (Rodrigues et al., 2008). According to Horta et al. (2010), Brazil is in consolidation in the international market, however, it goes through processes of instability and market impacts, such as, for example, external fluctuations, technical barriers of food security, recognition of health status, traceability, production costs , port cost and labor. The flow of slaughtering pigs is a complex process, with a series of operations, from receiving the animals to the final product (Buncic; Sofos, 2012). Despite all the procedures used to guarantee the quality of the product, there are possibilities of microbiological contamination at all stages of the process, as carcasses tend to reach the slaughterhouse contaminated by bacteria. However, this microbiota can be reduced or increased during the slaughter procedure (Contreras et al., 2003).

The slaughtering process consists of a sequence of steps, divided into a dirty area, which covers the stage of sensitization up to the carcasses toilet areas, and a clean area comprising the steps of opening and removing viscera until cooling in chambers (KICH) and SOUZA, 2015).

According to Algino et al. (2009), contamination can occur during the slaughter and processing stages, derived from the animal itself (contaminated skin, feet, feces and viscera) or even through cross contamination in the process or from the facilities, equipment and utensils. According to Choi et al. (2013), the slaughter line can be seen as an open process, which offers several contaminating sources, by numerous pathogenic bacteria, resulting from the animal's skin, the water used, equipment and utensils used in the process.

Microbiological analyzes performed on foods can be used as a method to assess the variety and quantity of the microbiota present, verifying the sanitary quality and hygiene conditions of the food manufacturing process. This evaluation serves as a reference for the consumer regarding the risks that the food may offer to their health, as

well as the intended useful life, taking into account the parameters of food security (Franco and Landgraf, 2005).

The count of aerobic mesophiles and bacteria from the *Escherichia coli* and *Enterobacteriaceae* group can also be used for general assessment of the process, related to hygienic conditions and as indicators of faecal contamination during the slaughter process (Ghafir et al., 2008).

Regulation (EC) No. 2073/2005, determines that the results of microbiological tests for *Salmonella* spp., *Enterobacteriaceae* and total count of aerobic mesophiles, are used as hygienic-sanitary indicators of the pig slaughter process.

Staphylococcus aureus has stood out among microorganisms that play an important role in causing food poisoning. Its presence maintains influence on the failure indicator in sanitary hygienic procedures (Franco; Landgraf, 1996).

Coliforms are also microorganisms that indicate hygienic-sanitary conditions in food manufacturing processes, as they have characteristics that contribute to the identification of flaws in hygiene procedures, and they are sensitive to the action of the chemicals used.

It is extremely important to evaluate the slaughter process of pigs, based on the hygienic-sanitary conditions of the slaughter stages, and to verify the influence on the microbiological contamination of the carcass surface. The stages of scalding, buckling, evisceration, toilet and cooling are the most addressed in research, regarding the assessment of the presence of *Salmonella* spp., incidence of *E. coli* and mesophilic aerobes (botteldoorn et al., 2003; lima et al., 2004; delhalle et al., 2008; arguello et al., 2012; mannion et al., 2012; hernández et al., 2013). Thus, the pig slaughtering process needs constant evaluations including general mapping, through greater detail in the process steps, until the final product, analyzing the indicators of other microorganisms besides *Salmonella* spp.

Therefore, the objective of the work is to evaluate the microbiological quality at different stages of the slaughter of pigs in a slaughterhouse in the Northwest Region of Rio Grande do Sul, to ensure compliance with the sanitary requirements of the importing markets.

2 MATERIAL AND METHODS

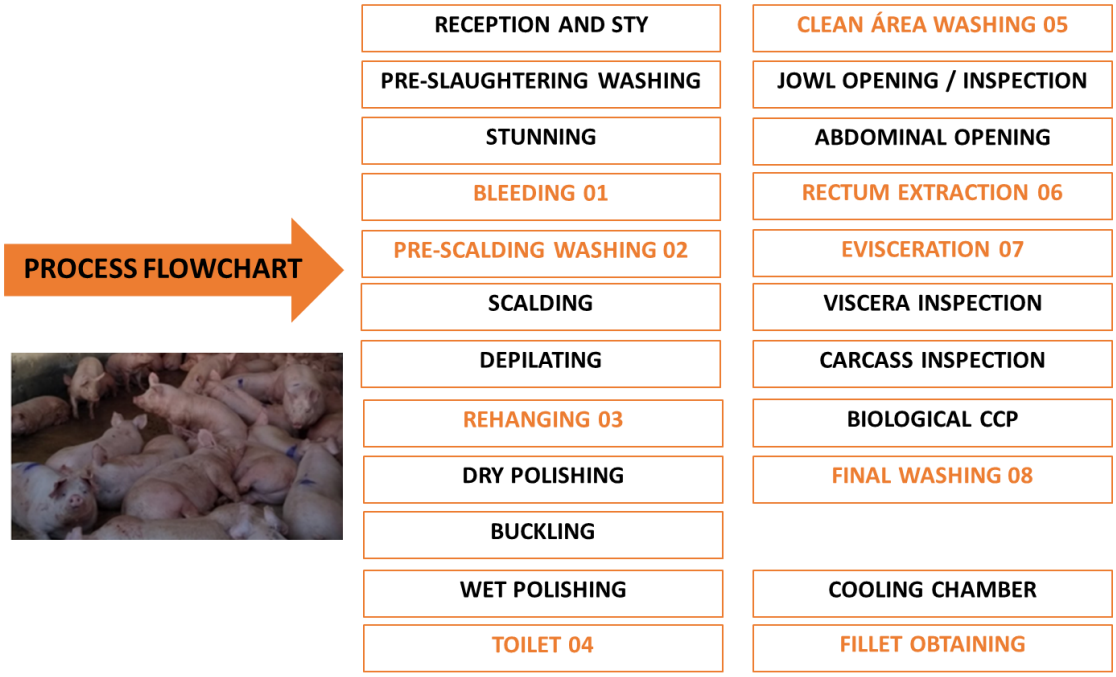
The research was carried out in a pig slaughterhouse located in the northwest region of the State of Rio Grande do Sul, from October 2017 to January 2018, whose

daily slaughter is greater than 2,000 animals. This slaughterhouse is licensed and exports to the Russian market, which requires a liberatory analysis to ship the products. The tests performed and the analysis methodology were defined according to the requirements of this market.

Samples of carcasses from the slaughter line, the final product, surfaces of utensils and equipment and water used in the process were collected.

For the collection of carcass samples and the final product, three pig carcasses were selected, in three different slaughter days, identified by tattooing and submitted to sample collection at eight points, as can be seen in (Figure 1): after bleeding, before scalding, rehanging, after toilet, after the clean entrance shower, after rectum occlusion, then removal of the viscera, immediately after the final carcass wash and a sample of the final product *in natura* porkfillet. Totaling 72 carcass samples and nine pork fillet samples.

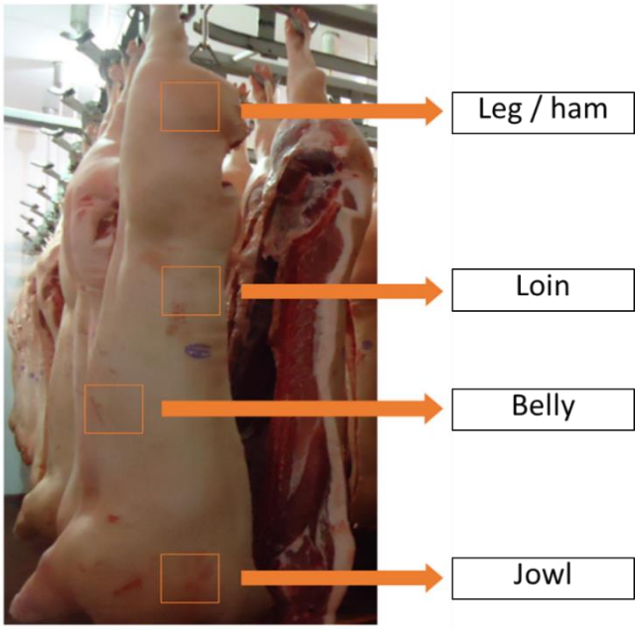
Figure 1. Steps in the pig slaughtering process, highlighting the steps for collecting samples.



Collections on the surface of the carcasses were performed using sponges (Nasco Whirl-Pak-Speci-Sponge Bag), previously packed in a polyethylene autoclave bag and hydrated with 10 mL of 1% buffered water (Merck, Darmstadt, Germany) and sterilized in an autoclave for 30 minutes at 121 °C, kept refrigerated until collection.

The samples were collected using the abrasive sponge technique, a non-destructive method as established by Brasil (2007). The smears were applied in four anatomical regions of the carcass: ham, loin, belly and jowl area (figure 2), totaling a sample area of 400 cm^2 in each carcass. The collected area was delimited using a sterile stainless steel template with an area of 100 cm^2 at each collection point, (Figure 2). At each point, the sponge was applied to the surface of the carcass positioned 10 times in the vertical direction and 10 times in the horizontal direction (Brasil, 2007), using the entire surface of the sponge. Then it was immediately deposited in sterile packaging.

Figure 2: Collection points on pig carcasses.



The samples of the final product were collected in sterile and intact plastic packaging in order to avoid any interference in the result, after finishing the standardization of the swine cut in the spine line. The sample was properly closed and sent to the laboratory for evaluation of the researched parameters.

Five collections of surface samples of utensils and equipment were also carried out: knives used for bleeding, toilet, evisceration and in the cutting room and surfaces of the rectus extractor cup. The collections of the surfaces of utensils and equipment were performed using the surface smear technique using sterile swabs. The total area sampled was 50 cm^2. The swab was placed in a test tube containing 10 mL of the inactivating solution (rinsing solution). A total of 15 surface swab samples were collected.

In the three study days, samples were collected with a volume of 100 mL of water from the scalding tank and three process showers, pre-scalding shower, clean area shower and final shower. The collection was performed using sterile bottles. A total of 12 water samples were collected. The samples were identified and sent for analysis at the Slaughterhouse Microbiology Laboratory.

The carcass swab samples were placed in 1% buffered peptide water (Merck, Darmstadt, Germany), with a final volume of 100 mL for each 400 cm^2 of sampled area of the carcass. In relation to the samples of the final product, weighings of 25 g ± 5 g were carried out and added to 225 mL of 1% buffered peptide water (Merck, Darmstadt, Germany). Then the samples were homogenized for 1 minute on a stomacker shaker and after the analyzes were performed according to the specific test method for each microorganism investigated.

The scalding water samples were pre-enriched using 100 ml of water sample in 50 ml of 3% peptone water.

The quantification of aerobic mesophilic microorganisms was performed using Petrifilm plates (3M, Saint Paul, MN, USA) according to the methodological guidelines of AOAC (2012a). Inoculation of 1 mL of the sample was performed in dilutions of 10-1 to 10-4, in plates of the Aerobic Cout Plate (AC) medium, which contains, in addition to nutrients, the 2,3,5-triphenyltetrazoic chloride. The samples were distributed in an area of 20 cm^2, kept in incubation at 35 °C ± 1 °C for 48 hours ± 3 hours; after the incubation period, all colonies with red growth characteristics were counted (Aoac, 2012a).

To determine the presence of total coliforms, 0.01 g undetectable, as required by Customs Union legislation (TP TC 34/13, TR CU 21/11, Memo. 381/13, Memo 152/14) in the samples, the inoculation of a 1 ml aliquot of the samples in test tubes containing 10 ml Lauryl Tryptose (LST) Broth and an inverted Durhan tube was performed. The presence of coliforms was evidenced by the formation of gas in the tubes of Durhan, after 48 hours of incubation at a temperature of 35 ± 0.5 ºC produced by the fermentation of lactose contained in the medium (Apha, 2015).

The confirmation of the presence of total coliforms was carried out by transferring a range of positive LST tubes to tubes with 2% bright green bile broth, subsequent incubation at 35 ± 0.5 ºC for 48 hours. The presence of gas in Durhan's tubes inside the bright green broth showed the fermentation of the lactose present in the medium and the positive result in the test.

The determination of the presence of thermotolerant coliforms, not detectable 1 g, as required by the Customs Union legislation (TP TC 34/13, TR CU 21/11, Memo. 381/13, Memo 152/14) in the samples was performed by transferring a range of positive LST tubes to tubes with Escherichia coli (EC) broth, which were subjected to incubation at a selective temperature of 45 ± 0.2 ºC for 24 hours. The presence of gas in Durhan's tubes showed the fermentation of lactose present in the medium and the presence of thermotolerant coliforms in the samples (Abnt, 2012).

The detection of *E. coli* was carried out by transferring 0.1 mL from the tubes that showed positive results in the EC broth to plates with L-EMB agar, which after inoculation were subjected to incubation at a temperature of 35 °C ± 1 °C for 24 hours. Typical *E. coli* colonies were those that showed green color with or without metallic luster, showing nucleated center.

Typical colonies were submitted to biochemical confirmation, where a loop was inoculated with a light inoculum of the culture and incubated at 35 ± 1 °C for 24 ± 2 hours. After incubation, 5 drops of Kovacs reagent were added to each 4 ml of culture and shaken slightly. When the presence of a red-violet ring was observed on the surface of the culture medium, the test was considered positive and when the ring remains yellow in color of the reagent the test was considered negative.

The samples were subjected to qualitative tests for the detection of *Salmonella* spp., first through the VIDAS® system, AOAC Method No. 2011.03, automated test for the detection of *Salmonella* spp. in food products, which uses a mixture of capture antibodies with great specificity, directed against O and H antigens and which allows the detection of mobile and immobile strains of *Salmonella* spp. The VIDAS® *Salmonella* system is an immunoenzymatic test, which allows the detection of *Salmonella* antigens by the ELFA technique (Enzyme Linked Fluorescent Assay) in 48 hours, according to Jay (2005), this methodology has been widely used to detect the presence of *Salmonella* spp. in samples of different foods.

Samples showing “positive” results in VIDAS® should be confirmed for the presence of *Salmonella* spp by transferring aliquots to tubes with the selective media Rappaport Vassiliadis broth with soy (RVS) with incubation at 41.5 °C ± 1 ºC for 24 hours ± 3 hours in a water bath and Muller-Kauffman tetrathionate/novobiocin broth (MKTTn) incubated at 37 °C ± 1 ºC for 24 ± 3 hours. Isolation and selection should be performed on plates with xylose lysine deoxycholate agar (XLD), incubated in the inverted position, at 37 ºC ± 1 ºC for 24 ± 3 hours. Samples that presented typical

morphological characteristics of *Salmonella* spp. in the XLD medium, should be submitted to biochemical confirmation in tubes with the triple sugar and iron (TSI) and lysine (LIA) media, incubated at 37 °C ± 1 ºC for 24 hours ± 3 hours.

Finally, samples that showed typical biochemical reactions in TSI and LIA media should be subjected to agglutination serum testing, through the antigen-antibody reaction, with consequent agglutination of the antigen against the antiserum for *Salmonella* (ISO, 2002).

Quantification of *Enterobacteriaceae* was performed by using Petrifilm plates (3M, Saint Paul, MN, USA) according to AOAC methodological guidelines (2012a). Inoculation of 1 mL of the sample was performed in dilutions of 10-1 to 10-4, in plates of the Enterobacteriaceae Count Plate medium, which contains the culture medium violet red bile agar (VRBA). 1 ml of sample was added on the plate, in an area of 20 cm^2, and subjected to incubation at 37 °C ± 1 °C for 24 hours ± 2 hours. After the incubation period, all colonies with growth characteristics were counted in the red color with yellow zones and/or red with air bubbles with or without yellow zones. (Aoac, 2012a).

The determination of *Staphylococcus aureus* was performed using Petrifilm plates (3M, Saint Paul, MN, USA) according to the methodological guidelines of AOAC (2016). Inoculation of 1 mL of the sample was performed in dilutions of 10-1 to 10-4, in plates with Staph Express Count Plate (STX), which is composed of the modified Baird-Parker chromogenic medium, being selective and differential for *S. aureus*. The samples were distributed in an area of 20 cm^2, kept in incubation at 35 °C ± 1 °C for 24 hours ± 2 hours. After the incubation period, the upper film of the Petrifilm plate was lifted and a reactive disk was placed in the appropriate place, lowering the upper film, so that the disk flap remained outside the 20 cm^2 area, the plates were incubated again from 1 - 3 hours at 37 ± 1 ºC. Colonies that had a pink halo that are characteristic of *S. aureus* were counted. For the counts of all colonies, the manufacturer's guidelines were followed:

a) Plate without colony growth - do not use the reactive confirmation disk.

b) Plate containing only red-violet colonies - do not use the disk. Count the red-violet aureus colonies and express as *S. aureus*.

c) Plate containing only blue-green colony - do not use the disk. Express as negative for *S. aureus*.

d) Any other situation, other than those described above - insert the reactive disk. Count the pink halos and express as positive *Staphylococcus*.

To perform the water analysis, it was necessary to use a Manifold® filtration system, coupled to the vacuum pump, by attaching to a 0.45 µm sterile cellulose nitrate membrane. After fixing the membrane in the funnel in the Manifold system, 100 mL of the water sample was poured, sucked through the membrane.

After filtration, the membrane was removed from the filter and accommodated on the plate containing the culture medium chromogenic coliform agar (CCA). The plates were incubated, inverted and incubated at 36 ± 2 °C, for 21 to 24 hours. After the incubation time, the filter membranes were examined and all colonies showing positive Beta Galactosidase reaction (pink to red coloration) were counted and these were identified as presumptive coliform bacteria. Colonies that showed a positive reaction of Beta galactosidase and B-D-glucuronidase (dark blue to violet color) were identified as *E. coli* colonies.

To confirm the presumptive coliform bacteria that are not *E. coli*, an oxidase test was performed on strips. The oxidase reaction was verified by the appearance of a dark blue color on the strip within 30 seconds.

For the calculation and expression of the results, the following criteria were used, all colonies were counted (sum of colonies pink to red, in addition to dark blue-violet), oxidase-negative as bacteria of the coliform group. And all dark blue-violet colonies, such as *E. coli*, were counted.

The determinations of heterotrophic bacteria were performed from the inoculation in a Petri dish of 1 ml of sample and in another dish of 0.1 ml of each selected dilution. Approximately 15 to 20 mL of PCA medium, previously melted and cooled to 44 to 46 °C, was poured into the inoculated plates. The inoculum was mixed with the culture medium by moving the plates gently in the form of eight, on a flat surface. To prevent the eventual occurrence of scattered surface colonies, a second layer of the same medium was added to the surface of the medium after solidification. After the solidified medium, incubation was carried out at 35 ± 1 °C for 48 ± 3 hours.

3 RESULTS AND DISCUSSION

The results of the total coliform survey can be seen in Table 1. Through the results presented, it can be seen that the bleeding stage and before scalding, form the sampling points with the highest incidence of total coliforms. The points with the lowest results identified were the points of the shower passing into a clean area with 0% in all samples followed by the final shower with 33.33% in the average of the three days sampled.

This group of total coliforms belonging to the *Enterobacteriaceae* family can be divided into environmental and fecal coliforms, depending on the natural habitat of the microorganism, and may be present in the environment, in the intestinal tract of man and animals (Sousa, 2006). According to Ghafir et al. (2008), Enterobacteria and *E. coli* can be used as indicators of enteric contamination because they are found in the digestive tract of animals. The literature reports that *E. coli* belongs to the group of coliforms, the same relationship can be extended to the level of total coliforms.

Table 1. Survey of total coliforms in the slaughter of pigs in three carcasses between the period of 2017 to 2018.

Sampled point	Total coliforms not detectable in 0,01 g (%)		
	Collection 1*	Collection 2*	Collection 3*
Bleeding	100	66,66	66,66
Before scalding	66,66	66,66	33,33
Rehanging	100	33,33	0
Toilet	33,33	0	66,66
Shower	0	0	0
Extraction of the rectum	66,66	33,33	33,33
Evisceration	33,33	0	66,66
Final shower	33,33	33,33	33,33

* Average of 3 repetitions

According to the data obtained in the present study, we obtained 100, 66.66 and 66.66% of the samples at the point after bleeding, this contamination may come from the raw material. The cleaning and disinfection protocols adopted in the farms are ineffective, as the residual count of total coliforms is very high, which may be associated with the high number of carcasses with the presence of total coliforms in the samples collected in the bleeding. Pearce et al., (2004) evaluated different stages of the pig slaughtering process and, specifically after bleeding, obtained an average of 10^6 CFU/cm^2 total coliforms. For this reason, it is often used as a hygiene indicator (Prendergast et al., 2008), since they are found in the environment and in the intestines of warm-blooded animals.

The point before scalding obtained 66.66, 66.66 and 33.33% of isolated samples for total coliforms, this decrease may be related to the reduction of the dirt load on the surface of the carcass through the passage of the shower prior to scalding, however the

prevalence of positive samples is an indication that contamination must necessarily pass through other control points that tend to reduce contamination.

The samples collected in the hanging obtained 100, 33.33 and 0% of samples with the presence of total coliforms, the reduction in the load is probably related to the time of exposure of the carcass to scalding at a minimum temperature around 60 °C. However, it is necessary to consider that the presence of bacteria after scalding can be justified by the excess of dirt from the pigs that can adhere to the skin even after treatment, due to pre-slaughter, or factors related to water temperature or scalding time are not controlled correctly.

Another aspect that may be related to the prevalence of Total Coliforms at this point is that the rectum is open, the leakage of fecal content being able to contaminate the carcass and all the equipment, representing a cross-contamination site (Kich; and Souza, 2015).

The prevalence of positive samples followed by the toilet for Total Coliforms was 33.33, 0 and 66.66%, a value that fell compared to the previous point. Among the factors that can contribute to the reduction of contamination, it is possible to mention the buckling which, according to Vivian, 2019 in addition to burning the remaining bristles, with the flame used in an appropriate way, aims to reduce microbiological contamination. Silva et. al. (2012) in their evaluation on the carcass surface demonstrated a significant reduction in positive carcasses for the buckling stage.

The point after the shower in the clean area, there was no finding for this microorganism. It is possible to observe that the collection points prior to this, showed that the number of positive samples has been decreasing due to the process control points that seek to reduce the microbiological load.

The stages after rectus occlusion showed 66.66, 33.33 and 33.33% of positive carcasses, a result that can be seen as a direct or indirect contamination of the surface with fecal material, which may be a better indicator of the level of sanitation, with the presence of enteric pathogens (Ray, 2005).

The slaughter process of the evaluated site currently performs the extraction of the rectum with just a pistol, preventing a longer time for cleaning and sterilization at each operation. This result may be associated with cross contamination of the equipment or operator's hand with the carcass due to the overload of fecal content due to the inefficiency of hygiene and sterilization of the rectal extractor, further studies are suggested that aim to expand the evaluation to include one more extraction gun from the rectum, improving

the process. On the other hand, the practice of tying the rectum with a bag can help reduce the cross contamination of the carcasses, in the later stages of slaughter, preventing the fecal content from contaminating other carcasses, equipment and hand of operators.

In this study, the points after the removal of the viscera and immediately after the final washing of the carcass, maintained an average of 33.33% in the positive samples for total coliforms, showing a small increase in contamination. Pearce et al. (2004), evaluated different regions of carcasses after evisceration and observed an increase for coliforms, and yet Cê (2016), obtained an increase in counts, in the step after evisceration. This increase in coliform levels was possibly due to the contact of materials of gastro-intestinal origin with the carcass surface. As the operation is performed manually, it is difficult to eliminate this occurrence during the processing of the carcasses (Pearce et al, 2004).

The predominance immediately of the final washing of the carcasses may be justified, because even though the carcass undergoing visual inspection at the CCP, and the visual fecal contamination being removed, the procedure may not ensure that the carcass is not contaminated by microorganisms. Another aspect that can lead to contamination after the final washing of the carcass is the manipulation of the operators to direct the carcasses to the cooling chamber, which can represent cross-contamination through the gloves.

The results of the research of thermotolerant coliforms in the slaughter of pigs in three carcasses, collected in three random days between the period of 2017 to 2018, are shown in Table 2. It is possible to verify that in the sampling points of the bleeding, before scalding and rehanging, form the points with the highest incidence of thermotolerant coliforms, all with 100% confirmation in all evaluated samples. The steps of the shower to the clean area and toilet, on the other hand, presented the lowest percentage of this microorganism with a result of 66.66% on two of the sampled days and 33.33% and 0% respectively on one of the research days.

Table 2. Research for thermotolerant coliforms in the slaughter of pigs in nine carcasses between the period of 2017 to 2018.

Sampled point	Total coliforms not detectable in 0,01 g (%)		
	Collection 1*	Collection 2*	Collection 3*
Bleeding	100	100	100
Before scalding	100	100	100
Rehanging	100	100	100
Toilet	66,66	33,33	66,66
Shower	0	66,66	66,66
Extraction of the rectum	100	66,66	66,66
Evisceration	100	66,66	100
Final shower	100	66,66	66,66

* Average of 3 repetitions

The presence of thermotolerant coliforms was observed in all carcasses evaluated after bleeding, at the point before scalding and rehanging. These points, are part of the dirty area, standing out with results superior to the other evaluated points, this fact can be associated with the permanence of microorganisms on the surface of the animals received. The receiving conditions significantly interfere with the contamination of the carcass, the contamination of the skin is directly related to the waiting area.

The following points, toilet and after the shower in the clean area showed a reduction in the number of samples contaminated with thermotolerant coliforms, compared to the previous points.

This reduction may be associated with the application of automatic buckling, a mandatory step in the process, which exposes the surface of the carcase to fire, causing high temperature, as a side effect to reduce the presence of coliforms, this step is contemplated in Brazilian legislation, with the purpose of eliminating the remainders of the depilating process (Brasil, 1995). Another procedure that probably influenced the reduction of the presence of thermotolerant coliforms was the passage of the pigs through the shower with water under pressure and with the addition of chlorine at 2 ppm of chlorine.

In the carcasses evaluated after rectal occlusion, 100, 66.66 and 66.66% of positive samples for thermotolerant coliforms were isolated, this increase in contamination proves that the extraction of the rectum is a critical point of cross

contamination and the lack of operational sanitary procedures can significantly increase contamination of the carcass. The result for this point may be associated with flaws in the practice adopted for tying the rectum with plastic packaging at the time of occlusion, Borch et al., (1996) states that this procedure significantly influences the microbial contamination of carcasses.

In the evisceration stage, there was also an increase in positive carcasses in the samples collected, 100, 66.66 and 100%, respectively, which can be associated with the rupture of the viscera and, consequently, the leakage of intestinal content on the carcasses, leading to a subsequent contamination (Zardeh, 2001). Contamination of the carcass can occur in this procedure, due to the fecal content of the animal itself or by cross-contamination, through knives and/or hands of employees responsible for evisceration.

The collections carried out immediately after the final washing of the carcasses were present in 100, 66.66 and 66.66% samples, this reduction may be associated with the CCP (Critical Control Point), which consists of identifying and removing parts of the carcass that can be contaminated in the act of evisceration and also by passing in the final shower helping to reduce microorganisms, by applying high pressure water over the entire surface of the carcass using chlorine at 2 ppm.

Salmonella spp. it is one of the most researched microorganisms in meat processing, being a relevant concern in the swine sector. These bacteria can be present in the gastrointestinal tract of infected animals, during the slaughter process they can spread and cause cross contamination of pig carcasses (Rostagno and Callaway, 2012).

To ensure control of the spread of *Salmonella* spp. during the production process, preventive measures should be adopted in the primary phase of the production chain, ensuring the control of animal infections (Methner et al., 2011).

In the work developed, no results were identified with the presence of *Salmonella* spp. in any of the carcasses analyzed at any of the collection points. This result has great relevance considering the pathogenic character of this microorganism, often associated with outbreaks due to ingestion of contaminated animal products, showing that the process is being effective in inhibiting this microorganism. Although this result is very positive, it does not exempt measures to be taken and followed against contamination by this pathogen in the slaughterhouse, since Corbellini et al. (2016) report that contamination by *Salmonella* spp. can originate directly or indirectly from contact with the feces of infected pigs or by contact with the microbiota present in the process environment.

Table 3. Average in the counts of aerobic mesophilic microorganisms in the slaughter of pigs in three carcasses between the period of 2017 to 2018.

Sampled point	Aerobic mesophilic microorganisms (**CFU/g^{-1})		
	Collection 1*	Collection 2*	Collection 3*
Bleeding	1,3x10^4	1,4x10^3	2,1x10^3
Before scalding	6,7 x10^2	2,6 x10^2	6,5 x10^2
Rehanging	3,9 x10^2	3,1 x10^2	5,2 x10^2
Toilet	5,1 x10^2	4,3 x10^2	9,9 x10^2
Shower	2,03 x10^2	6,9 x10^2	4,9 x10^2
Extraction of the rectum	9,6 x10^2	7,4 x10^2	1,5 x10^3
Evisceration	5,4 x10^2	8,8 x10^2	8,6 x10^2
Final shower	1,08 x10^3	8,06 x10^2	9,7 x10^2

* Average of 3 repetitions, ** CFU: Colony Forming Unit

Even though *Salmonella* spp was not found, it is relevant to analyze all points where there could be contamination with this bacterium in the slaughterhouse, according to Thorberg and Engvall (2001), in the slaughter of pigs, the stages of evisceration, toilet, scalding and carcass division are particularly involved in the risk of contamination by *Salmonella* spp and that in these stages may introduce microorganisms that result in greater contamination at the end of the slaughter line.

The scalding system carried out in the slaughterhouse, in tanks by immersion in hot water at around 62 °C, results in a significant reduction in the swine skin microbiota, which can eliminate Salmonella spp. and obtain 2 logs of reduction of indicator microorganisms (Rodrigues et al., 2017)

In the depilating stage, if not monitored, some aspects may lead to an increase in contamination by *Salmonella* and other microorganisms. In this process, the swine rectum is still open, this condition allows the leakage of fecal content, which can contaminate the carcass, the equipment, which start to cross-contaminate (Kich and Souza, 2015). Other factors that can influence the incidence of this microorganism in the epilator are related to the sanitary profile of difficult cleaning, insufficient temperature and water flow, providing a bacterial proliferation inside the machine (Buncic and Sofos, 2012).

Sequencing the slaughterhouse process, the carcass is subjected to a temperature in the buckler that can exceed 700 °C, acting on the elimination of bacteria present,

presenting this step as relevant from a microbiological point of view (Kich and Souza, 2015).

In similar studies carried out, buckling reduced the incidence of *Salmonella* spp., as reported by Hernández et al. (2013), for research on *Salmonella* spp. there was a prevalence of 2.5%, in turn Seixas, Tochetto and Ferraz (2009) and Pearce et al. (2004), did not detect any positivity in carcasses at this stage. All research attributed the result to the fact that the carcasses are exposed to high temperatures as a result of direct contact with the flames of the buckling, the microorganisms present are reduced or eliminated by the action of heat.

After the buckling is carried out, the next stage is the polishing stage, applied in order to remove hair and hair fragments remaining from previous operations. According to Sánchez-Rodríguez (2018) polishing is an important source of microbial contamination in pig carcasses, the prevalence of *Salmonella* spp. in pig carcasses varies a lot and is mainly related to cross contamination produced during slaughter and to pigs carrying this pathogen.

Subsequently, the toilet operations are carried out, the carcasses proceed to final polishing and are washed in a shower with chlorinated water at 2 ppm. At this stage, according to the results of the study by Lima et al. (2004), there was a reduction in the incidence of *Salmonella* to 6.67%, when analyzing carcasses after rectus occlusion, detecting a *Salmonella* prevalence of 6.70%.

After the rectus occlusion activity, the results of the research carried out by Neitzke et al. (2017) presented one of the lowest isolation frequencies of *Salmonella* spp., 3.1% of the total samples collected. The attribution of this result was associated with the practice of occlusion of the rectum with plastic bag isolation. Berends et al. (1997) also found that the rectus occlusion procedure prevents 75% of carcass contamination with *Salmonella* spp.

The prevalence of *Salmonella* after evisceration is quite varied between the different studies conducted. Among the works carried out, there are reports that during the execution of the evisceration procedure it can cause the rupture of the viscera with the release of intestinal contents on the surface of the carcasses, this rupture can trigger cross contamination (Zardeh, 2001).

Lima et al. (2004) found a frequency of 16.70% of *Salmonella* spp. in the evisceration stage, justifying that the evisceration process is one of the main factors of carcass contamination. Hernández et al. (2013) did not detect any positive samples for

Salmonella spp. McDowell et al. (2007) reported a prevalence of 40% for the evisceration stage in their study. Seixas, Tochetto and Ferraz (2009) detected 4 positive samples out of a total of 18 sampled, which results in a prevalence of 22.2%. Pearce et al. (2004) obtained 7% of the samples, being positive after this stage.

The presence of a pig carrying *Salmonella* spp. is not always associated with carcass contamination by this pathogen, if the evisceration procedure is well conducted. However, pig carcasses without the presence of *Salmonella* sp. can acquire this pathogen through cross contamination. (Van Der Gaag et al., 2003 apud Ducas; Silva, 2011).

Thus, the presence of *Salmonella* sp. in animals received in the slaughterhouse represents a risk factor, but it does not mean an index of contamination of the final product. The greater the number of animals arriving with *Salmonella* sp. at the time of slaughter, the greater the difficulty of controlling critical points in the industry. The number of carrier animals received at the slaughterhouse has been considered the first critical processing point for *Salmonella* sp. (Bessa et al., 2004).

The counting of aerobic mesophilic microorganisms in the different stages of the process is an important tool for understanding the level of hygiene of the environment in which the food was processed, in addition to providing the provision of information to improve procedures from the sanitary point of view along the slaughter and boning line (Carvalho, 2010).

According to the results obtained in the evaluations on the surface of the sampled animals, during the slaughter line, we can see in Table 03, oscillations of the microbial levels, with the highest count point showing for the collections carried out in the bleeding and the lowest count showing in the collections performed in the rehanging.

Table 4. Average in the counts of *Escherichia coli* in the slaughter of pigs in three carcasses between the period of 2017 to 2018.

Sampled point	*Escherichia coli* (**CFU/cm^2)		
	Collection 1*	Collection 2*	Collection 3*
Bleeding	2,0x10^1	2,3x10^1	2,0x10^1
Before scalding	1,6 x10^1	0,6 x10^1	5,3 x10^1
Rehanging	1,6 x10^1	4,0 x10^1	1
Toilet	0	0	0
Shower	0	0	0
Extraction of the rectum	0	0	0
Evisceration	0	0	1,6x10^1
Final shower	0	0	0

* Average of 3 repetitions, ** CFU: Colony Forming Unit

The results obtained in this study are inferior to those observed by Irish researchers, who in a similar work obtained an average of 10^6 CFU/cm^2 for mesophilic aerobes after bleeding (Pearce et al., 2004).

The contamination of the carcasses seems to be directly related to the contamination of the skin of the live pigs before the stunning stage, because when analyzing the results of mesophiles at the collection point, just after the shower before the scalding, it is possible to verify a reduction of a log in average.

The current Brazilian legislation, Consolidation Ordinance No. 5 of 2017, does not allow the addition of antimicrobial agents in the water used in the showers of the carcasses, allowing only a maximum content of free residual chlorine of 2 mg/L. However, if the percentage is met and the structural conditions of the shower, as well as the water pressure maintained to the point that reaches all regions of the carcasses, the action of washing carcasses can contribute to the reduction of microorganisms in this stage.

The analyzes performed right after the scalding and depilating process showed the best results, with the lowest concentrations of mesophilic aerobes with averages of 3.9 x102, 3.1 x102 and 5.2 x102 CFU/g^{-1}. This result is in line with the results obtained by Pearce et al. (2004) and Spescha et al. (2006), who showed a reduction of aerobic bacteria in the pig carcasses that were scalded at a temperature between 59-62 °C.

Other authors have also found that the high temperature of the scalding water acts on the carcasses acting on the microbial reduction, decreasing the levels of mesophilic aerobes above 2 log, and Salmonella levels varying between 0% and 5.6%. (Buncic and Sofos, 2012).

The results obtained after performing the toilet using knives, showed an increase in the number of aerobic bacteria, with averages of 5.1 x10^2, 4.3 x10^2 and 9.9 x10^2 CFU/g^{-1}. This fact can be explained by Kich and Souza, (2015) who found that the toilet, with manual removal of hair from the surface of the carcass by means of knives, offers microbiological risks, as the knife with failures in sterilization can cause recontamination of carcasses.

Another important aspect to be considered is the polishing step that precedes the toilet, as the machine responsible for this process does not have a sanitary layout for hygiene, making it act as a potential source of recontamination of the carcasses in the slaughter line (Buncic and Sofos, 2012). The study by Pearce et al. (2004) demonstrated

a significant increase at the microbiological level in the passage of the carcasses through the polishing step.

Table 5. Average in the counts of *Staphylococcus aureus* in the slaughter of pigs in three carcasses between the period of 2017 to 2018.

Sampled point	*Staphylococcus aureus* (**UFC/g 10^{-1})		
	Collection 1*	Collection 2*	Collection 3*
Bleeding	$2,1x10^3$	$1,4x10^3$	$2,8x10^2$
Before scalding	$4,2x10^2$	$1,4x10^2$	$1,5x10^2$
Rehanging	$6,3x10^1$	0	1
Toilet	0	0	0
Shower	0	0	0
Extraction of the rectum	0	0	$1,6\ x10^1$
Evisceration	0	0	0
Final shower	0	0	0

* Average of 3 repetitions, ** CFU: Colony Forming Unit

After the polishing and toilet operations have been carried out, the carcasses are subjected to a wash, from which stage they enter an area known as a clean slaughter area. At this point, immediately after cleaning the carcasses, there was a considerable reduction in aerobic mesophilic microorganisms to 2.03 $x10^2$, 6.9 $x10^2$ and 4.9 $x10^2$ CFU/g^{-1}. This reduction is probably due to the same reason evidenced in the shower before the scalding, where the number of aerobics were also reduced, due to the action of water pressure, as determined by Ordinance 711 of 1995 and free residual chlorine activity of 2 mg/L at levels permitted by Consolidation Ordinance No. 5 of 2017 operating under the carcasses.

In the rectus occlusion stage, there was a significant increase in the presence of aerobic mesophilic microorganisms 9.6 $x10^2$, 7.4 $x10^2$ and 1.5 $x10^2$ UFC/g^{-1}, unlike the results found by Bolton et al. (2002), in which there was no increase in the total bacterial count, attributing this result to good manufacturing practices in the process, regarding the training of operators and hygiene. In addition, the use of a plastic bag to isolate the rectum, prevents the spread of pathogens through the feces (Hald et al., 2003).

Corbellini et al. (2016), emphasizes that the levels of microbiological presence are related to the prevalence of batches, environmental contamination and hygiene, cleaning,

disinfection and operational training practices. Therefore, we can consider that the increase in contamination identified in the research is related to noncompliance with good practices or operational and hygiene failures, during the execution of the rectal extraction task.

In this research, the evisceration step provided a small reduction in the mesophilic aerobic level counts to 5.4 $x10^2$, 8.8 $x10^2$ and 8.6 $x10^2$ CFU/g^{-1}. In the study by Pearce et al. (2004), evaluating different regions of carcasses after evisceration, a reduction in the levels of mesophilic aerobes was observed similar to the present study (0.12 log).

The evisceration stage provides carcasses with a high criticality regarding microbiological risk (ARGUELLO et al., 2012). According to Kich and Silva (2015), the slaughter stage of the clean area is more critical of operations, in relation to microbiological aspects, being characterized as a critical control point, being monitored through visual assessment of eviscerated carcasses, checking for presence of gastrointestinal content. Carrying out the hand hygiene of the operators and sterilizing the knives, are efficient ways to avoid cross-contamination at this stage (Buncic; Sofos, 2012).

The final wash provided an increase in the levels of indicator microorganisms, presenting a result of 1.08 $x10^2$, 8.06 $x10^2$ and 9.7 $x10^2$ CFU/g^{-1}. Increases in the number of mesophilic aerobes were also observed at this stage in a similar survey conducted by Ce (2016), showing an increase of 3.62; to 3.92; log CFU/cm^2.

Kich and Souza (2015) attributed the increase in the levels of indicator microorganisms, due to the mechanical action of water exerted on the carcasses during the washing step, disseminating and distributing the bacteria on the surface of the carcasses.

Ordinance 711 (Brazil, 1995) determines the use of 3 atmosphere pressure in the showers of the slaughter line, however failures in the process may occur, such as a lower pressure than recommended, or misalignment and obstruction of the jets, negatively impacting washing efficiency.

When observing the results for *E. coli* (Table 4), we can see that the highest results obtained are from the point sampled after the bleeding, and most of the collected samples did not present counts for this microorganism. This result is very similar to those observed by another researcher, who evaluated the *E. coli* count at different stages of the slaughter of pigs and also found 10^1 CFU/cm^2 in the bleeding stage (Ce, 2016).

The shower before scalding proved to be efficient steps in microbial reduction, decreasing E. coli counts to zero. The scalding stage plays an important role in microbial reduction, due to the high temperature applied to the carcasses (BUNCIC and SOFOS, 2012). Cê (2016), developed a work similar to the present study, obtaining an average of 0.86 CFU/cm^2 in the bleeding stage, and after the scalding stage practically eliminated the incidence of *E. coli* with an average of 0.01 CFU/cm^2.

According to Belluco et al. (2015) the scalding process allows an effective reduction in the count of *E. coli* and *Enterobacteriaceae* in pig carcasses, reaching a reduction greater than 3 log CFU/cm^2 for both microorganisms.

In the toilet stage and after the shower at the entrance to the clean area, there was no incidence of *E. coli* in the evaluated carcasses. After these operations, the carcass undergoes a wash which led to a decrease in the microbial load in it. A similar result was obtained by Cê (2016), who obtained a reduction in the counts of total coliforms, *E. coli* and enterobacteria in the toilet stage and after the shower at the entrance to the clean area. Namvar and Warriner (2006), stated that the polishing process reduced the *E. coli* count, but the operation was not evaluated in isolation, but in combination with the washing and evisceration stage, making the cause and effect relationship between the stage and the counting.

In the evisceration stage, there was a small increase in *E. coli* counts, with results of 0.0 and 1.6x10^1 CFU/cm^2, respectively. Lindblad et al. (2007) also showed levels of E. coli in samples of pig carcasses after evisceration. This increase may be related to cross contamination during the manual operation of the removal of the viscera, which occurred through the contact of materials of gastrointestinal origin with the surface of the carcass (Ce, 2016).

At the sampling point after the final shower, the presence of *E. coli* was not identified, this result is in line with the behavior of other microorganisms researched in the present study, where the action of water pressure and the guarantee of adding chlorine at 2 ppm, influenced the reduction in count and presence.

Staphylococcus aureus plays an important role among the microorganisms that cause food poisoning and for being recognized as an indicator of food hygiene (Lima et al., 2004). This microorganism is an enterotoxin-producing bacteria and able to survive in adverse conditions.

The count of *Staphylococcus aureus*, (table 5) shows the greatest representativeness in bleeding, this number being reduced in the steps before scalding, followed by re-opening until its counts are zeroed in the other evaluated points.

The bleeding evaluation point showed a higher concentration of *Staphylococcus aureus*. These concentrations can be related to the fact that these microorganisms are considered symbionts, composing the microbiota of the skin and respiratory tract of several species of animals. These bacteria are present on various surfaces in diverse environments from water to dust and may be temporarily contained in the gastrointestinal tract (Biberstein and Hirsh, 1999).

In a similar study conducted in Germany, it was also found that the greatest risk of contamination of carcasses by isolated *S. aureus* was observed after the stunning of pigs (MEYER et al., 2010). In the pre-scalding shower we observed a small reduction in the averages of the results, going to $4.2x10^2$, $1.4x10^2$ and $1.5x10^2$ CFU/g 10^{-1}. This small reduction can be attributed to the application of chlorinated water at 2 ppm under pressure, during the carcass washing procedure.

According to Cardoso et al. (2011) there are often pigs entering the slaughter line, harboring microorganisms, which do not show clinical signs and have no evidence of loss of productive performance because they are contaminated. Lima et al., (2004) emphasizes the importance of such contamination being considered important, because even though the count is low in the beginning, this pathogen can multiply during the slaughter stages, and therefore, it is characterized as a risk parameter in the slaughter process.

In the other analyzed points, during the process, high counts of *Staphylococcus aureus* were no longer identified. In other similar studies, with evaluation at different points and stages of the slaughter of pigs, the presence of positive coagulase *Staphylococcus* was found, including in the scalding, buckling and refrigeration stage, which may increase throughout the day and spread between the stages of the process (Spescha et al., 2006).

Similar works are also found in Brazil, in the state of Minas Gerais (Lima et al., 2004). No significant differences were detected between slaughter steps evaluated, so the probability of occurring this pathogen was statistically the same in the different stages of the pork process. In the state of São Paulo (Baker et al., 2008), there were evidences of *S. aureus* isolated at various points of the slaughter line in a slaughterhouse.

Considering the results obtained and the consulted works, we can consider that during the whole production of pork, hygienic care related to animals, collaborators,

environment, equipment and utensils used is necessary, which can be sources of contamination by *S. aureus*.

The results of the microbiological analyzes performed on the fillet, (table 6), show that the presence of total coliforms in food, in some cases, may not be indicative of fecal contamination, as bacteria in this group are included where the direct origin is not exclusively enteric. The group of coliform microorganisms is capable of environmental colonization, especially in the soil. Therefore, their presence may be related to inadequate hygiene and product processing practices, or to cross-contamination, after this procedure (Souza, 2006).

Table 6. Survey of microorganisms from fillet samples collected in the quartering room from 2017 to 2018.

Microorganisms	Fillets		
	Collection 1*	Collection 2*	Collection 3*
Thermotolerant coliforms (%)	33,33	33,33	0
Total coliforms (%)	33,33	0	0
Salmonella spp. (%)	0	0	0

* Average of 3 repetitions

The samples collected in the swine carcass did not show the occurrence of *Salmonella* spp., aspect that can explain the absence in the final product. Kich and Souza (2015) suggest that the application of a thermal shock on the carcasses, promoting rapid cooling, may contribute to the reduction of the prevalence of *Salmonella* ssp. in case of presence in the carcass due to cross contamination in the slaughter process, this occurs due to the desiccation of the carcass surface due to the passage of cold air at high speed. The same effect can be extended to other contaminating microorganisms in the carcasses. This thermal shock is not carried out in the slaughterhouse, but the carcasses are cooled for 24 hours until reaching a temperature of ≤ 7 °C inside the carcasses.

The results of the samples collected in the pig carcass after the final shower, made explicit the presence of thermotolerant coliforms and this contamination may have reached the final product. However, there was no presence of bacteria in all of the analyzed fillet samples, which reinforces the importance of cooling the carcasses for 24 hours until a temperature of ≤ 7 °C is reached inside, which has been showing to be an effective practice in microbiological control, reducing the levels of all the indicators

surveyed, as well as the prevalence of pathogens, which is in line with what was reported by Buncic and Sofos (2012).

Other authors have also found coliforms in swine cuts: Sales et al. (2013) obtained positive results for thermotolerant coliforms in all samples of pork analyzed in their study. The Brazilian legislation RDC nº 12/2001, currently does not refer to requirements for the analysis of coliforms for *in natura* pork, however it establishes as a standard the absence for *Salmonella* spp. in 25g of sample (Brasil, 2001).

The choice of the analysis of the coliforms group in this study was to evaluate the hygienic and sanitary conditions, for they are microorganisms that indicate hygienic-sanitary conditions, they are generally used to monitor, detect changes in quality; classify or restrict the use of water and food (Sousa, 2006).

The results of the final fillet product for aerobic mesophilic microorganisms, *Escherichia coli* and *Staphylococcus aureus* are in Table 7, and it can be seen that the samples presented average counts for Aerobic Mesophiles, however for *Escherichia coli* and *Staphylococcus aureus* they did not present any incidence in the analyzed samples.

Table 7. Microbiological evaluation of samples of fillets collected in the quartering room between 2017 to 2018.

Microorganisms	Fillets		
	Collection 1*	Collection 2*	Collection 3*
Aerobic mesophilic microorganisms (**CFU/g 10^{-1})	3,3 x10^{1}	3,6x10^{1}	6,3x10^{1}
Escherichia coli (CFU/cm^2)	0	0	0
Staphylococcus aureus (CFU/g 10^{-1})	0	0	0

* Average of 3 repetitions, ** CFU: Colony Forming Unit

Sales (2013) observed in his research with in natura pork lower values for aerobic mesophilic microorganisms, with an average of 2.68 CFU/g 10^{-1}. Zweifel, Fisher and Stephan (2008) evaluated the levels of pig carcasses within three hours of cooling, where the levels of aerobic mesophiles varied between 10^2 and 10^4 CFU/cm^2. Ghafir et al. (2008) evaluated the performance of indicator microorganisms, with results 10^3 CFU/cm^2 for aerobic mesophiles. The presence of aerobic mesophilic bacteria is expected to some extent in pork, since part of the food is subject to several potential sources of

microorganisms, and meat is an excellent medium for the multiplication of microorganisms (Arguello, 2013).

The microbiological quality of a food can be maintained at an acceptable level, permitted by current legislation and which will not cause harm to the health of the consumer, through proper handling, knowledge and use of factors that influence the growth of microorganisms in food, among other actions (Lima and Souza, 2001).

Table 8. Evaluation of aerobic mesophilic microorganisms in water used to slaughter pigs from 2017 to 2018.

Sampled point	Aerobic mesophilic microorganisms (**CFU/g 10^{-1})
	Collection*
Pre-scalding shower	$2{,}0x10^{1}$
Scalding tank	$4{,}8x10^{2}$
Pigs wash shower after polisher	0
Final wash shower of carcasses	$4{,}3x10^{1}$

** CFU: Colony Forming Unit

The absence of *Escherichia coli* and *Staphylococcus aureus*, in the final fillet product, consists of satisfactory results, considering the pathogenic character of these bacteria. This absence can be associated with hygienic procedures, the correct cooling of the carcass, acting in the reduction of the microbial load on the carcass surface and, consequently, presenting a final product free from contamination.

Lima et al. (2004), obtained a lower frequency in samples collected in carcasses after 24 hours of refrigeration for *Staphylococcus aureus*, and attributed this reduction to the refrigeration process, which inhibits the multiplication of these pathogens. Lee et al. (2009) showed that fresh pork, not properly refrigerated, was the most important source (14.9%) of pathogenic *E. coli*.

Delhalle et al. (2008), in carcasses after cooling, reported a reduction in *E. coli* counts of 0.61 log CFU/cm^2 and Ghafir et al. (2008) describe results of 0.55 log CFU/cm^2 for *E. coli* in refrigerated meat products. The counts found by these authors were higher than those obtained in this research. Considering the control of this bacterium of great importance for the consumer's health, the absence of *Escherichia coli* verified in the samples evaluated in this work favors the quality of the pork produced in the slaughterhouse.

In the tests carried out to check microorganisms in the water used in the slaughter of pigs, shown in Tables 8 and 9, we can see that results were not presented outside the expected limit for aerobic mesophiles, *E. coli* and *Salmonella* spp. at any point.

Table 9. Research of microorganisms in water samples used in the slaughter of pigs from 2017 to 2018.

Sampled point	Microsrganisms (%)		
	Total Col. *	*E.coli**	*Salmonella* spp.*
Pre-scalding shower	33,3	0	0
Scalding tank	0	0	0
Pigs wash shower after polisher	0	0	0
Final wash shower of carcasses	33,33	0	0

Table 9 shows the results of the evaluation of microorganisms in samples of water used in the slaughter of pigs. Among the results presented for total coliforms at the scalding tank points and swine washing shower after polishing, there was no presence of these microorganisms, however, in the pre-scalding shower and final washing of the carcasses, 33.33% percentage presence was obtained. This finding of total coliforms may be related to cross contamination at the time of collection, as the structure of the equipment does not allow aseptic care to guarantee the integrity of the sample. Another factor that makes this hypothesis possible is that in most of the researched microorganisms they presented reduction and even elimination in the points of the pre-scalding showers and final washing of carcasses.

As for the water in the scalding tank, one of the factors that can interfere or guarantee the absence of contamination is the water temperature. According to Hald et al. (1999), maintaining the temperature of this scalding water above 60 °C prevents bacterial contamination of the carcasses.

In the utensils used (table 10), (with the exception of the rectum extractor) there was no presence of these microorganisms. The rectal extractor was the tool with the highest number of aerobic mesophiles and enterobacteria.

Table 10. Evaluation of microorganisms in utensils used to slaughter pigs from 2017 to 2018.

Sampled point	Microorganisms	
	Aerobic mesophilic microorganisms (**CFU/g 10^{-1})	Enterobacteria (CFU/g 10^{-1})
Bleeding knife	0	0
Hanging knife	1,3 $x10^{1}$	0
Pig toilet knife	0	0
Rectum extractor	1,7$x10^{2}$	2,6$x10^{1}$
Knife removed from white viscera	0	0

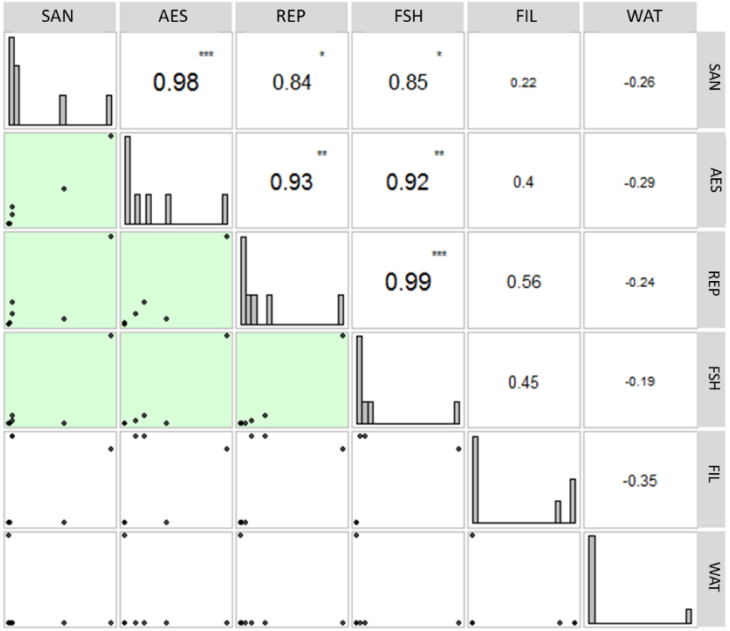
** CFU: Colony Forming Unit

Among the utensils used in the slaughtering process, which were sampled, we can see that all the knives used are in accordance with the expected result, with regard to the operational sanitary procedure for cleaning and sterilization during the process, thus, these tools do not offer risks of cross contamination between the carcasses.

The equipment for extraction of the rectum, in turn, had the highest count of aerobic mesophiles and for Enterobacteria, this result may be related to the fact of the direct contact of the extraction pistol with the feces, as well as the time to perform the cleaning procedure and sterilization, carried out with each extraction operation, which despite occurring quickly, can often be ineffective. According to Swart et al., (2016) insufficient disinfection of knives or cutting machines can even lead to cross contamination from one carcass to another.

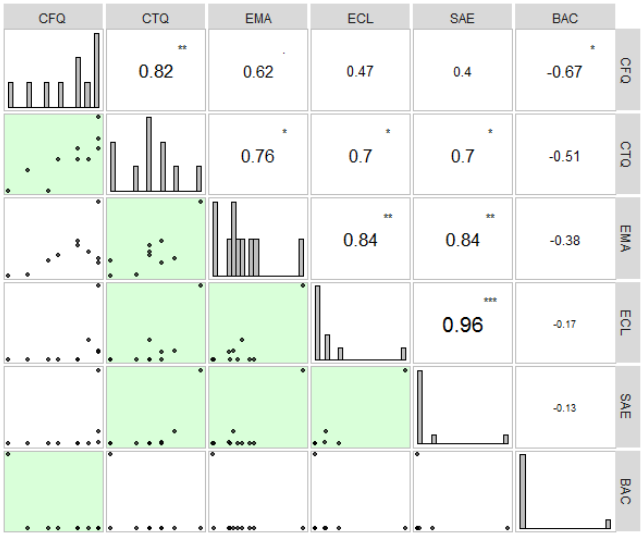
The linear correlation (figure 3) shows that, regardless of the contaminating microorganisms, some processes showed significance for the increase in this number of microorganisms. Those related to the increase in the bleeding stage, the processes before scalding, rehanging and final shower. For the process before scalding, the increase is related to the rehanging and final shower and the rehanging is also related to the final shower. This increase may be related, due to the order in which each process is carried out, where there is a high index of microorganisms at the beginning of the procedure, it tends to maintain until the carcass is completely clean.

Figure 3: Linear correlation for the different processes in the slaughter of pigs.



For the microorganisms evaluated, according to the linear correlation (figure 4), it shows that for the increase in the number of fecal coliforms (CFQ) there is an increase in the number of total coliforms (CTQ) and a reduction in the number of bacteria present. One of the factors that can justify is that at the point of rehanging, where the rectum is open, with the leakage of fecal contents, the carcass and all the equipment can be contaminated, representing a place of cross contamination.

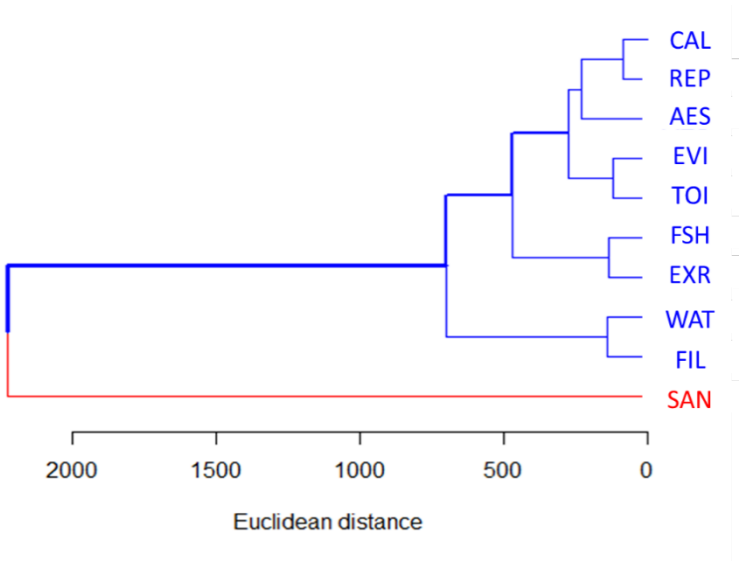
Figure 4: Linear correlation for microorganisms independent of the process step.



Relating the increase in the number of total coliforms, there is an increase in aerobic mesophilic microorganisms (EMA), *E. coli* (ECL) and *Salmonella* (SAE). The increase in aerobic mesophilic microorganisms (EMA) is related to the increase in *E. coli* (ECL) and *Salmonella* (SAE), which are also related to the increase in (ECL), there is an increase in SAE. These factors occur due to hygienic conditions and as indicators of faecal contamination during the slaughter process (Ghafir et al., 2008).

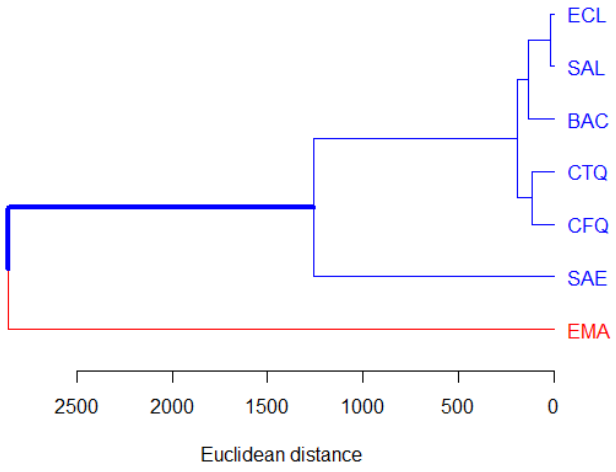
The dendrogram (figure 5) shows that regardless of the microorganisms, the process steps show similarities, mainly between the shower in the clean area entrance (CAL) and the re-opening (REP), eviseration (EVI) and toilet (TOI), final shower (FSH) and extraction of the rectum (EXR), water (WAT) and fillet (FIL). This may have occurred because the counting number of the microorganisms was similar between these locations. For the bleeding stage, it had an isolated effect, due to its higher number of microorganisms, which are present in the blood and in the animal.

Figure 5: Dendrogram for the slaughter times of the pigs, using the average Euclidean algorithm with all variables measured.



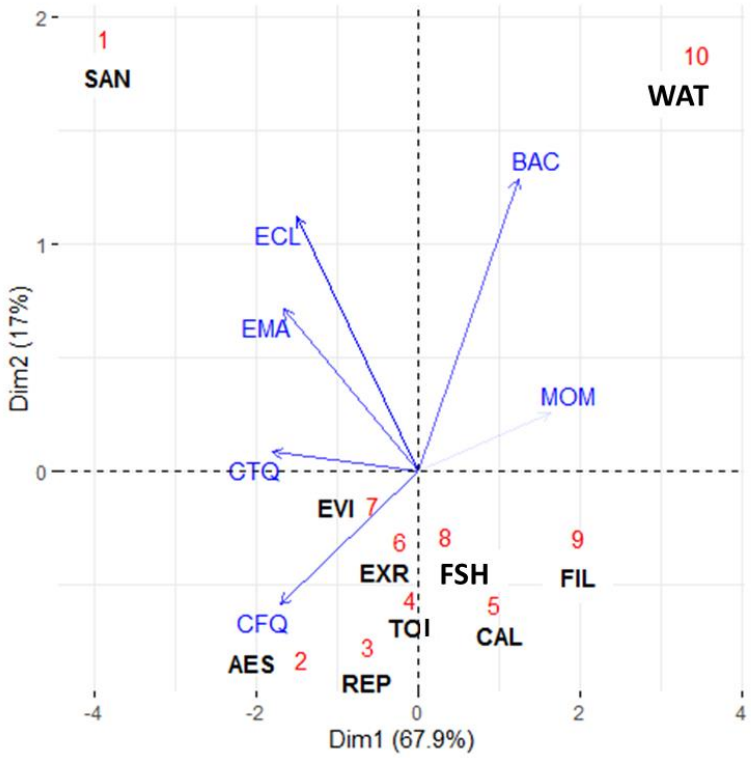
When we consider the microorganisms, the dendrogram (figure 6) represents similarity between certain groups of microorganisms, being *E. coli* (ECL) and *Salmonella* (SAL), for the former, it presents a high quantity of these microorganisms, in the bleeding stage that tends to be normal, but as the carcass advances in the processes, the amount tends to decrease, for *Salmonella* there was no presence in the carcasses of the animals. The relationship between both is related to the reduction of the amounts of microorganisms. Another relationship occurred between the amount of total coliforms (CTQ) and fecal coliforms (CFQ), this occurred because one factor influences the other. And for aerobic mesophilic microorganisms (EMA) they were not related to any other type of contaminating microorganisms, this may be related to the fact that they do not present high values of these microorganisms.

Figure 6: Dendrogram for the microorganisms present in the slaughter stages, using the average Euclidean algorithm with all variables measured.



The BPLOT main component graph, (figure 7) shows in which moments of the slaughter stage it is related to the quantity of microorganisms, and for the bleeding stage, which presents a greater amount of *E. coli* (ECL), aerobic mesophilic microorganisms (EMA), total coliforms (CTQ). This contamination resulting from the raw material, since the disinfection and cleaning processes are not very effective, thus the residual count of total coliforms becomes high, associated with the high number of carcasses with the presence of total coliforms in the samples collected in the bleeding. For the water analysis, it presented higher levels of bacteria, because water is used to clean the carcasses in each slaughter process, and for the process before scalding, it presented higher values of fecal coliforms.

Figure 7: Biplot principal components based on the interaction between the slaughter process and the microorganisms present.



From the results presented in this study, it was possible to obtain an assessment of the influence of each of the stages of the process studied, with regard to the prevalence of pathogens and the levels of microorganisms that indicate hygiene. According to Sofos and Geornaras (2010), the presence of microorganisms may be due to contamination of animals coming from the field, equipment, handlers or may be related to the processing environment.

4 CONCLUSION

The contamination index found during the pig slaughter meets the microbiological limits in force.

A high proportion of microorganisms occurred in bleeding in relation to groups of microorganisms, being aerobic mesophiles, *E. coli*, total coliforms and thermotolerants.

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