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EVALUATION OF THE MICROBIOLOGICAL QUALITY OF PORK MEAT FROM THE SLAUGHTERHOUSE IN THE MUNICIPALITY OF IMPERATRIZ, MARANHÃO

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ABSTRACT

Nowadays, pork has been consumed more and more each year, so the interest in raising animals and meat production has increased. The microbiological alteration of the product, caused by the presence of undesirable microorganisms can lead to toxinfections for those who eat it. The objective of this work is to evaluate the hygienic quality and microbiology of pork in natura and was carried out in the Municipal Slaughterhouse of Imperatriz. Samples of pork samples randomly selected from the belly, rib and pallet regions of the animal carcass were randomly selected from 10 different animals. The samples were transported to the Laboratory of Microbiology and Health of the State University of the Tocantina Region of Maranhão, in iceboxes, soon after the collections, their analysiswere started. There were microbiological quality evaluation for total coliforms, and thermotolerant coliforms, *Escherichia coli* and *Salmonella spp*. Positive samples of microorganisms were found in all analyzes. In view of the results achieved, such as total and thermotolerant coliforms, it has been found that measures such as the standard operating procedure should be applied more rigidly to improve the quality of the product in question.

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INTRODUCTION

In circumstances of the growing consumption of pork in Brazil, in the 4th quarter of 2016, 10.81 million head of pigs were slaughtered, representing increases of 0.8% compared to the immediately previous quarter and 5.8% compared to thesameperiodof 2015 [IBGE, 2017]. Contaminations must be avoided in all meat production processes.The various microorganisms that alter meat come to it through the infection of the live animal (endogenous contamination) or by the *post mortem* invasion (exogenous contamination). Both are highly harmful, but changes due to exogenous contamination are the most frequent and not infrequently lead to serious illnesses for consumers. The main potential sources of contamination in slaughterhouses are: The animals' skins and furimpregnated with dirt and feces, that can carry millions of aerobic and anaerobic bacteria; air and dust; the water used to wash the carcass, equipment, utensils (knives, saws and hooks) and the various containers used; The labor used [Carvalho, 2010]. These factors can determine cross-contamination, resulting in meat with a high bacterial load and possible contamination from fecal origin, where *Escherichia coli*stands out on the national and international scene as a microorganism of importance in animal health, hygiene and public health [Franco, 2002]. *Salmonella* sp. it is among the main agents involved in human-borne foodborne toxins. Pork is one of the linkers of this bacterium, which reaches the food due to procedural errors in the slaughterhouses, excessive handling during meat processing, contact of processed meat with raw meat, anderrors in storage temperature [Turci, BegottiandMerlini, 2013]. According to the specific legislation of the National Health Surveillance Agency – ANVISA, resolution - RDC nº 216, of December 15th, 2004, regulation establishes the procedures of Good Practice for food services. These apply to food services that perform some of the following activities; handling, preparation, fractionation, storage, distribution, transport, display for sale and delivery of prepared food for consumption, in order to guarantee the hygienic and sanitary conditionsofthepreparedfood [BRASIL, 2004]. The present work aims to evaluate the slaughtered pork meat hygienic, sanitary and microbiological quality and to evaluate the results ofthechecklistthatwillbecarried out in the municipal slaughterhouse in thecityof Imperatriz – MA.

MATERIALS AND METHODS

Checklist of Physical-Structural and Hygienic-sanitary Conditions of the Slaughterhouse (*Check List*): To investigate the physical conditions of the handling environment, a checklist was carried out at the Municipal Slaughter house of Imperatriz, in order to verify that the establishment was within the standard according to RDC legislation nº 275/20027 and RDC nº 216/20048 of ANVISA [BRASIL, 2003]. We used the environment observation criteria for such verification. The checklist that was applied is divided into blocks, where they were: Block1) Buildings and Facilities; Block 2) Facilities and Equipment Sanitation; Block 3) Vector and Urban Pest Control; Block 4) Water supply; Block 5) Waste Management; Block 6) manipulators; Block 7) Food Storage and Transport; Block 8) Documentation and Registration. The establishments classification is divided into groups, which, according to their sum of conformity, are classified for any of them. The groups are: Group 1) 76% to 100% of item service; Group 2) 51% to 75% of compliance with the items and finally, Group 3) 0% to 50% of compliance with the items.

Material Collection: Collections of 10 samples of pork meat from the Municipal Slaughterhouse of Imperatriz were carried out, where these collections were divided into two stages, and then submitted to analysis. At each stage, 5 samples were collected from 5 different animals, where these samples were taken from the belly, ribs, shoulder blade and jowls of the carcass regions, chosen at random. All care was taken in relation to hygiene and food safety, so that there was no contamination of the meat after its collection. The samples were collected aseptically, stored in individual and original transparent plastic containers, then deposited in previously sanitized thermoboxes containing sanitized ice pack, and taken immediately to the Laboratory of Microbiology and Health of the State University of the Tocantina Region of Maranhão. The samples were refrigerated at -20°C until the moment of analysis [BRASIL, 2004].

Physicochemical analysis: To carry out the physicochemical analyses, the measurement of the hydrogenic potential (pH) of the collected pork meat was carried out, using a benchtop phmeter, brand AKSO, model AK88, numbering 10200630, to analyze whether it was in accordance with the legislation. The phmeter was calibrated with pH 4 and pH 7 buffer solutions. For pH analysis, the procedure was performed by weighing 10g of the sample in a beaker and diluting with the aid of 100 mL of water. The content was stirred until the particles, ifany, were uniformly suspended [BRASIL, 2005].

Microbiological analyzes - *Swab*: During material collection, samples of aseptic *swabs* were collected from the hands of slaughterhouse handlers, using the *swab-test* method to count possible contaminants. Before the visits to the Municipal Slaughterhouse to collect the *swabs*, they were previously prepared at the Laboratory of Microbiology and Health, Federal University of the Tocantina Region of Maranhão – UEMASUL. Were moistened in peptone water, deposited in test tubes and in the course of collection were passed in rotational movements in the hands of the handlers and deposited back in the sealed test tubes. The culture used was Standard Count Agar and stored in a bacteriological incubator at 35ºC for 24 hours. According to [BRASIL, 1991/1992] Expression of counting results. In calculating the counts, the final result will be expressed in UFC/g or mL, taking into account the dilution used.

Microbiological analysis - Total, thermotolerant coliforms and *Escherichia coli*: Samples were homogenized, preceded by an initial dilution of 1:10 (10^{-1}), adding to the 25g of the sample, 225mL of a suitable diluent (peptone water 0,1%). For the preparation of the second dilution (10^{-2}), will be transferred aseptically 1,0 mL of the dilution 10^{-1} to 9,0 mL of diluent, the subsequent dilutions being obtained in the same way. For the presumptive test, three appropriate sample dilutions will be selected and, with a pipette of at most 10 mL, inoculate a series of three tubes of Lauryl Sulfate Tryptose Broth (STB) by dilution, adding up 1,0 mL of the dilution per tube with 10 mL of broth. Then the STB tubes will be incubated to 35ºC for 24 hours and observe if there is growth with gas production. If yes, go to subsequent items. If not, reincubate until 48 hours and repeat the reading [Tortora, Funke and Case, 2010]. The confirmatory test for Total Coliforms was performed from each positive tube of the previous step, a well-loaded elevation of the culture will be transferred to tubes with Brillant Green Bile Broth (BG). Tubes will be incubated at 35ºC for 24-48 hours and it will be observed if there is growth with gas production. The Most Probable Number of total coliforms will be determined MPN/g or mL in an appropriate table to the inoculated dilutions. The thermotolerant Coliform test was performed from each positive tube from the previous step. A loaded elevation of the culture will be transferred to *E. coli* Broth tubes (EC). The tubes were incubated at 44,5ºC (most foodfor 24 hours and it will be observed if there is growth with gas production, confirmation of thermotolerant coliforms. The Most Probable Number of total coliforms will be determined MPN /g or mL in an appropriate table to the inoculated dilutions [Tortora, Funke and Case, 2010]. The *E. coli* Count test was done from the previous step. An elevation of each culture obtained in each EC tube with gas production was taken and streaked on Eosin Methylene Blue Agar plates (EMB). Plates will be incubated at 35ºC/24 hours and will be observed if there is development of *E. coli* typical colonies (nuclei with black center, with or without metallic shine) [Pelczar, Chan and Krieg, 1996].

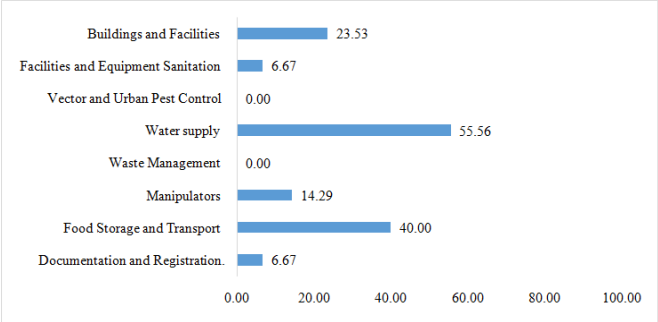
Microbiological analysis - *Salmonella spp*: Initially, the Pre-enrichment step was carried out, which aims to recover injured cells, achieved by incubating the sample under non-selective conditions for at least 18 hours. It was used buffered peptone water, recommended by *International Commission on Microbiological Specifications for Food* (ICMSF), *International Organization for Standardization* (ISO) and Brazilian Association of Technical Standards (ABNT). This step consisted of removing 25g or 25mL of the analytical unit and transferring it to a homogenization bottle, previously sterilized. Then 225mL of pre-enrichment broth was added and the sample homogenized. The vials were incubated at 35ºC/18-24 hours, with the lids slightly loosened. The selective enrichment step aimed to inhibit the multiplication of the accompanying microbiota and promote preferential increase in the number of cells of *Salmonella* sp., incubating the pre-enriched sample in selective broth, for 18 to 24 hours. The use of two ways of different enrichment is recommended, as the resistance of Salmonella to selective agents varies from strain to strain. In this step, the bottle with pre-enrichment broth was gently shaken and transferred 1,0 mL to 10 mL of tetrathionate broth (TT) and 1,0 mL to 10 mL of Selenite Cystine Broth (SC). Tubes were incubated at 35ºC for 24 hours [Pelczar, Chan and Krieg, 1996]. The differential plating step aimed to promote the preferential development of Salmonella colonies, with typical characteristics that differentiate them from competitors, for further serological and biochemical confirmation. In this step, the selective enrichment tubes were shaken in a "vortex" mixer and and striated with an elevation of TT broth on Hektoen's Enteric Agar plate (HE), Bismuth Sulfite Agar (BS) and Xylose Lysine Deoxycholate Agar (XLD). This procedure was repeated with SC or RV broth. Plates were incubated upside down to 35ºC for 24 hours and verified if there was development of typical colonies of *Salmonella* [Tortora, Funke e Case, 2010].

Tabulation of data: All analyzes were performed in triplicate. The results tabulated in Microsoft Excel spreadsheets, with mean and standard deviation, calculated according to the BRAZIL method (1991/1992) presented in UFC/ml.

RESULTS AND DISCUSSION

Checklist of Physical-Structural and Hygienic-sanitary Conditions of the Slaughterhouse (*Check list*): For the evaluation of the physical-structural and hygienic-sanitary conditions of the Municipal Slaughterhouse of Imperatriz, a checklist was used based on the RDC nº 275/20027 and RDC nº 216/20048 of the ANVISA. Overall, out of 100%, the establishment obtained only 19% compliance, falling into group 3. According to the results obtained in the evaluation, the water supply block had the best compliance index

(55,56%), however, the waste management and vector and urban pest control block had the worst compliance rate (0.00%) (Graphic 1).



Graphic 1. Blocks Conformity index (%) evaluated on the Physical-Structural and Hygienic-sanitary conditions of the Municipal Slaughterhouse of Imperatriz-Ma

Microbiological Analysis – *Swabs*: Counting of aerobic and mesophilic microorganisms in plates (inoculation). The results of the microbiological analysis of the *swabs* from the first collection were all countless. In the second collection performed, the results ranged from 0.45x10² to 2.85x10², as shown in Table 1.

Table 1. Plate counting by the *swab* method of surfaces in the swine wing of the municipal slaughterhouse in Imperatriz – Ma

Samples	Counting	
	1° Collect UFC/cm²	2° Collect UFC/cm²
1	Inc	2,8 x 10^2
2	Inc	0,45 x 10^2
3	Inc	0,8 x 10^2
4	Inc	1,22 x 10^2
5	Inc	2,85 x 10^2

Inc = countless.

In the work of Malagueta Junior, Silva and Souza (2012), who carried out the hygienic-sanitary assessment of the hands of handlers, equipment and utensils in the meat market in the city of Limoeiro do Norte, Ceará, observed in the count 5.2x10³ UFC/cm² of aerobic mesophilic bacteria, indicating a poor sanitary hygienic quality for food preparation. Comparing with the results of these authors, all samples from the 2nd stage had a lower result than what they found in their research. Only the results of the first stage indicate poor sanitary hygienic quality, where all were countless. Another work that performed swab analysis using the Colony Forming Unit (UFC) method was the work of Rubin et. al. (2012), where he evaluated the microbiology of the hands, utensils, and surface of food handlers in food bank entities in the city of Cruz Alta. In their results, only 1 of the 7 entities evaluated obtained a positive result for the handlers' hands, with 520 UFC/ml. In the results obtained at the Municipal Pork Slaughterhouse of Imperatriz, all 10 samples collected from the handlers' hands were positive, where all 5 samples from the 1st stage were countless, however, the 5 samples from the 2nd stage did not have values that exceeded 2,85 x 10^2.

pH analysis: The results of the pH analysis of the five pork samples collected at the Municipal slaughterhouse of Imperatriz – MA, ranged from 6,08 to 6,50 (Table 2).

Table 2. Determination of the pork meat pH from the municipal slaughterhouse of Imperatriz – Ma

Samples	Ph
01	6,50
02	6,37
03	6,39
04	6,47
05	6,08

According to Santos (2008), the pH 6.0 has been conceptualized as the dividing line between the normal cut and the "dark-cutting", but some authors also use values from 6,2 to 6,3. The high pH makes the meat more prosperous for the development of microorganisms, therefore, with a shorter shelf life. The origin of these meats is inappropriate *ante-mortem* handling, for example, prolonged transport. In comparison with Santos (2008) and the other authors that he cites, meat pork from the municipal slaughterhouse of Imperatriz, 3 samples were within the average with a pH of up to 6.39 and only two samples (pH 6.47 and 6.50) were above the normal cut and fit the "dark-cutting", also known as DFD meat. According to Medina (2009), pH faz a fundamental importance in the process of transforming muscle into meat. When a muscle's pH does not decline to a level below 6.0, it becomes a perfect environment for the growth and accumulation of spores and microorganisms, reducing its shelf life. Comparing the results obtained with the author above, 100% of the samples were in a favorable situation for the accumulation of spores and microorganisms. No samples with pH less than 6.0. For Caldara et. al. (2012) the accelerated pH drop in the *post mortem* period directly influences the water retention capacity of meat, compromising on other characteristics, such as color. According to Moura et. al. (2014), during the process of converting muscle into meatthe pH gently reduces in the following hours after slaughter, then stabilizing around 6,0. The results obtained compared to Moura et. al. (2014), 1 sample was properly stabilized (with 6,08 pH) and the other samples with similar values (6,37 to 6,50 of pH), with a pH around 6.0.

Total, thermotolerant coliforms, E. coli (NMP/g) and *Salmonella* 25g: In the first collection, the results of *E. coli* showed 100% absence of the microorganism in all samples. However, in the second collection, 20% of the samples were positive for *E. col* and 80% negative for the presence of the bacteria. For *Salmonella spp*, the results in the first collection were present for 40% of samples and absence for 60% of samples. In the second collection performed, 100% of the samples showed the presence of *Salmonella spp* (Table 3).

Table 3. Total, thermotolerant coliforms, *E. coli* (NMP/g) and *Salmonella* (25g) of the pork samples from the municipal slaughter house of Imperatriz – Ma

Samples	Coliforms 35°C (NMP/g)	Coliforms 45°C (NMP/g)	*E. coli* (NMP/g)	*Salmonella* (25g)
1	400	300	Absence	Presence
2	300	300	Absence	Presence
3	400	300	Absence	Absence
4	300	300	Absence	Absence
5	300	300	Absence	Absence
6	400	300	Absence	Presence
7	300	300	Absence	Presence
8	400	400	400	Presence
9	300	300	Absence	Presence
10	400	300	Absence	Presence

Resolution RDC nº 12 of 2001 [BRASIL, 2001] constitutes as a parameter of microbiological quality of "in natura" meat the total absence of *Salmonella spp* in 25g of sample. According to the aforementioned resolution, only 30% of the total samples met the parameters of total absence of *Salmonella spp* in 25g of sample. In the slaughter process, skinning and evisceration are considered critical control points that can cause a greater risk of contamination of the carcasses. For Dainty and Mackey, (1992), after skinning, the surface of the carcass may contain a microbiota of up to 10^4 UFC/cm². Magnani et. al. (2000) conducted a study on the incidence of *Salmonella* and *Escherichia coli* in fresh pork and colonial salami, consumed by the population of Chapecó - SC. A total of 50 meat samples were analyzed. The results of this study revealed the presence of *Salmonella* in 6,0% of fresh pork samples, 82% percent of the pork samples were contaminated by *E. coli*, where, 44% were above the maximum number allowed for human consumption. Compared to the results of these authors, only 10% of *E. coli* (1

sample) were contaminated by and for *Salmonella spp* 70% (7 samples) were contaminated. Another work that performed the analysis using the Most Probable Number (MPN) method was the work of Ferreira and Simm (2012) which analyzed ground beef from a butcher shop in the central region of the municipality of Pará de Minas in the State of Minas Gerais, analyzed 6 samples, where 16,67% showed the presence of *Salmonella spp.* A particularly very good rate when compared to the 70% positive samples (7 out of 10 samples) collected from the municipal slaughterhouse of Imperatriz-MA. In the work of Hangi et. al. (2015), the microbiology of ground beef sold in the city of Anápolis, Góias was analyzed, where the total coliform population was estimated, coliforms at 45ºC using the MPN (Most Probable Number) method. Of the 24 samples of ground beef, of 150 grams each, 100% of the samples collected were positive for total coliforms, where 37,5% had amazing values, above 103 NMP/g. From the meat obtained at the slaughterhouse, from the 10 samples of fresh pork, 5 samples varied around 400 NMP/g for coliforms at 35ºC and 5 samples varied around 300 NMP/g. For coliforms at 45ºC, only one sample reached 400 NMP/g and the remaining 9 ranged between 300 NMP/g. In the work of Soares, Sousa and Silva (2016), total and thermotolerant coliforms in refrigerated beef steaks treated with lactic acid and sodium lactate were evaluated, using the determination of the NMP, analyzed on days one, three, six and nine of the study. The data of interest for this work was the evaluation of untreated meat from day 0 (zero) in natura, which presented a frequency of 100% of total coliforms in the evaluated samples. Compared with the results obtained, samples from the municipal slaughterhouse had a similar result, where all samples had the presence of coliforms, with values ranging from around 300 to 400 NMP/g. In the research by Costa e Tanamati (2018), which evaluated the sanitary and physical-chemical hygiene of fresh ground beef sold in Campo Mourão - PR, the total coliforms and thermotolerants present in the meat ranged from 28 NMP/g to 251,50 NMP/ g, considering a very low amount. In comparison with fresh pork from the slaughterhouse, this value is close to the values obtained, which ranged from 300 and 400 NMP/g.

In the work of Oliveira and Salvador (2011), who determined the microbiological contamination of chicken meat sold in the city of Apucarana and Califórnia - PR, analyzed 10 samples, where the results for total coliforms ranged from 23 to 240 NMP/g, whereas for thermotolerant coliforms they ranged between 7,4 and 43 NMP/g, and the authors concluded that the values found were satisfactory. Compared to fresh pork that ranged from 300 to 400 NMP/g, the values were not so different in relation to total coliforms and can be considered satisfactory, however, the results of thermotolerant coliforms have already diverged a little from the results obtained by these authors. Based on the results found in all analyzes performed, out of the 10 fresh pork samples, 100% were contaminated by fecal coliforms, 100% contaminated by thermotolerant coliforms, however, many of them in satisfactory quantities when compared with the results of other authors, 10% by *Escherichia coli* and 70% by *Salmonella spp*. For *Salmonella spp*. in which the absence or presence is considered, 70% of the samples were positive for the presence of the same, which is a worrying situation. Another worrying result was the *swabs* from the hands of meat handlers, which showed the presence of microorganisms that could be the cause of contamination of the carcasses, in addition to other factors in buildings and facilities.

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