Avaliação microbiológica de *Escherichia coli* em cortes de carne bovina (moída) comercializada nos Municípios de Valença e Vassouras, RJ

Microbiological evaluation of *Escherichia coli* in cuts of beef (ground) sold in the Municipalities of Valença and Vassouras, RJ

Evaluación microbiológica de *Escherichia coli* en cortes de carne (molida) vendidos en los Municipios de Valença y Vassouras, RJ

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Resumo

A importância da carne bovina (moída) na alimentação humana e a preocupação de oferecer um alimento inócuo e incapaz de transmitir doenças induziram ao desenvolvimento deste trabalho, que teve por objetivo avaliar a qualidade microbiológica, o perfil de sensibilidade antimicrobiano e as condições higiênico-sanitárias em amostras de carne bovina (moída), comercializadas nos Municípios de Valença e Vassouras, RJ. Foram realizadas amostragens em 20 estabelecimentos comerciais, que oferecem carne moída em condições de consumo e analisados segundo a técnica de quantificação de coliformes em NMP/g. O perfil antimicrobiano dos isolados de *Escherichia coli* foi submetido aos testes de suscetibilidade à tetraciclina (30μg), ampicilina (10μg), cefalotina (30μg), gentamicina (10μg) e cefotaxima (30μg), através do método de disco-difusão. Já, a avaliação das condições higiênico-sanitárias, deu-se a partir de critérios de observações pré-estabelecidos em questionário modelo. Os índices de coliformes totais foram superiores a 240 NMP/g em 90% das amostras analisadas e 55% das amostras apresentaram índices superiores a 240 NMP/g para coliformes termotolerantes, sendo que o Município de Valença revelou 40% de contaminação dentro do universo amostral. A avaliação do perfil antimicrobiano para o antibiótico cefotaxima revelou

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maior espectro de atuação para as estirpes de *Escherichia coli* isoladas das amostras, enquanto o maior índice de resistência foi observado para o antibiótico cefalotina. A avaliação das condições higiênico-sanitárias foi considerada insatisfatória em relação à manipulação da carne, o que torna esse tipo de alimento um potencial causador de toxinfecções alimentares.

Palavras-chave: Carne bovina; Coliformes totais e termotolerantes; *Escherichia coli;* Intoxicações alimentares; Perfil antimicrobiano.

Abstract

The importance of beef (ground) in human food and the concern to offer an innocuous food that is incapable of transmitting diseases led to the development of this study, which aimed to evaluate the microbiological quality, the antimicrobial sensitivity profile and the hygienic-sanitary conditions in samples of beef (ground), sold in the Municipalities of Valença and Vassouras, RJ. Samples were carried out in 20 commercial establishments, which offer ground meat under conditions of consumption and analyzed according to the coliform quantification technique in MPN/g. The antimicrobial profile of *Escherichia coli* isolates was submitted to susceptibility tests to tetracycline (30μg), ampicillin (10μg), cephalothin (30μg), gentamicin (10μg) and cefotaxime (30μg), using the disk-diffusion method. The assessment of hygienic-sanitary conditions, on the other hand, was based on pre-established observation criteria in a model questionnaire. The total coliform indices were greater than 240 MPN/g in 90% of the analyzed samples and 55% of the samples had indices greater than 240 MPN/g for thermotolerant coliforms, and the Municipality of Valença revealed 40% of contamination within the sample universe. The evaluation of the antimicrobial profile for the antibiotic cefotaxime showed a greater spectrum of action for strains of *Escherichia coli* isolated from the samples, while the highest index of resistance was observed for the antibiotic cephalothin. The evaluation of hygienic-sanitary conditions was considered unsatisfactory in relation to the handling of meat, which makes this type of food a potential cause of food poisoning.

Keywords: Antimicrobial profile; Beef; *Escherichia coli*; Food poisoning; Total and thermotolerant coliforms.

Resumen

La importancia de la carne de res (molida) en la alimentación humana y la preocupación por ofrecer un alimento inocuo que no sea capaz de transmitir enfermedades condujo al desarrollo de este trabajo, cuyo objetivo era evaluar la calidad microbiológica, el perfil de sensibilidad antimicrobiana y las condiciones higiénico-sanitarias en muestras de carne de res

(molida), vendida en los municipios de Valença y Vassouras, RJ. Las muestras se llevaron a cabo en 20 establecimientos comerciales, que ofrecen carne molida en condiciones de consumo y se analizaron de acuerdo con la técnica de cuantificación de coliformes en NMP/g. El perfil antimicrobiano de los aislados de *Escherichia coli* se sometió a pruebas de susceptibilidad a tetraciclina (30 μg), ampicilina (10 μg), cefalotina (30 μg), gentamicina (10 μg) y cefotaxima (30 μg), utilizando el método de difusión en disco. La evaluación de las condiciones higiénico-sanitarias, por otro lado, se basó en criterios de observación preestablecidos en un cuestionario modelo. Los índices de coliformes totales fueron superiores a 240 NMP/g en el 90% de las muestras analizadas y el 55% de las muestras tenían índices superiores a 240 NMP/g para coliformes termotolerantes, y el Municipio de Valença reveló el 40% de la contaminación dentro del universo de la muestra. . La evaluación del perfil antimicrobiano para el antibiótico Cefotaxima reveló un mayor espectro de acción para las cepas de *Echerichia coli* aisladas de las muestras, mientras que se observó el mayor índice de resistencia para el antibiótico cefalotina. La evaluación de las condiciones higiénico-sanitarias se consideró insatisfactoria en relación con el manejo de la carne, lo que hace que este tipo de alimentos sea una posible causa de intoxicación alimentaria.

Palabras clave: Carne de res; Coliformes totales y termotolerantes; *Escherichia coli*; intoxicación alimentaria; Perfil antimicrobiano.

1. Introduction

The world meat market is of great economic importance in several countries, especially in Brazil. In 2018, beef cattle in the country generated around 597.22 billion in gross domestic product (GDP). Brazil has a cattle herd of 214.7 million head in an area of 162.19 million hectares, slaughtering 44.23 million head per year (ABIEC, 2019).

The quality of meat intended for consumption is a matter of constant concern worldwide, according Fehrenbach (2017) beef is a food of great importance in the human diet, due to its nutritional composition, so there was the need to qualify livestock farming throughout the country and for that it was necessary to evaluate the production efficiency as an important factor to generate improvement in productivity (ABIEC, 2019).

The ground beef stands out among meat products, for its good commercial acceptance and for being characterized as a popular and accessible product, being used in meals in a practical and variable way. However, ground beef is generally from pieces of other meats, making it a potential source of contamination for people who consume it, because its quality

can be compromised due to the fact that it undergoes fractionation processes that, if carried out without proper hygiene conditions, they become a favorable means for the proliferation of microorganisms (Costa & Tanamati, 2018).

This contamination can happen throughout the processing chain, from the moment the animal is slaughtered for sale in commercial establishments. In addition to hygienic deficiencies during slaughter and handling, the greater contact surface of ground meat favors the growth of aerobic bacteria, which often cause deterioration at low temperatures (Lopes et al., 2017).

The microbiological analyzes aim to diagnose an etiological agent that causes an outbreak of food origin, as well as to verify the microbiological quality of the raw product and the final product, contaminating microorganisms and to determine corrective measures and Critical Control Points (CCP) (ABERC, 2015) .

In Brazil, microbiological standards are followed according to the importing market, and for domestic trade the standards are established by ANVISA, in accordance with RDC nº 12 of January 2, 2001 (BRASIL, 2001).

With the increase in the world population, the need to produce more food has increased considerably, and with that there was a need to serve a more critical consumer with higher levels of demand in relation to the quality of the food consumed.

Considering the social and economic relevance of the consumption of ground meat from meat cuts, its importance on the Brazilian diet, as well as the maintenance of its quality until reaching the consumer, it is necessary to understand factors (intrinsic and extrinsic) that influence the quality characteristics of the meat. Within this context, the objective of the present study was to evaluate the microbiological quality, the antimicrobial sensitivity profile and the hygienic-sanitary conditions in samples of ground beef, marketed in the Municipalities of Valença and Vassouras, RJ.

2. Material and Methods

The research was based on laboratory analysis (quantitative-qualitative) according to the methodology proposed byPereira, Shitsuka, Parreira, & Shitsuka (2018).

Samples were carried out in 20 commercial establishments (10 in Valença/RJ and 10 in Vassouras/RJ) that offer ground beef under consumption conditions, including butchers and supermarkets, from october 2011 to april 2012. In each of the 20 commercial points were acquired, 400 g of fresh beef. The samples were previously identified and packed in

isothermal boxes, and transported to the Microbiology Laboratory at the Vassouras University.

In the laboratory, 25g of the beef sample (ground) was weighed, added to 225 mL of 0,1% peptone solution in the flask, and subsequently homogenized to obtain the 10^{-1} dilution. Then, a 1 mL aliquot was removed from this dilution, transferring it to a tube containing 9 mL of 0,1% peptone saline solution, thus obtaining the other decimal dilutions.

The decimal dilutions were sown in three series of three test tubes each containing 9 ml of selective culture media, and incubated in a bacteriological incubator at 35°C - 37°C for 24-48 hours. The reading of the inoculations made in the tubes allowed to detect the presence of total and thermotolerant coliforms present in the sample.

2.1. Enumeration of total, thermotolerant coliforms and *Escherichia coli*

Total and thermotolerant coliforms were listed according to the Most Probable Number method (MPN) (Vanderzant&Splittstoesser, 1992), using Broth LaurilTriptose (presumptive assay), Brilliant Green Broth at 2% Bile (confirmatory assay) and *Escherichia coli* broth (EC) (thermotolerant coliforms).

The samples were sown with the aid of sterile 1 mL pipettes in 3 series of 3 test tubes with ground-glass stoppers, containing 9 mL of the Lauryl Sulfate Tryptose Broth (LST) for analysis of total coliforms (presumptive assay), which were incubated in bacteriological oven at 35 ºC/24-48 hours. After that period, from the positive tubes (turbid and with gas production inside the Durham tubes), in the presumptive test, a scope was transferred to tubes containing Brilliant Green Broth (BG), confirmatory test. The incubation of tubes with BG broth was performed at 35ºC for 24/48 hours, with tubes that showed bacterial development and gas production being considered positive.

From the tubes considered positive, an aliquot with a platinum handle was removed and transferred to tubes containing 9 mL of broth *Escherichia coli* (EC) with Durham tubes. The samples were incubated in a water bath for 24-48h at a controlled temperature and regulated at 45,5 ºC, since these microorganisms are thermotolerant. Pipes that presented turbidity and gas production were considered positive.

From the positive tubes of broth EC, *E. coli* was identified by culturing on Eosyn Methylen Blue agar (EMB). From each plate with characteristic *E. coli* growth, two colonies were selected to perform the biochemical tests composed of Simmons Citrate Agar,Indol Motility Sulfide agar (IMS) and Methyl Red Broth (MR) and VogesProskauer (VP). The

typical strains of *E.coli* present as positive results the tests for MR, Indole and Motility, with negative results for citrate, VP and H_2S production.

2.2. Antimicrobial susceptibility testing

Escherichia coli species isolated from beef samples (ground) were suspended in saline solutions and diluted in the concentration of the 0.5 far tube on the McFarland scale equivalent to 1.5×10^6 cells/mL.

The disks impregnated with antibiotics were placed in petri dishes containing Mueller-Hinton agar, previously inoculated with samples. The isolated strains characterizing microbiologically and biochemically as belonging to the *E. coli* species were subjected to the susceptibility test to Tetracycline (30μg), Ampicillin (10μg), Cephalothin (30μg), Gentamicin (10μg) and Cefotaxime (30μg), using the method of disk-diffusion and then incubated for 24 hours in the bacteriological oven at 35 °C.

After incubation, readings and respective measurements of the diameter of the inhibition zone were made in mm (Table 1), and evaluated as resistant, intermediate or sensitivemicroorganisms for each antibiotic tested, according to the disc-diffusion methodology established by the Clinical and Laboratory Standard Institute (CLSI, 2011).

Table 1 - Values of inhibition halos in diameter zones measured in mm, as established by CLSI (2011).

Antibiotics	Sensitive	Intermediate	Resistant
Ampicillin 10 μg	≥ 17	14 – 16	≤ 13
Cephalothin 30 μg	≥ 18	15 – 17	≤ 14
Gentamicin10 μg	≥ 15	13 – 14	≤ 12
Cefotaxime30 μg	≥ 26	23 – 25	≤ 22

Source: CLSI, 2001.

2.3. Assessment of hygienic-sanitary conditions

For the evaluation of hygienic-sanitary conditions, the criterion for making a model questionnaire (Chart 1) was filled out based on observations made by the authors,regarding the hygienic-sanitary conditions of establishments, equipment, utensils and handlers, as well as the storage of products exposed for sale. Based on the observations made, it was possible to

establish evaluation parameters according to the average hygienic-sanitary conditions in each municipality, based on the following criteria: (A) Excellent 100% to 90%, (B) Good 89% to 70 %, (C) Regular 69% to 50%, (D) Bad 49% to 30% and (E) Poorly less than 30%.

Chart 1 - Model questionnaire used to investigate the hygienic-sanitary conditions of beef (ground) sold in butchers and supermarkets in Valença and Vassouras, State of Rio de Janeiro (Adapted from Fortuna & Fortuna, 2008).

<table>
<tr><td colspan="4">Hygienic-sanitary observation of beef handlers from the city of Valença and Vassouras / RJ.

Meat .... Sample: .... Location: ....
Opening hours: from .....to .....hours.

Date of collection: ____ / _____ / 2012. Collection time: ____: _____ hours.</td></tr>
<tr><td colspan="2">Hygienic-sanitary observation of handlers</td><td>Yes</td><td>No</td></tr>
<tr><td>01</td><td>Do you wear jewelry or adornment on your hands?</td><td></td><td></td></tr>
<tr><td>02</td><td>Clean and trimmed nails?</td><td></td><td></td></tr>
<tr><td>03</td><td>Hair protected by a cap, cap or net?</td><td></td><td></td></tr>
<tr><td>04</td><td>Do you use meat handling gloves?</td><td></td><td></td></tr>
<tr><td>05</td><td>Who manipulates meat, manipulates money too?</td><td></td><td></td></tr>
<tr><td>06</td><td>Is the machine cleaned to serve other customers?</td><td></td><td></td></tr>
<tr><td>07</td><td>Do you clean the meat preparation counter for each customer?</td><td></td><td></td></tr>
<tr><td>08</td><td>Is the balance cleaned before weighing?</td><td></td><td></td></tr>
<tr><td>09</td><td>AreIndividual Protection Equipment (IPE's) such as boots, lab coats, gloves clean?</td><td></td><td></td></tr>
<tr><td>10</td><td>Are the meats conditioned in a clean refrigerator?</td><td></td><td></td></tr>
</table>

Source: Authors

## 3. Results and discussion

3.1. Enumeration of total, thermotolerant coliforms and *Escherichia coli*

Of the 20 commercial establishments investigated, all showed high levels of contamination by total and thermotolerant coliforms. In relation to total coliforms, all samples (100%) from the

municipality of Valença presented values above 240 MPN/g. Similar results were also found in the municipality of Vassouras, when they revealed 80% of the sample universe with values above 240MPN/g, and 20% with levels below 240MPN/g (figure 1A).

The analysis of the thermotolerant coliforms showed that the municipality of Valença presented values above 240 MPN/g in 8 (80%) of the analyzed samples, with only 2 samples (20%) with values below 240MPN/g, whereas the municipality of Vassouras presented levels above 240 MPN/g in 30% of the samples, 70% of which revealed levels below 240 MPN/g (Figure 1B).

Figure 1 - Enumeration of total coliforms (A) and thermotolerants (B) using the multiple tube (MPN/g) method of beef samples (E1, E2, E3, E4, E5, E6, E7, E8, E9 and E10) sold in the municipalities of Valença and Vassouras, State of Rio de Janeiro.

A   B
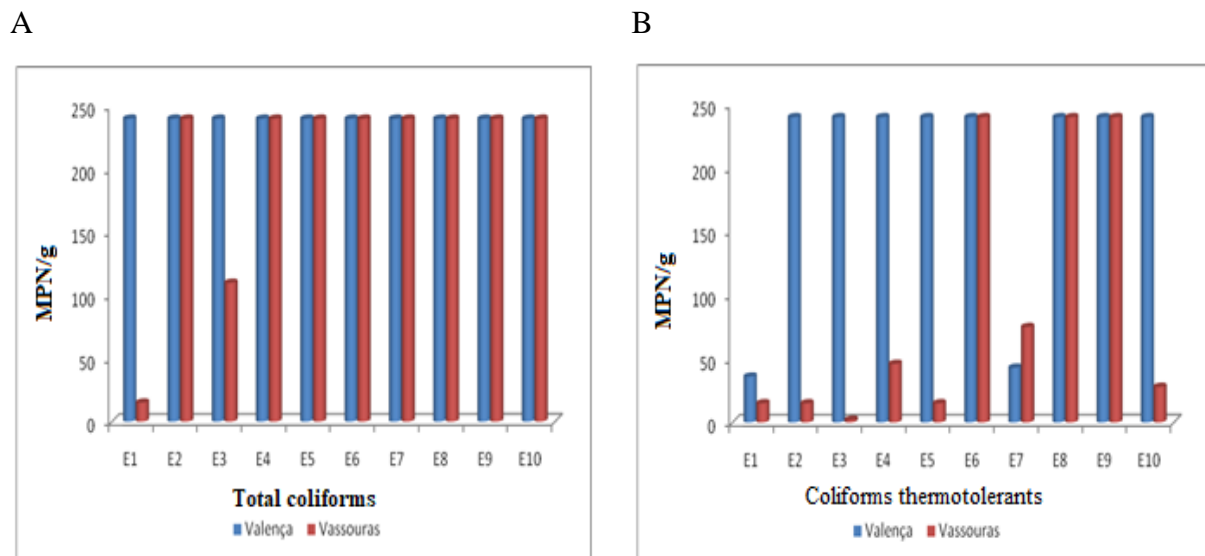
Source: Authors

According to the limit established by the ANVISA Brasil resolution (2001) on microbiological standards for food, it stipulates that raw meat has maximum tolerance for indicative and representative samples of 104 thermotolerant coliforms at 45 ° C / g, the values of thermotolerant coliforms in both cities are above what was established by the resolution.

The same results were also observed in the study by Silva et al. (2004) who analyzed 20 samples of ground beef in the city of João Pessoa/PB, where 18 (90%) of the samples had a level of contamination by total coliforms greater than 240 MPN/g and all samples showed contamination by superior thermotolerant coliforms at 240 MPN/g. Corroborating with our study, Carneiro& Santos (2010) revealed that of the 20 samples of ground beef from

commercial establishments, butcher shops in the city of Brasília/ DF, the values found for total coliforms were greater than 240 NMP/g in all samples analyzed, for thermotolerant coliforms, the values found were higher than 240NMP/g in 55% of the analyzed samples, with only 4 samples with low levels of thermotolerant coliforms.

3.2. Antimicrobial susceptibility testing

Regarding the antimicrobial profile tests, we found that of the 20 strains of *E.coli* isolated and tested in both municipalities, 11 (55%) were sensitive to Tetracycline, 12 (60%) sensitive to Ampicillin, 8 (40%) sensitive to Cephalothin, 15 (75%) sensitive to Gentamicin and 16 (80%) sensitive to Cefotaxime. According to the results found, the antibiotic Cefotaxime would be the antibiotic that presented a greater spectrum of action in relation to strains of *E.coli* isolated in the investigated municipalities.

E. coli strains from the municipality of Valença, when compared to the samples from the municipality of Vassouras, showed greater resistance to the tested antibiotics, thus representing a greater risk to Public Health. Antibiotics, Tetracycline and Cephalothin, were those that showed the highest resistance index in the municipality of Vassouras (Figure 2A, Figure 2B).

Figure 2 - Antimicrobial sensitivity profile of *E.coli* strains isolated from samples of beef (ground) from commercial establishments in the municipalities of Valença and Vassouras, State of Rio de Janeiro. Lengend: S=sensible, R=resistant, I=Intermediate.

A
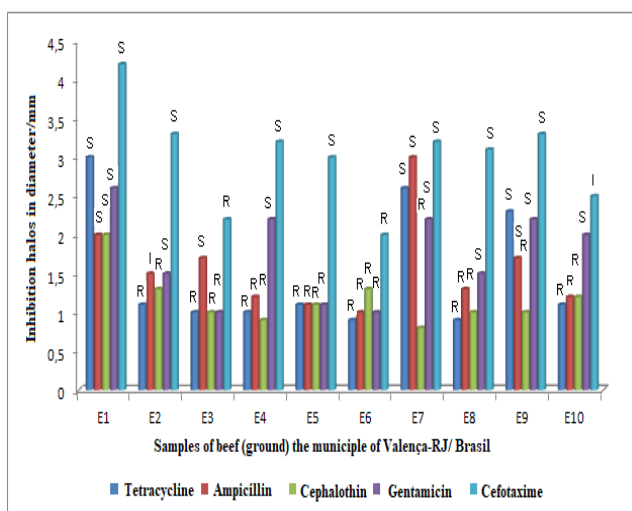
B
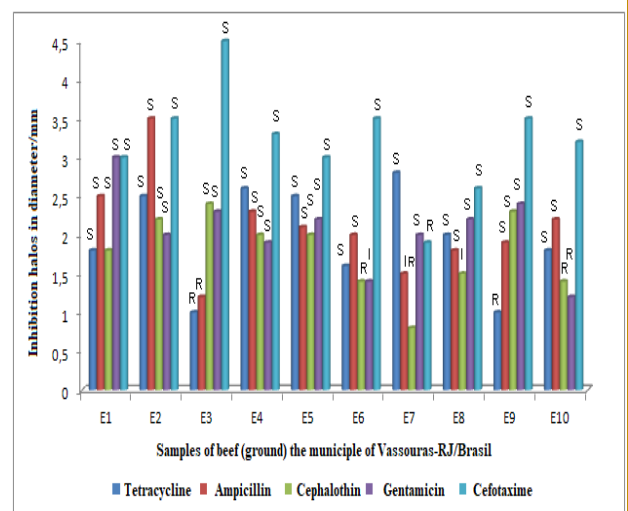
Source: Authors

The results found for the antimicrobial resistance profile are in agreement with the work of Franco (2002), who revealed that the majority of *E. coli* pathogenic strains or not showed a large spectrum of resistance to the tested antimicrobials.

According to Kasnowski (2004), the literature warns that the most isolated *E.coli* strains are resistant to most antimicrobials. The increase in these resistance indices can be attributed to the selection imposed by the indiscriminate use of antimicrobials in the therapy of humans and animals, associated with the transfer of genes responsible for antimicrobial resistance.

The isolation of *E.coli* (thermotolerant coliform) in beef samples (ground) highlights the importance of heat treatment given to the food, since this microorganism can cause mild diarrhea or in more severe cases, hemorrhagic colitis increasing the risk of death mainly in children. It is always important to emphasize the need to investigate the antimicrobial profile, as we can see that in the present study *E.coli* isolates have high levels of resistance to the tested antibiotics, which makes it difficult both to treat and recover patients affected by toxinfections caused by these microorganisms. The probability of the appearance of multidrug-resistant pathogenic microorganisms may be due to the indiscriminate use of antibiotics in the treatment of humans and animals (Queiroz, 2017).

3.3 Assessment of hygienic-sanitary conditions

In general, the occurrence of total coliforms in food indicates poor hygienic conditions and that of thermotolerant coliforms in food is considered as an indicator of thermotolerant contamination and the possibility of the presence of pathogenic bacteria that have their habitat in the intestinal tract Florentino & Leite(1997), Costa, Alves & Monte (2000).

From the observations made and with the aid of the model questionnaire we can observe that the results of the hygienic-sanitary conditions of the commercial establishments in the municipality of Valença, reveal that 60% of the establishments did not keep the conditioned meat in a clean refrigerator and all establishments did not performed cleaning of the grinding machine, benches and scales to serve other customers.

Regarding the manipulators of establishments in Valença-RJ, it was observed that 80% wore jewelry or adornments on their hands, 70% did not have clean and trimmed nails, none had hair protected by burrows, caps or hammocks, as well as not wore gloves to handle meat, 50% handled meat and money and 60% did not have clean IPE´s.

According to the pre-established parameters for the evaluation of hygienic-sanitary conditions, the municipality of Valença obtained the Bad rating for meeting only 36% of satisfactory results from the criteria of the model questionnaire.

Regarding handlers in the municipality of Vassouras, it was observed that 100% wore jewelry or ornaments on their hands, 30% did not have clean and trimmed nails, 40% did not have hair protected by burrows, caps or hammock, 80% did not wore gloves to handle meat, 60% handle meat and money and 90% did not have clean IPE´s.

Regarding the parameters found for the establishment in the municipality of Vassouras, we can see that 80% did not keep the conditioned meat in a clean refrigerator and all did not clean the machine, benches and scale to serve other customers.

In accordance with the pre-established parameters for the evaluation of hygienic-sanitary conditions, the municipality of Vassouras obtained the Bad rating for meeting only 40% of satisfactory results from the criteria of the model questionnaire.

Regarding the evaluation of hygienic-sanitary conditions, both municipalities investigated presented unsatisfactory conditions for the cleaning of commercial establishments. The same results were described in the research carried out by Loguercio, Silva & Aleixo (2002) to evaluate the hygienic-sanitary conditions of beef (ground) sold in butchers in Pelotas / RS.

The work of Campagnol et al. (2009), Loguercio, Silva & Aleixo (2002), Silva, Souza & Souza (2004)corroborate with the results of the present research, and make an association of the high rates of microbiological contamination in beef (ground) with the poor hygienic-sanitary conditions.

According to Oliveira, Brasil & Taddei (2008), the hygienic-sanitary conditions of meat grinding machines, handlers and microbiological quality of ground meat, evaluated in 5 establishments in the municipality of Lavras/MG, indicated inadequate hygiene of meat grinding machines, which would be responsible for significant increase in the number of spoilage microorganisms and pathogens in most meat samples after grinding and handling, and they are often unfit for consumption.

According to Pigatto & Barros (2003) the quality of the product sold comes from the industry and people involved in meat processing, requiring a more rigorous inspection of products of animal origin. In this way, beef (ground) can be considered as a potential source of pathogenic microorganisms, acting as a trigger for foodborne diseases Campagnol et al. (2009).

4. Final Considerations

The high count of total coliforms signals inadequacy regarding the processes of handling, cleaning or breaking the cold chain and the possible encounter of pathogens. Research in the municipalities of Valença and Vassouras, both for the total coliform contamination index and for the thermotolerant coliform index, indicate a deficiency in the quality of (ground) beef sold in butchers and supermarkets. Thus, this high consumption product, characterized by its practicality of preparation and use through various forms of preparation, can act as a trigger for toxinfections resulting from the action of pathogenic microorganisms.

Such findings confirm the relationship between good sanitary hygiene practices and the quality of food offered to the consumer, highlighting the urgent need for action by surveillance agencies in the quality of the supply of food products.

References

ABERC (2015). Manual Aberc de práticas de elaboração e serviço de refeições paracoletividades. São Paulo, p. 136.

ABIEC (2019) Associação Brasileira das Indústrias Exportadoras de Carne, Exportação Brasileira de Carne Bovina. http://www.abiec.com.br/. Acesso em 01 de maio de 2020

BRASIL (2001). ANVISA - Agência Nacional de Vigilância Sanitária. Resolução - RDC n° 12. Aprova o Regulamento Técnico sobre padrões microbiológicos para alimentos. Diário Oficial da União, Brasília.

Campagnol, P. C. B., Padilha, A. D. G., Santos, B. A., Fries, L. L. M., & Terra, N. N.(2009). Qualidade higiênico-sanitária da carne bovina moída comercializada na cidade de Santa Maria, RS. *Higiene Alimentar* 23: 170 -171.

Carneiro, L. A., & Santos, P. F. B. (2010). Avaliação microbiológica de carne moída comercializada em açougues de Brasília/DF. *Ciências da Saúde*, Brasília 8(1), 33-43.

CLSI (2011). Performance standards for antimicrobial susceptibility testing.m100-s21th. Clinical and Laboratory Standards Institute, Wayne, PA.

Costa, F. N., Alves, L. M. C., & Monte, S. S. (2000).Avaliação das condições higiênico-sanitárias de carne bovina moída, comercializada na cidade de São Luís, MA. *Higiene Alimentar*14 (77), 49-52.

Costa, L. C., & Tanamati, A. (2018). Avaliação higiênico-sanitária e físico-química de carne in natura comercializada em Campo Mourão-PR. *Revista UNINGÁ Review* 33 (1), 55-65.

Fehrenbach, R. B.(2017). Desenvolvimento de sistema automatizado para controle de dosagem de ração animal. Trabalho de Conclusão de Curso (Engenharia de Controle e Automação) – Centro de Ciências Exatas e Tecnológicas do Centro Universitário – UNIVATES, Lajeado, Rio Grande do Sul.

Florentino, E. R. & Leite, J. F. (1997). Avaliação da qualidade microbiológica da carne comercializada em Campina Grande-PB. *Revista Higiene Alimentar*11 (47).

Fortuna, D. B. S. & Fortuna, J. L. (2008). Avaliação da qualidade microbiológica e higiênico-sanitária da água de coco comercializada em carrinhos ambulantes nos logradouros do Município de Teixeira de Freitas (BA). *Revista Baiana de Saúde Pública* 32 (2), 203-217.

Franco, R. M. (2002). *Escherichia coli*: Ocorrência em Suínos abatidos na grande Rio e sua viabilidade experimental em LingüiçaFrescal tipo toscana. Niterói-RJ. Tese (Doutorado em Medicina Veterinária)- Universidade Federal Fluminense, UFF, Niterói, 153 p.

Kasnowsky, M. C. (2004). *Listeria* spp. *Escherichia coli*: isolamento, identificação, estudo sorológico e antimicrobiano em corte de carne bovina (alcatra) inteira e moída. Dissertação (Mestrado)–Higiene Veterinária e Processamento Tecnológico de Produtos de Origem Animal, Universidade Federal Fluminense, Niterói, 110p.

Loguercio, P. A., Silva, W.P, & Aleixo, J. A. G. (2002). Condições higiênico-sanitária no processamento de carne bovina moída, *Higiene Alimentar* 16 (98).

Lopes, M. A., Maia, E. M., Bruhn, F. R. P., Custódio, I. A., Rocha, C. M. B. M., & Faria, P. B. (2017). Fatores associados à percepção e atitude de consumidores de carne bovina com certificação de origem em Uberlândia, Minas Gerais. *Revista Ceres* 64 (1), 31-39.

Oliveira, M. N., Brasil, A. L. B., & Taddei, J. A. (2008). Avaliação das condições higiênico-sanitárias das cozinhas de creches públicas e filantrópicas. *Ciência e Saúde Coletiva*, Rio de Janeiro 13 (3), 1051-1060.

Pereira, A. S., Shitsuka, D. M., Parreira, F. J. & Shitsuka, R. (2018). Metodologia do trabalho científico. [e-livro]. Santa Maria. Ed. UAB / NTE / UFSM. Disponível em:https://repositorio.ufsm.br/bitstream/handle/1/15824/Lic_Computacao_Metodologia-Pesquisa-Cientifica.pdf?sequence=1.. Acessado em10/05/2020

Pigatto, C. P.; & Barros, A. R. (2003). Qualidade da carne moída bovina resfriada, comercializada em açougues da região de Curitiba. *Higiene Alimentar*, Itapetininga 17 (108): 53-57.

Queiroz, I. K. A. (2017). Carne moída bovina em Araguaína: qualidade higiênico-sanitária e perfil de resistência antimicrobiana de *E.coli* e *Salmonella* sp. Dissertação de Mestrado – Programa de Pós-graduação em Ciência Animal Tropical da Universidade da Universidade Federal de Tocantins, 79p.

Silva, C. A.; Souza, E. A.; & Souza, C. P. (2004). Estudo da qualidade sanitária da carne moída comercializada na cidade de João Pessoa, PB. *Revista Higiene Alimentar*18 (121), 90-94.

Vanderzant, C.; & Splittstoesser, D. F. (1992).Compendium of methods for the microbiological examination of foods. ed. 3, Washington: *American Public Helth Association*(APHA), 1219 p.

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