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Microbiological analysis of surfaces in butchers of the municipal market of Cuiabá

Análisis microbiológico de superficies en carnicerías del mercado municipal de Cuiabá

Análise microbiológica de superfícies em açougues do mercado municipal de Cuiabá

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ABSTRACT

Objective: *to isolate enterobacteria, especially the thermotolerant, present on the surfaces of knives, trays and cutting boards used in the handling of meat in butchers.* ***Method:*** *cross-sectional study, conducted from April to October 2017 in nine establishments that sell meat products in Cuiabá – Mato Grosso, Brasil. Surface samples were collected from three different utensils: knife, board and tray.* ***Results:*** *Among utensils used on handling poultry meat, the knife got greater growth of enterobacteria (4,36x10^4 UFC/cm^2). The cutting board intended for pork also reported higher 7,44x10^4 UFC/cm^2. For beef, the tray and the board respectively, had scores of 2,5x10^5 UFC/cm^2 and 2,41x10^5 UFC/cm^2. The high growth of enterobacteria is justified by the grooves contained in these tools, poor maintenance and hygiene. It was also observed the presence of 27% of fecal coliforms and 73% of total coliforms.* ***Conclusion:*** *from the data obtained it is inferred that the hygienic and sanitary conditions are not in accordance with the RDC No. 216/2004.*

Descriptors: *Commerce; Meat; Hygiene.*

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RESUMEN

Objetivo: *aislar enterobacterias, especialmente los termotolerantes, presentes en las superficies de cuchillos, bandejas y tablas de cortar utilizadas en el manejo de carne en carnicerías.* ***Método:*** *estudio transversal, realizado de abril a octubre de 2017 en nueve establecimientos que venden productos cárnicos en Cuiabá – Mato Grosso, Brasil. Se recogieron muestras de superficie de tres utensilios diferentes: cuchillo, tablero y bandeja.* ***Resultados:*** *entre los utensilios utilizados para manipular carne de aves, el cuchillo consiguió un mayor crecimiento de enterobacterias (4,36x10^4 UFC/cm^2). La tabla de cortar destinada a carne de cerdo también reportó mayores 7,44x10^4 UFC/cm^2. Para la carne de res, la bandeja y el tablero, respectivamente, tuvieron puntajes de 2,5x10^5 UFC/cm^2 y 2,41x10^5 UFC/cm^2. El alto crecimiento de las enterobacterias se justifica por las ranuras contenidas en estas herramientas, el mantenimiento deficiente y la higiene. También se observó la presencia de 27% de coliformes fecales y 73% de coliformes totales.* ***Conclusion:*** *de los datos obtenidos se infiere que las condiciones higiénicas y sanitarias no están de acuerdo con el RDC No. 216/2004.*

Descriptores: *Comercio; Carne; Higiene.*

RESUMO

Objetivo: isolar enterobactérias, principalmente as termotolerantes, presentes nas superfícies de facas, bandejas e tábuas utilizadas no manuseio de carne em açougues. **Método:** estudo transversal, realizado de abril a outubro de 2017 em nove estabelecimentos que comercializam derivados de carne em Cuiabá - Mato Grosso, Brasil. Coletaram-se amostras da superfície de três utensílios diferentes: faca, tábua/tabuleiro e bandeja. **Resultados:** entre os utensílios utilizados no manejo de carne de aves, a faca obteve um maior crescimento de enterobactérias (4,36x10^4 UFC/cm^2). A tábua de cortar carne de porco também reportou 7,44x10^4 UFC/cm^2. Para a carne bovina, a bandeja e o tabuleiro, respectivamente, apresentaram valores de 2,5x10^5 UFC/cm^2 e 2,41x10^5 UFC/cm^2. O alto crescimento de enterobactérias é justificado pelos sulcos contidos nessas ferramentas, falta de manutenção e higiene. Também foi observada a presença de 27% de coliformes fecais e 73% dos coliformes totais. **Conclusão:** a partir dos dados obtidos, infere-se que as condições higiênicas e sanitárias não estão de acordo com a RDC nº 216/2004.

Descritores: Comércio; Carne; Higiene.

INTRODUCTION

Foodborne diseases may be one of the most significant contemporary public health problems, not only because of the large number of cases reported and the associated economic costs, but also, because many of the causative organisms are newly recognized[1].

Among the factors that contribute for the increased incidence of foodborne illness stand out from the increasing population, the process of disorderly urbanization, the need for food production on a large scale and the consumption of food on public roads together with the dietary habits

changed. Other factors like unsafe food storage conditions and poor hygiene practices are major contributing factors to food associated illnesses. Coupled to this there is the poor control of public and private agencies to maintain the quality of food offered to the population[2,3].

Fairs, municipal markets, retail market and other of same segment are considered traditional in the marketing of food, being a fixed trade with high turnover of people[4]. In many of them, it is observed that the sanitary conditions related to the sale and disposal of food products are unsatisfactory, becoming a key factor in the contamination and proliferation of foodborne illness process[5].

At butcher shops located on this places, meat contamination can occur due to different possible reasons: storing food in unclean utensils, holding food at a temperature that would permit microbial growth, utilization of water of questionable hygienic quality, using packaging materials that is not of food-grade quality, vending site that had no facilities for waste disposal and utilization of unclean utensils.

In addition, lack of awareness in basic personal cleanliness and safe food handling of butchers enhances contamination of meat by microbes[6].

As we know, meat sold in a unhygienic condition can pose threat to the health of the consumers. This Contamination of meat can result from contaminated working surfaces, equipments and the workers' hands used in the processing[7].

Foodborne diseases control and prevention has improved considerably in Brazil in recent years. However, Brazil is still facing problems in controlling foodborne illnesses on its whole territory with a population of more than 195 million habitants. For instance, between 2000 and 2013, the Brazilian Ministry of Health has registered 8,857 foodborne outbreaks, 163,425 infected people and 112 deaths due to foodborne illnesses. Even though these were the numbers officially reported, it is believed that the real number of foodborne cases is much higher. In fact, among the twenty-six Brazilian States, only few have consistent statistics and data published on the most common etiological agents and foods most frequently involved in foodborne outbreaks[8].

With regard to the number of outbreaks of waterborne diseases and

food recorded by the Diseases of the National System Notification - SINAN - NET in 2007 - 2010, Mato Grosso is fourth in the Midwest region totaling 17 cases and Goiás, the first on the list with 69 cases[9].

Although the state of Mato Grosso does not present alarming data with regard to disease outbreaks waterborne and food, the need for regular health surveillance in public markets there is, as are many possibilities for contamination. In this scenario, this study aimed to the isolation of enterobacteria, especially the thermotolerant, present on the surfaces of knives, trays and cutting boards used in the handling of meat in butchers.

METHOD

A cross-sectional study was carried out from april to october 2017. The sampling was conducted in nine butcher shops located in a municipal market Cuiaba, Mato Grosso, Brasil and were collected the written authorization of the owners. Of these, three are intended for sale to the final consumer of beef, three pork and three chicken meat. Samples were obtained from three different surfaces: knife blade, plastic cutting board and plastic meat packaging trays in refrigeration counters. All experiment was performed in duplicate under aseptic conditions with negative controls.

For microbial isolation, it was used a mold made from polypropylene totalizing 9 cm^2 in order to delimit the area to be analyzed. With sterile swabs soaked in peptone solution, it is rubbed on the surface test at an angle of 30 degrees, performing three times upward and downward ziguezag movements. After the friction swabs were placed individually into previously identified tubes containing 10 mL of sterile peptone solution, and put in icebox cooler and taken to the laboratory for further study.

With the finality to realize the enumeration of total viable count and isolation of bacteria, immediately upon arrival at the laboratory, the tubes were mechanically shaken on Vortex for 10 seconds. Subsequently we selected three dilutions (10^{-2}, 10^{-3}, 10^{-4}) inoculating 1 mL of the sample by pour plate method on MacConkey agar Petri dishes and sterilized properly identified[10]. Plates were incubated at 37ºC for 24-48 hours. The number of enterobacteria lactose

fermenting and non-fermenting of that carbohydrate on each plate was enumerated using a colony conter. Colony Forming Units (CFU) per mL or cm^2 of sample was calculated, using the dilution factor of each. Mean values of total aerobic viable counts were determined and reported.

The lactose positive colonies were subcultured into fresh MacConkey agar plates aseptically to obtain pure cultures of the isolates. Concomitantly these colonies were submitted to Gram stain to certify its purity and only then be stored at 4°C. Later, colonies were inoculated on an Eosin Methylene Blue Agar (EMB) at 45° C/24 hours. Following the described by Winn[11] the colonies with morphological caracteristics similar to *Escherichia coli* were submitted to indole test. For achieving this, the strain was inoculated in test tubes containing 2 mL tryptophan broth (Himedia), incubated at 37°C/24 hours being added five drops of Kovacs reagent (NewProv) on tube wall by the end of this period. The development of a bright red color in the contact surface between the reactive and tryptophan broth indicates the presence of indole. According to the literature, strains that are experiencing growth at 45 °C and the positive indole test are considered presumptive coliform[11,12].

For the analysis of surfaces were accepted standards of Silva Jr[13], which establish compliance with sanitary conditions values $\leq 10^2$ CFU/cm^2 of total coliforms and absence of fecal coliform in the sampled surfaces. The data obtained on this work were entered into MS-Excel spreadsheets and analyzed.

RESULTS AND DISCUSSION

Checking the hygiene and sanitary conditions of the nine butcher shops surveyed was verified notoriously the poor physical structure for the sale of perishable products such as meat. In addition to the storage conditions present themselves inadequate, it was evident the lack of protection of these environments against insects. The physical structure there are no walls, ceilings and floors coated easy to clean materials. The lighting and ventilation are inadequate and the lack of organization of their own food. We found that good hygiene and sanitation practices were not in compliance, confronting the RDC Resolution 275/2002 of the Ministry of

Health[14], which establishes the technical regulations regarding sanitary conditions and best practices for developers/industrializers food establishments. The surfaces analyzed, were isolated enterobacteria lactose fermenting in all establishments. For the group of enterobacteria lactose non-fermenting, only knife and tray of Shop B and C showed no isolation.

Table 1 - Lactose fermenting enterobacteria isolated of the utensils of the nine butcher shops sellers of different meat on a municipal market (CFU/cm^2).

Shop	POSITIVE LACTOSE			NEGATIVE LACTOSE		
	K	T	C	K	T	C
Shop A	1,14 x 10^4	4,51 x 10^3	1,01 x 10^4	0,33 x 10^2	4,6 x 10^2	5,91 x 10^3
Shop B	4,36 x 10^4	8,5 x 10^3	5,35 x 10^3	1,33 x 10^3	-	2,16 x 10^2
Shop C	9 x 10^2	1,69 x 10^4	1,10 x 10^4	-	-	1,6 x 10
Shop D	1,14 x 10^4	4,51 x 10^3	1,01 x 10^4	3,3 x 10	4,6 x 10^2	5,91 x 10^3
Shop E	2,5 x 10^3	2,62 x 10^4	2,35 x 10^3	2,5 x 10^3	7,71 x 10^3	3,36 x 10^3
Shop F	2,31 x 10^4	1,61 x 10^4	7,44 x 10^4	4,5 x 10^3	1,15x 10^3	1,42 x 10^4
Shop G	1,66 x 10^4	4,85x 10^4	7,41 x 10^3	1 x 10^2	3,03 x 10^3	1,6 x 10
Shop H	1,15 x 10^4	6,26 x 10^4	2,41 x 10^5	6,91 x 10^2	1,29 x 10^5	4,67 x 10^5
Shop I	1,14 x 10^4	2,0 x 10^5	2,65 x 10^4	6 x 10	3,81 x 10^3	6,46 x 10^3

K: knife; T: tray; C: cutting board; Shop A, B e C: chicken meat; Shop D, E e F: pork; Shop G, H e I: beef; - : no growth.

The propably Enterobacteriaceae lactose positive reported in the literature are: *Citrobacter* sp; *Enterobacter* sp; *Klebsiella* sp. and some species belonging to the genera *Escherichia* sp. and *Serratia* sp. Enterobacteriaceae lactose non-fermenting may include the following genera: *Proteus* sp; *Providencia* sp; *Salmonella* sp; *Shigella* sp. and *Yersinia* sp. These Gram-negative bacteria are known to cause diarrhea, infectious gastroenteritis, emetic, dysentery, enterocolitis, among others[11].

There are several works which relate meats and surfaces bringing the presence of gram negative microorganisms as well as to the present study[3,15-19]. There is great concern when these microorganisms are isolated from food and/or equipment intended, due to the high possibility of causing disease causing so great problems of public order. According to data of the Brazilian Ministry of Health, during the period between 2000 and 2013, *Salmonella* sp. was identified as the major causative agent of reported foodborne diseases (39.39%), followed by *Staphylococcus aureus* (19.71%),

Escherichia coli (12.40%), and *Bacillus cereus* (7.62%)[8].

Among the tools used in the handling of chicken meat, coming from the knife Shop B obtained the highest growth of enterobacteria, totaling 4,36x10^4 CFU/cm^2. A cutting board destined for pork also showed high rates (7,44x10^4 CFU/cm^2) compared to other appliances of the same type meat. With respect to beef, the tray and cutting board used in Shop I and H, respectively, the counts of 2.5x10^5 CFU/cm^2 and 2,41x10^5 CFU/cm^2. High counts of knives' microorganism were also observed in the studies of Gurmu and Gebretinsae[18], Sudhakar et al[20], Haimanot et al[16]. During the sampling was noted that the knives used are not been changed for the entire day hence the higher microbial level due to the accumulation of microorganisms and likely, biofilms on their surfaces. As well as has highlighted Kusumaningrum et al[21], we should be attent to the the fact that pathogens could be remain viables on dry stainless steel surfaces and present a contamination hazard for considerable periods of time, being dependents of the contamination levels and type of pathogen.

Cutting boards when destinated to food manipulation also point to the high microorganism counts. Certainly the amount of grooves and the frequency of sanitizing contributed as to obtain as high. Pork in particular has nutritionally greater nutritional support for microorganisms[22].

As can see on literature, several studies have evaluated the contamination of cutting boards, as well as issues related to the material used in the production of these utensils, and the ease of cleaning cutting boards. Ak et al[23] assessed possible differences in the decontamination of cutting surfaces and observed that more bacteria were recovered from plastic than from wooden cutting boards. Moore et al[24] studied the recovery of S. Typhimurium from formica, stainless steel, polypropylene and wood and observed greater recovery from formica and stainless steel than from polypropylene and wood.

In a study conducted by Ravishankar et al[25], the rate of transfer of *Salmonella enterica* from poultry to lettuce handled with knives and on plastic cutting boards was studied under different scenarios.

When utensils were not cleaned after they were used, the transfer rate was 1.25% from poultry to plastic and 45.62% from plastic and knives to lettuce. According Gurmu and Gebretinsae[18], the high microbial load obtained from the butchers table is an indication of the ineffectiveness of the method used in cleaning the tables, which are usually washed with water only. The presence of bacterial pathogens in meat contact surfaces may contribute to the contamination of meat.

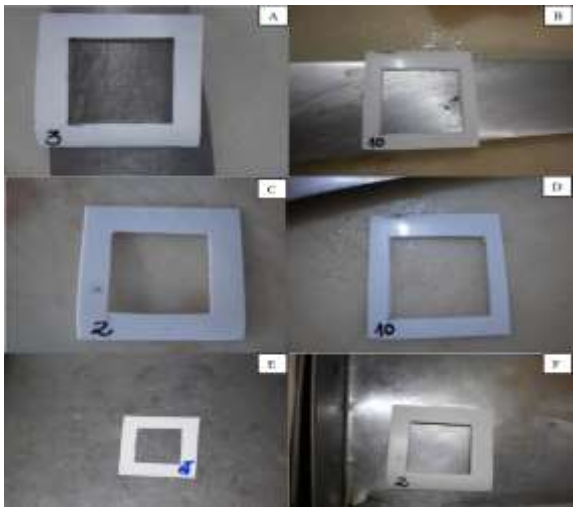
The presence of bacterial pathogens in meat-processing equipment and associated surfaces may contribute to the contamination of meat[26]. Typical food contact surfaces in municipal markets may include handlers hands and outer garments, wooden or plastic cutting boards, cutting knives, weighing scales, cleaning sponges/brushes, aprons and water-holding utensils such as metal buckets or plastic containers. These food handling equipment should therefore be maintained and stored in a way that will minimise the chance of food becoming contaminated as their contamination can contribute to cross-contamination of non-contaminated meat.

Is known that the grooves caused on stainless steel, wood or plastic may shelter microorganisms that persist in the inner structure, many times forming biofilms that it is not removed when improperly washed. Unless such equipment are thoroughly sanitized, they may continue to contaminate foodstuff as noted[17,27]. Hence, uncontaminated meat will become contaminated by the time it comes in contact with such surface. On the other hand, contaminated meat is able also to disseminate food-borne pathogens to clean contact-surfaces[28].

The ordinance n. 368 of 09.04.1997 of the Ministry of Agriculture, Livestock and Food Supply (MAPA)[29] provides that the surfaces should be free and free of imperfections such as cracks, crumpled, grooves and others that can compromise food hygiene. In our study the reverse can be clearly seen in Figure 1.

The grooves present on these utensils can contribute to the high count CFU's. Direct contact with the hands of manipulative, low hygiene conditions and poor hygiene utensil corroborate this fact.

Figure 1 - (A) Knife of Shop B, (B) Knife of Shop G, (C) cutting board of Shop A e (D) cutting board of Shop G, (E) tray of Shop F e (F) tray of Shop A.



In knife case, each new cut exposes a new surface with the increase of potential of more micro-organisms in exposed tissue. As can be seen in Figure 1C and Figure 1D, the cutting boards have a great number of grooves and blood, which makes a focus conducive to the growth of micro-organisms. It is recommended that these should be changed periodically, remaining smooth, favoring its cleanliness and avoiding waste accumulation. The same can be considered for the plastic trays, where the meat product remains in contact with the surface for longer, contributing to the development of a bacterial biofilm[30].

The high microbial load on the processing facility surfaces in this study underscores the poor level of personnel hygiene and poor sanitation at the butcher shops. The personnel working in the butcher shops did not apply hygienic practices which is mainly due to lack of knowledge. Based on the bacteria isolated and bacterial load on different surfaces in the butcher shops, meat could be contaminated by contact with contaminated surfaces and equipments in the butcher shops to pose public health hazards. Thus to safeguard the public against the risks of food borne bacterial infections, there is need to educate and advocate for practicing good sanitation and meat handling techniques in the butcher shops[18].

Sodium hypochlorite has an important biocidal activity and is widely recommended worldwide for the disinfection process, however its biocidal activity depends on several factors such as chlorine concentration, pH, temperature and the presence and quantity of organic matter, besides the time of exposure to the solution[21]. Vegetative bacteria are susceptible to chlorine concentrations of 2 to 500 ppm in environments with low organic matter[31].

For microbiological evaluation

of areas where food is handled are considered three groups of microorganisms indicators: a) general; b) hygienic-sanitary c) potentially pathogenic[13]. As for the isolation of the indicators microorganisms hygienic-sanitary comprising total coliforms, these accounted for 73%, and may be linked to failures in hygienic aspect in processing. Already the fecal coliform refer to contamination of fecal origin acting as health indicators, present in 27% of butcher shops studied. It is known that the presence of total coliform bacteria in most samples indicates unsatisfactory hygienic conditions. Studies reported by Gurmu and Gebretinsae[18] indicated that *E. coli* was the predominant isolate (32%) of a total of 72 swab samples obtained from the butcher's knives, processing tables and worker's hands[18]. Hassan Ali et al[17] observed that of 342 bacterial pathogens isolated from meat samples, 120 (35%) were identified as *Escherichia coli* and 51 (15%) of these *E. coli* isolates were characterized as serotype 0157:H7. In this same study were found to this same microrganism 50 (24%) environmental surface swabs, including knives, weighing scales, wooden boards, meat mincer, customer platforms, floors, walls, inch long steel meat anchors.

The coliform comprise enteropathogenic bacteria such as *E. coli*, from the intestine and human warm-blooded animals. When found in foods or even in the hands of manipulators, indicate unsatisfactory sanitary conditions[32]. A study conduced by Zerabruk e cols[33] in contact surfaces in selected butcher shops in Ethiopia, showed that from a total 40 samples, 14 (35%) of them were presumptively designated as positive for the presence of E. coli. Before the data presented it appears that the hygiene and sanitary conditions of the environment are not in accordance with the RDC 216/2004 that emphasize the absence of fecal coliform[34].

Before the results presented here becomes evident the need to educate the owners and manipulators for practicing good sanitation of utensils, surfaces and meat handling techniques in the butcher shops to safeguard the public against the potential risks of foodborne bacterial infections.

CONCLUSION

The limitation present in this study is about the great distrust regarding the academic character of the research observed mainly when approaching the owners of the establishments for the due general clarifications of the study. Most were believed to be a health inspection visit by some official government agency. For this reason, many declined to participate in the survey. This fact directly impacts the amount of samples analyzed which could be much larger and comprehensively portray the hygienic-sanitary profile of establishments in this municipal market destined to the sale of meat products.

Considering the RDC Resolution No. 216/2004 of good practice for food service we note a poor hygienic condition of the nine butcher shops analyzed. Become necessary facilities suitable for the production of a sanitary safe food. Resents the Brazilian legislation for surface analysis, the lack of standards for total coliforms and thermotolerant, since these parameters are important and essential to evaluate the quality of food.

The results of this study allowed to infer that it is necessary to create shares in order to prevent contamination and food poisoning. Thus it is necessary to train both the owners as the handlers belonging to hygiene and health care, and basic health education and food hygiene courses. Good manufacturing practices and HACCP can be applied to decreased or control this microrganisms for these handlers collective establishments, and instruments significant for prevention of food poisoning outbreaks, a serious public health problems.

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