ORIGINAL ARTICLE

https://doi.org/10.21708/avb.2024.18.2.12325

Microbiological evaluation of beef sold in Brazil and consumer knowledge about food safety

Avaliação microbiológica de carne bovina comercializadas no Brasil e conhecimentos de consumidores sobre segurança de alimentos

Caroline Pereira das Neves[1]*, Kelly Lameiro Rodrigues[1], Jozi Fagundes de Mello[1], Tatiane Kuka Valente Gandra[1], Eliezer Avila Gandra[1]

ABSTRACT: Meat is highly perishable and is often associated with foodborne disease outbreaks. The risk associated with handling food at home is underestimated, as consumers generally associate foodborne disease outbreaks with food from food services. This study aimed to quantify *Escherichia coli*, coagulase-positive staphylococci, mesophilic aerobic bacteria, and *Salmonella* spp. in ground beef samples sold at commercial establishments in Pelotas, Rio Grande do Sul State, Brazil, and to evaluate the knowledge of food safety among consumers in the region. Microbiological analyses were performed on 70 ground beef samples, and the knowledge of 92 meat consumers was analyzed using an online questionnaire based on the World Health Organization (WHO) safe food guidelines. Fifty percent of the meat samples had aerobic mesophilic microorganism counts above the limit established by Brazilian legislation, indicating unacceptable food quality. Only two samples (2.86%) had counts of coagulase-positive staphylococci above those allowed by legislation. The consumer knowledge regarding food safety showed that items related to "cook thoroughly" and "keeping food at safe temperatures" had the lowest percentage of correct answers. Dissemination of information about temperatures that ensure food safety is essential for consumers, especially considering that even with high levels of contamination, most microorganisms are destroyed by heat.

KEYWORDS: coagulase positive staphylococci; *Escherichia coli*; *Salmonella* spp.; aerobic mesophilic microorganisms; food quality.

RESUMO: Carnes são alimentos altamente perecíveis e estão frequentemente associados a surtos de doenças transmitidas por alimentos. Existe uma subestimação de risco associado a preparação de alimentos em casa, associando geralmente os surtos a alimentos preparados em serviços de alimentação. Objetivo desse estudo foi quantificar *Escherichia coli*, estafilococos coagulase positiva, aeróbios mesófilos e realizar a pesquisa *Salmonella* spp. em amostras de carnes bovinas moídas comercializadas em estabelecimentos comerciais do sul do Brasil, além de avaliar o nível de conhecimento sobre segurança de alimentos de consumidores da região. Foram feitas análises microbiológicas de 70 amostras de carne moída e foram analisados o conhecimento de 92 consumidores de carne a partir de um questionário online criado com base nas diretrizes de alimento seguro da Organização Mundial da Saúde. Cinquenta por cento das amostras de carne estavam com contagens de microrganismos mesófilos aeróbios acima dos limites máximos estabelecidos pela legislação brasileira indicando qualidade inaceitável do alimento e apenas duas amostras (2,86%) tiveram contagens de estafilococos coagulase positiva acima do permitido pela legislação. O conhecimento geral da população em relação a segurança dos alimentos foi considerado razoável, sendo os itens relacionados ao "cozimento adequado dos alimentos" e "manter alimentos a temperaturas seguras" os pontos mais fracos. Disseminação de informação sobre temperaturas que assegurem a segurança dos alimentos é essencial para consumidores, especialmente considerando que mesmo em altos níveis de contaminação, a maioria dos micro-organismos são destruídos com o calor.

PALAVRAS-CHAVE: estafilococos coagulase positiva; *Escherichia coli*; *Salmonella* spp.; micro-organismosaeróbios mesófilos; qualidade do alimento.

[1]Universidade Federal de Pelotas, Pelotas/RS, Brasil
*Corresponding author: neves_caroline@ymail.com
Received: 01/24/2024. Accepted: 02/29/2024

INTRODUCTION

Meat and meat products are high-quality protein sources as they contain all the essential amino acids in sufficient quantities to meet the daily requirements and provide iron, zinc, and some B vitamins (Basso, 2021; Salter, 2018). However, the richness of nutrients combined with high water activity makes meat a highly perishable product because these intrinsic characteristics facilitate the survival and multiplication of various microorganisms, including pathogens, and lead to rapid deterioration of food (Mcwilliams, 2016; Germano, 2019). Another factor related to meat safety is the high risk of contamination throughout the production chain, from slaughter and evisceration to handling during processing, storage, and sale (Forsythe, 2013).

Previous studies have shown that establishments selling fresh meat under inadequate hygienic and sanitary conditions, which favor meat contamination, consequently put consumer health at risk (Araújo; De Moura; Da Luz, 2021; Silva *et al.*, 2016; Khanal; Poudel, 2017; Gebeyehu; Tsegaye, 2022). Araújo; De Moura; Da Luz (2021) evaluated the hygiene conditions in butcheries in municipalities of the Vale do Guaribas, PI, Brazil. Of the ten establishments evaluated, eight (80%) were classified as poorly adapted, with unsatisfactory hygiene, physical and structural conditions. Silva *et al.* (2016) evaluated the hygiene and safety of the utensils and equipment in three commercial establishments in Viçosa, MG and found inadequate sanitary conditions in the meat product processing environment; they identified tables, grinders, and kneaders as the main sources of contamination with the microorganisms evaluated in the study, highlighting the need for greater attention to hygiene in these areas.

Foodborne diseases are generally infectious or toxic and are caused by bacteria, viruses, fungi, parasites, or chemical substances that enter the body through food or water (WHO, 2022). Every year, approximately 600 million people worldwide are estimated to fall ill after consuming contaminated food, causing a burden on health systems with consequences for the economy, tourism, and trade. Notably, cases are underreported and establishing causal links between food contamination and the resulting illness or death is difficult (WHO, 2022; WHO, 2015).

Foods of animal origin, such as beef and chicken, are among those most frequently associated with foodborne disease outbreaks (Brasil, 2023; Germano, 2019). Among microorganisms commonly found in meat, *Salmonella* spp. can cause salmonellosis with symptoms of diarrhea, nausea, abdominal pain and fever; *Staphylococcus aureus (S. aureus)* forms enterotoxins in food and ingestion of these enterotoxins can cause staphylococcal food poisoning; *Escherichia coli (E. coli)* has groups such as enterohemorrhagic *E. coli* (EHEC) that can cause bloody diarrhea, enterotoxigenic *E. coli* (ETEC) associated with travelers' diarrhea, and enteropathogenic *E. coli* (EPEC) known for causing diarrhea, mainly in children (Bhunia, 2018a; Bhunia, 2018b; WHO, 2018a; FDA, 2022).

In Brazil, fresh beef, offal, and processed meat accounted for 4.4% of foodborne disease outbreaks between 2013 and 2022. Notably, the most frequent place of occurrence for the outbreaks in this period were households (35.1%) (Brasil, 2023). The risks associated with preparing food at home are underestimated, which can result in consumers often neglecting safe food handling. People who cook at home do not believe that the food they handle can cause disease (Sivaramalingam *et al.*, 2015; Zanetta *et al.*, 2022), as they usually associate outbreaks with food prepared by food services. Food can be contaminated by inappropriate preparation and/or storage, because consumer knowledge of food safety is often lacking (Motta *et al.*. 2014; Mello, 2017).

This study aimed to quantify *E. coli*, coagulase-positive staphylococci, mesophilic aerobes bacteria, and *Salmonella* spp. in samples of ground beef sold in commercial establishments in Pelotas, a city in the southern region of Brazil, and to assess the level of knowledge regarding food safety among consumers in the region.

MATERIAL AND METHODS

MICROBIOLOGICAL ANALYSES

An electronic database (Google®) was searched to quantify the butchery stores in Pelotas, a city in the southern region of Rio Grande do Sul State, Brazil. Based on these data, 25% (n = 36) of the establishments were randomly selected for purchasing ground beef samples to simulate actual purchase situations.

Two samples were collected from each establishment. In total, 70 samples of ground beef, qualitatively classified as "second-rate beef", were collected. Samples were collected during two different seasons (the first collection in late summer/early autumn and the second collection in the winter) to investigate whether the ambient temperature influenced the microbiological quality of the meat. In the first period (February, March and April of 2022), 36 samples were collected, whereas in the second period (July and August of 2022), 34 samples of the same type were collected because in the second collection period, two commercial establishments had closed, resulting in a total of 70 samples.

The samples were properly identified and immediately transported in an isothermal box with recyclable ice to the Laboratory of Food Microbiology at the Faculty of Nutrition of the Federal University of Pelotas (UFPel), Pelotas, Brazil, for the quantification of *E. coli*, aerobic mesophilic microorganisms, positive-coagulase staphylococci, and *Salmonella* spp. Microbiological analyses were performed in accordance with the methodologies recommended by the Bacteriological Analytical Manual of the Food and Drug Administration (FDA, 2001) and the American Public Health Association (APHA) (Downes; Ito, 2001). To evaluate the results of the analyses, the microbiological standards for the food group

"beef, pork and other meat" and the specific category "ground meat, molded raw meat products, seasoned or not, refrigerated or frozen (hamburgers, meatballs, kibbeh)" in the Normative Instruction No. 161 of July 1, 2022, of the National Health Surveillance Agency of the Brazilian Ministry of Health (Brasil, 2022) were considered.

For statistical analysis, microbiological results were converted into log values. Analysis of variance (ANOVA) was carried out, followed by Fisher's least significant difference Test ($p < 0.05$) to identify significant differences between each commercial establishment and collection period.

EVALUATION OF CONSUMER KNOWLEDGE REGARDING FOOD SAFETY

To evaluate consumer knowledge of food safety, a survey was conducted with a self-administered online questionnaire using Google Forms. Email lists and social networking sites were used to approach the consumers. The inclusion criteria were as follows: consumers who handled food at home at least twice a week, were over 18 years old, and lived in Pelotas, Rio Grande do Sul State, Brazil. This study was approved by the Ethics Committee of the Faculty of Medicine at the Federal University of Pelotas (UFPel) (no. 4.881.940); all participants provided informed consent prior to participation.

The questionnaire included questions regarding demographics (sex, age, educational level, and monthly income) and general knowledge of food safety. These questions were taken from the "five keys to safer food" manual of the WHO (WHO, 2006). The answer options for each question were true, false, or I do not know. Figure 1 presents the questions based on the manual.

The answers to the questionnaires were coded and descriptive statistics were used to examine the general characteristics and frequency of the demographic questions and questions about general food safety knowledge.

RESULTS AND DISCUSSIONS

MICROBIOLOGICAL ANALYSES

Table 1 shows the results of the analyses of coliforms at 35°C, coliforms at 45°C, aerobic mesophiles, and coagulase-positive staphylococci in the 70 ground beef samples from commercial establishments in Pelotas, Rio Grande do Sul State, Brazil.

Brazilian legislation does not define microbiological standards for coliforms at 35°C and coliforms at 45°C for ground beef; therefore, the results for these microorganisms were used only as an indication of product hygiene.

Fifty-one samples (72.86%) had counts above 1100 MPN/g for coliforms at 35°C, which is a group of facultative anaerobic bacteria capable of fermenting lactose with gas production at 35°C. This group includes bacteria from the gastrointestinal tract of humans and other warm-blooded animals as well as non-enteric bacteria. Most coliforms at 35°C are found in the environment and their detection is used as a general indicator of the hygienic and sanitary conditions of the food-processing environment; further, they are easily eliminated using commercial sanitizers. Another important factor affecting coliforms at 35°C is that they are easily destroyed by heat (Silva *et al.*, 2017; Feng *et al.*, 2020). Therefore, proper cooking is essential to ensure the final quality of meals prepared using ground beef. Among

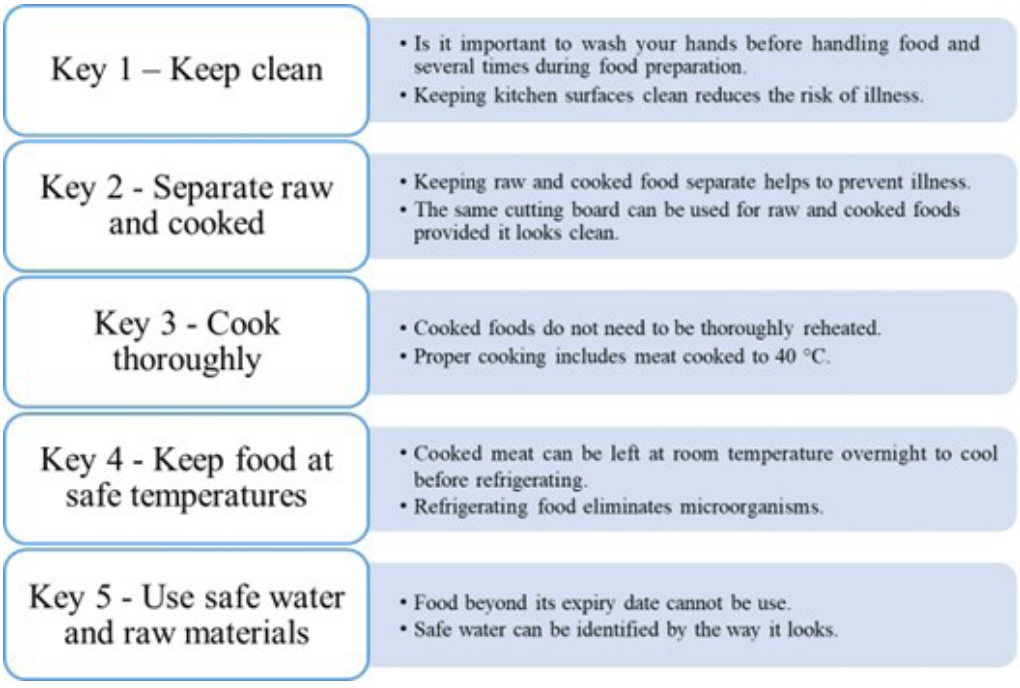

Figure 1. Evaluation of consumer knowledge about food safety. Retrieved from: WHO, 2006.

Table 1. Results of microbiological analyses of ground beef from 36 commercial establishments in the city of Pelotas, Rio Grande do Sul State, Brazil.

C. E.	Coliforms 35° [a]		Coliforms 45° [a]		AM[b]		CPE[b]	
	C 1	*C 2*	*C 1*	*C 2*	*C 1*	*C 2*	*C 1*	*C 2*
1	> 1100	> 1100	43	7.4	1.17×10^8	7.00×10^4	< 0.01	< 0.01
2	> 1100	> 1100	< 3.0	9.2	3.95×10^7	5.60×10^6	< 0.01	< 0.01
3	> 1100	> 1100	1100	93	1.14×10^8	9.00×10^4	< 0.01	< 0.01
4	> 1100	> 1100	3.0	< 3.0	2.06×10^8	9.00×10^4	< 0.01	< 0.01
5	> 1100	330	< 3.0	< 3.0	2.85×10^6	2.10×10^5	< 0.01	< 0.01
6	> 1100	*	240	*	5.75×10^6	*	< 0.01	*
7	> 1100	> 1100	93	23	1.36×10^6	9.24×10^6	< 0.01	< 0.01
8	> 1100	1100	> 1100	3.6	3.30×10^6	3.65×10^7	< 0.01	< 0.01
9	> 1100	> 1100	240	< 3.0	1.52×10^7	1.50×10^5	< 0.01	< 0.01
10	> 1100	> 1100	> 1100	1100	9.15×10^6	3.35×10^6	< 0.01	< 0.01
11	> 1100	> 1100	15	3.6	6.00×10^6	1.12×10^7	< 0.01	< 0.01
12	> 1100	> 1100	15	3.6	9.60×10^6	5.60×10^5	< 0.01	< 0.01
13	> 1100	> 1100	9.2	93	4.30×10^7	2.45×10^6	< 0.01	< 0.01
14	> 1100	1100	< 3.0	< 3.0	3.20×10^6	6.15×10^5	< 0.01	< 0.01
15	> 1100	> 1100	15	23	4.55×10^6	2.41×10^6	< 0.01	< 0.01
16	> 1100	1100	29	< 3.0	2.95×10^6	4.15×10^5	< 0.01	< 0.01
17	1100	460	23	< 3.0	1.25×10^5	1.50×10^5	< 0.01	< 0.01
18	> 1100	> 1100	1100	< 3.0	2.55×10^6	5.05×10^6	< 0.01	< 0.01
19	> 1100	> 1100	460	< 3.0	9.25×10^5	1.05×10^5	< 0.01	2.70×10^4
20	> 1100	460	240	< 3.0	6.45×10^5	<0.004	< 0.01	< 0.01
21	460	460	7.4	< 3.0	1.35×10^5	9.5×10^4	< 0.01	< 0.01
22	460	> 1100	43	240	3.35×10^5	6.70×10^6	< 0.01	< 0.01
23	3.6	> 1100	< 3.0	9.2	3.00×10^5	1.40×10^6	< 0.01	< 0.01
24	1100	> 1100	93	< 3.0	3.75×10^7	8.35×10^5	< 0.01	< 0.01
25	1100	> 1100	3.6	35	1.00×10^5	2.02×10^6	< 0.01	< 0.01
26	> 1100	*	23	*	4.30×10^6	*	< 0.01	*
27	310	> 1100	< 3.0	< 3.0	6.65×10^4	2.00×10^4	< 0.01	4.00×10^2
28	> 1100	> 1100	23	< 3.0	6.00×10^4	6.5×10^4	< 0.01	< 0.01
29	> 1100	1100	43	460	4.40×10^5	1.20×10^5	< 0.01	< 0.01
30	240	240	93	< 3.0	7.50×10^4	2.00×10^3	< 0.01	< 0.01
31	> 1100	> 1100	9.2	< 3.0	1.10×10^5	2.00×10^5	< 0.01	< 0.01
32	> 1100	> 1100	9.2	< 3.0	7.35×10^5	8.10×10^5	< 0.01	3.30×10^5
33	460	320	< 3.0	< 3.0	9.00×10^5	1.02×10^6	< 0.01	< 0.01
34	> 1100	> 1100	93	< 3.0	1.90×10^7	1.47×10^6	< 0.01	< 0.01
35	> 1100	> 1100	> 1100	3.6	2.85×10^6	4.50×10^5	< 0.01	< 0.01
36	> 1100	> 1100	15	< 3.0	1.36×10^6	4.60×10^5	< 0.01	< 0.01

Abreviations: C.E. = commercial establishments; AM = aerobic mesophiles; CPE = coagulase-positive staphylococci; a = most probable number per gram; b = colony-forming units per gram; C = collection * = second collection not performed.

the evaluated samples, commercial establishments 23 and 30 had significantly lower counts ($p < 0.05$) of coliforms at 35°C, indicating that the environment in which meat is stored and handled may have better hygiene conditions than those at other commercial establishments.

The majority of coliforms at 45°C (35.71%) had counts less than 3.0 MPN/g. This group of microorganisms is also an indicator of hygiene conditions, especially the sanitary conditions of food manufacturing processes, and includes enterobacteria from the gastrointestinal tract as well as some

microorganisms of non-fecal origin, which have the characteristic of lactose fermentation with gas production; however, this occurs at temperatures of 44.5–45.5°C (Silva *et al.*, 2017). In general, the results for coliforms at 45°C were better compared to those of contamination by coliforms at 35°C; however significantly higher counts ($p < 0.05$) than most commercial establishments were found in establishments 3 and 10. At the time of sample collection, the structural conditions and hygiene at these establishments appeared visually adequate, indicating that the higher contamination levels compared with those found in the majority of other commercial establishments may be related to contaminated raw materials or flaws in meat handling.

In addition to the analyses of coliforms at 35°C and 45°C, INViC tests were performed, which did not confirm the presence of *E. coli* in this study. Normative Instruction no. 161 of July 1, 2022, states that *E. coli* values equal to or higher than 10^2 NMP/g indicate unacceptable product quality (Brasil, 2022); therefore, all the samples in this study were acceptable in terms of this microorganism.

E. coli presence has been used to indicate recent fecal contamination or unsanitary food processing. Several strains of this microorganism are pathogenic and can cause disease. Cattle are carriers of *E. coli*, and undercooked ground beef products are among the main foods containing pathogenic *E. coli* (WHO, 2018b; Feng *et al.*, 2020). According to the Brazilian Ministry of Health, between 2013 and 2022, *E. coli* was the most prevalent etiological agent (32.3%) identified in foodborne disease outbreaks in Brazil (Brasil, 2023). The absence of this microorganism in the analyzed ground beef samples is a positive result, considering its risk to public health, as several serogroups of *E. coli* are pathogenic.

Thirty-five samples of ground beef (50%) had counts of aerobic mesophilic microorganisms equal to or greater than 10^6 CFU/g, and were considered to have unacceptable quality according to Normative Instruction no. 161, of July 1, 2022 (Brasil, 2022). The count of aerobic mesophilic bacteria is a general indicator of bacterial populations in food and is associated with characteristics such as the hygienic quality of products and raw materials as well as their processing and handling conditions, and is directly related to shelf-life (Silva *et al.*, 2017). Mesophilic microorganisms grow at moderate temperatures between 20–45°C, and the optimum temperature for multiplication is 30–39°C (Schiraldi; De Rosa, 2014). The high counts of these microorganisms may be associated with a failure to control the refrigeration temperature of meat in commercial establishments, influencing the microbiological quality of these samples.

Based on the results, commercial establishment 20 had significantly lower counts of aerobic mesophilic bacteria than those in the majority of other commercial establishments (10^2 CFU/g, $p < 0.05$), except in commercial establishments 17, 21, 27, 28, 30, and 31, indicating better hygienic control. Further, commercial establishment 20 is a store owned by the slaughterhouse itself; therefore, the meat is directly taken from the slaughterhouse to the cold chamber. Consequently, the meat is handled less, does not need transportation between the slaughterhouse and store, and is possibly subjected to fewer temperature fluctuations.

Staphylococci can be found in air, dust, sewage, and water, and are commonly present in the environment. Humans are reservoirs for *S. aureus*, a common microorganism associated with foodborne diseases and representative of coagulase-positive staphylococci, which are present in the nasal passages, throat, skin, and hair of humans. Consequently, handlers are a frequent source of food contamination; however, equipment and handling surfaces can also cause contamination. These microorganisms produce enterotoxins in food, which can cause food poisoning upon ingestion. Although bacteria are vulnerable to destruction by heat, the enterotoxins produced by these microorganisms are highly thermostable. It is thus important to avoid food contamination by *S. aureus* and to avoid keeping food at risky temperatures (between 10 and 46º) that allow multiplication of this micro-organism and the production of enterotoxins (Bhunia, 2018a; Silva *et al.*, 2017; Tallent *et al.*, 2019). Unrefrigerated or inadequately refrigerated meats are among the food sources of *S. aureus* contamination that cause foodborne disease outbreaks (FDA, 2022).

Two ground beef samples (2.86%) had coagulase-positive staphylococci counts higher than 10^4 CFU/g and were considered to be of unacceptable quality according to Brazilian legislation (Brasil, 2022). These samples were obtained from commercial establishments 19 and 32, which had significantly higher counts ($p < 0.05$) than those from the majority of establishments for this microorganism. These commercial establishments may have failed in handling ground meat appropriately, and contamination may have originated from the handlers themselves or from contaminated surfaces and equipment, such as meat grinders. The low number of samples unfit for consumption is important, especially considering that *S. aureus* is the third most frequent agent causing foodborne disease outbreaks in Brazil between 2013 and 2022 (10.8%) (Brasil, 2023). Regardless of this positive result, the necessity of reinforcing hygiene measures of handlers in commercial establishments to avoid meat contamination should be emphasized, especially considering that cooking temperatures do not inactivate the toxins that cause illness.

None of the ground beef samples contained *Salmonella* spp. in 25 g, which was in accordance with Brazilian legislation requirements (Brasil, 2022). *Salmonella* spp. were the second most recurrent etiological agents (10.9%) of foodborne disease outbreaks in Brazil between 2013 and 2022 (Brasil, 2023).

The absence of this microorganism in ground beef is a good result, especially considering that most human infections by *Salmonella* spp. are contracted by consuming contaminated food of animal origin, with the intestinal tract of

humans and animals being its main habitat. Food contamination by this microorganism is mainly associated with the quality of raw material, inadequate temperature control, improper handling practices, or cross-contamination of food (Bhunia, 2018c; FDA, 2019; FDA, 2022; Forsythe, 2013; Silva *et al.*, 2017; WHO, 2018a).

Problems in the hygiene and sanitary quality of meat from commercial establishments are not restricted to southern Brazil and have been reported in different regions of the country. De Almeida; Massago; Boni (2018) aimed to evaluate the hygienic quality of ground beef from 20 butcheries in Sarandi, PR, and found 17 (85%) samples had counts 2,4 x 10^3 MPN/g for coliforms 45º. All samples showed absence of *Salmonella* spp. Pelayo et al. (2019) with 100 ground beef samples collected from 25 commercial establishments in Londrina, PR, two enteropathogenic *E. coli* strains, three Shiga toxin-producing *E. coli* strains, and five enteroaggregative *E. coli* strains were isolated, indicating a possible risk to population health.

Ventura *et al.* (2020) evaluated 40 butcher shops in Uberlândia, MG. The commercial establishments presented 36.5% nonconformities regarding the adoption of good manufacturing practices, and *Salmonella* spp. was found in samples of ground beef from two establishments.

Bier *et al.* (2022) aimed to evaluate the hygienic quality of beef sold at commercial establishments in Campo Grande, MS. Seventeen establishments were selected and 71 sample were analyzed. *Salmonella* spp. was found in 7.04% of samples and a total of 25.35% of the samples were positive for *S. aureus*, with counts ranging from 1.0 x 10^2 to 4.3 x 10^4 CFU/g. In addition, 70% of *Salmonella* spp. isolates were sensitive to the antimicrobials tested, meanwhile *S. aureus* isolates exhibited resistance to penicillin, tetracycline and chloramphenicol.

Problems with the hygiene and quality of meat obtained from commercial establishments have also been reported in other countries. Bersisa; Tulu; Negera (2019) found meat samples contaminated with *Salmonella* spp. and *S. aureus* from commercial establishments and slaughterhouses in Bashoftu, central Ethiopia. Cardona-Lopez *et al.* (2020) found *E. coli* in 15 of 100 ground beef samples collected from commercial establishments in Guadalajara, Mexico. Ruiz *et al.* (2021) evaluated the quality of ground meat and facilities in 100 commercial establishments in Tandil, Argentina. Seventy-five percent of the establishments had at least one microorganism above the permitted level, and the counts of aerobic mesophiles, *E. coli*, and *S. aureus* exceeded 2.3%, 22.9%, and 40.2% of the 100 ground meat samples, respectively.

Differences in the results of coliforms at 35°C, coliforms at 45°C, aerobic mesophiles, and coagulase-positive staphylococci between the two collections carried out in commercial establishments are shown in Table 2.

There was no significant difference between the samples for coliforms at 35°C ($p > 0.05$). As for coliforms at 45°C and aerobic mesophiles, the ground beef samples from the second collection showed significantly lower average contamination than those from the first collection ($p < 0.05$). This result may have been influenced by the season of the year in which the collection was carried out; as previously discussed, mesophilic microorganisms have an optimum multiplication temperature between 30°C and 39°C (Schiraldi; De Rosa, 2014); as the first collection was carried out in late summer/early autumn and the second collection was in the winter, the lower environmental temperature during the second collection may have influenced the multiplication of bacteria in ground beef. Another factor that may be associated with this is that meat suppliers could differ depending on the time of year; therefore, raw materials from the second collection may have better microbiological quality than that of raw materials from the first collection.

Gutiérrez *et al.* (2020) reported that the probability of identifying meat samples containing *Salmonella* spp. is significantly higher during the warm season. Cardona-Lopez *et al.* (2020) also reported that all ground beef samples with *E. coli* showed its detection in the warm months of the year.

In contrast to the previous microorganisms, coagulase-positive staphylococci counts in ground beef were significantly higher during the second sample collection ($p < 0.05$). Their higher contamination in the winter may be related to the higher prevalence of viral infections, which increases the likelihood of handlers coughing and sneezing; food may thus be contaminated directly by droplets, indirectly by contaminated hands, or by contamination of the surface and equipment.

Considering these results, failures in the control of storage temperature, handling of meat, and hygiene of the equipment and facilities in commercial establishments can be suggested. These failures need to be corrected in commercial establishments so that the meat sold does not pose a risk to consumer health. Specificities related to the season of the year must also be considered, especially related to temperature control in warmer months and *S. aureus* contamination in colder months, which is probably related to the greater possibility of failures by handlers. Even if ground beef is cooked in consumer households and most of the bacteria analyzed are eliminated by heat, the risk to consumer health remains in cases such as contamination with *S. aureus*, which can produce a thermostable toxin

Table 2. Microbiological averages of ground beef from commercial establishments in Pelotas, Rio Grande do Sul State, Brazil.

Microorganism	Collection 1	Collection 2
Coliforms at 35°C (MPN/g)	10^{2a}	10^{2a}
Coliforms at 45°C (MPN/g)	10^{a}	1^{b}
Aerobic mesophiles (CFU/g)	10^{6a}	10^{5b}
Coagulase positive staphylococci (CFU/g)	0^{a}	1^{b}

Numbers followed by different lowercase letters indicate significant differences ($p < 0.05$) between columns.

in food, or cross-contamination due to direct contact of raw meat with food to be eaten raw and/or ready for consumption, and indirect contact from contamination of the utensils used in food handling.

FOOD SAFETY KNOWLEDGE

The demographic data of consumers from Pelotas, Rio Grande do Sul State, Brazil, who answered the questionnaire about food safety knowledge are shown in Table 3. The majority of consumers who participated in the research were female (66.3%), aged between 18–29 years (38.0%), with an undergraduate degree as the highest educational level achieved (44.6%), followed by a postgraduate degree (43.5%). This may be related to the method of disseminating the questionnaire as university email lists and social networks were used to invite consumer participation. Further, according to the Brazilian Institute of Geography and Statistics (IBGE), Brazil has a higher prevalence of females in higher education courses (IBGE, 2021), which may also have influenced the higher female participation. Most consumers (45.7%) reported having a monthly income of two minimum wages, which was approximately 2,200 Brazilian reais at the time of this study.

Table 3. Demographic characteristics of consumers from Pelotas, Rio Grande do Sul State, Brazil (n = 92).

Demographics characteristics	n	%
Sex		
Female	61	66.3
Male	31	33.7
Age (years old)		
18–29	35	38.0
30–39	29	31.5
40–49	18	19.6
50–59	6	6.5
≥ 60	4	4.3
Education (Highest Level Achieved)		
No qualification	0	0.0
Middle or Elementary School	0	0.0
High School	11	12.0
University (undergraduate)	41	44.6
University (postgraduate)	40	43.5
Monthly income		
≤ 2 minimum wages	42	45.7
2 to 4 minimum wages	21	22.8
4 to 10 minimum wages	23	25.0
10 to 20 minimum wages	5	5.4
> 20 minimum wages	1	1.1

The WHO recommends that consumers understand how to handle food safely and practice the "five keys to safe food" when handling food at home (WHO, 2022; WHO, 2006). Results of consumers' food safety knowledge are shown in Table 4.

Questions related to the key "keep clean" had the highest percentage of correct answers from consumers, with 97.8% of consumers affirming the importance of hand hygiene and 100% affirming that kitchen surface hygiene reduces the risk of foodborne diseases. Hand and surface hygiene are related to contamination by microorganisms, such as *E. coli* and *Salmonella* spp. (WHO, 2006; WHO, 2018a; WHO, 2018b).

In the "separate raw and cooked" key, 81.5% consumers reported that keeping raw and cooked food separate helps prevent illness and 70.7% answered that the information of the same cutting board can be used for raw and cooked foods provided it looks clean is false. Food contamination by *Salmonella* spp. is related to factors such as improper food handling and cross-contamination (Bhunia, 2018c; Silva *et al.*, 2017), and knowledge about the separation of raw and cooked foods is essential to avoid cross-contamination.

If knowledge related to the keys "keep clean" and "separate raw and cooked" is well disseminated among the general population, this may have reflected in the absence of *E. coli* and *Salmonella* spp. in the ground meats analyzed, considering that commercial establishments workers may also have this knowledge as part of the general population.

"Cook thoroughly" was the key with the lowest level of consumer knowledge, with 38% consumers answering "I don't know" and 14.1% answering that it was true that cooked foods do not need to be thoroughly reheated. Furthermore, 58.7% consumers did not know whether proper cooking includes meat cooked to 40°C and 10.9% said that this information was true. According to the WHO, for food safety a temperature of 70°C must be reached when cooking, because even high levels of microorganisms are destroyed within 30 seconds at this temperature. The same applies to the reheating of food (WHO, 2006).

The questions in the "keep food at safe temperatures" key had a better performance from consumers compared to those in the "cook thoroughly" key, but the level of knowledge was lower when compared to those in the other keys. The statement that cooked meat can be left at room temperature overnight to cool before refrigeration was rated false by 64.1% of consumers, while the statement about refrigerating food to eliminate microorganisms was claimed to be false by 62% of consumers.

Studies from Brazil (Motta *et al.*, 2014), Africa, and Asia (Odeyemi *et al.*, 2018) revealed that consumers have poor knowledge regarding the risk of leaving food at room temperature. Considering that the wide temperature range for mesophilic microorganism growth includes room temperature and that microorganisms can multiply very quickly

Table 4. Food safety knowledge of consumers from Pelotas, Rio Grande do Sul State, Brazil (n = 92).

Statements	True n (%)	False n (%)	I do not know n (%)
Key 1 – Keep clean			
Is it important to wash your hands before handling food and several times during food preparation.	90 (97.8)	0 (0.0)	2 (2.2)
Keeping kitchen surfaces clean reduces the risk of illness.	92 (100.0)	0 (0.0)	0 (0.0)
Key 2 - Separate raw and cooked			
Keeping raw and cooked food separate helps to prevent illness.	75 (81.5)	2 (2.2)	15 (16.3)
The same cutting board can be used for raw and cooked foods provided it looks clean.	8 (8.7)	65 (70.7)	19 (20.7)
Key 3 - Cook thoroughly			
Cooked foods do not need to be thoroughly reheated.	13 (14.1)	44 (47.8)	35 (38.0)
Proper cooking includes meat cooked to 40 °C.	10 (10.9)	28 (30.4)	54 (58.7)
Key 4 - Keep food at safe temperatures			
Cooked meat can be left at room temperature overnight to cool before refrigerating.	16 (17.4)	59 (64.1)	17 (18.5)
Refrigerating food eliminates microorganisms	18 (19.6)	57 (62.0)	17 (18.5)
Key 5 - Use safe water and raw materials			
Food beyond its expiry date cannot be use.	89 (96.7)	2 (2.2)	1 (1.1)
Safe water can be identified by the way it looks.	18 (19.6)	69 (75.0)	5 (5.4)

in this range, keeping food at temperatures below 5°C and above 60°C is necessary to slow down and/or prevent the multiplication of microorganisms (Schiraldi; De Rosa, 2014; WHO, 2006).

Finally, in the "use safe water and raw materials" key, 96.7% consumers said that it is true that food beyond its expiry date cannot be use and 75% said that it is false that safe water can be identified by the way it looks.

According to the Brazilian Ministry of Health, households are the most frequent places associated with foodborne disease outbreaks in the country (Brasil, 2023), suggesting consumer failures when handling food at home. The need for spreading food safety knowledge to the general population, especially that related to thorough cooking and safe temperatures at which food should be kept. These two keys were those in which the consumer samples showed the lowest level of knowledge and were essential for preventing foodborne disease outbreaks, especially considering that microbiological analyses showed that half of the meat samples collected had high counts of aerobic mesophilic microorganisms. The spread of adequate information about safe temperatures and thorough cooking of food is essential so that even with a high level of contamination, these microorganisms are destroyed and do not put consumer health at risk.

CONCLUSIONS

Fifty percent of ground beef samples from commercial establishments in Pelotas, Rio Grande do Sul State, Brazil, had counts of aerobic mesophilic microorganisms above the limits established by Brazilian legislation, indicating unacceptable food quality. Only two samples had coagulase-positive staphylococci counts above those permitted by law, again posing a risk to consumer health. Failures in handling, temperature control, and hygiene at commercial establishments, as well as the quality of raw materials may be responsible for these results. Consumer food safety knowledge showed that the items related to "cook thoroughly" and "keeping food at safe temperatures" have the lowest knowledge points among consumers. Thus, dissemination of information about temperatures that ensure food safety is essential for consumers, especially considering that even with high levels of contamination, most microorganisms are destroyed by heat. Future studies should analyze the surfaces of equipment and facilities in commercial establishments, in addition to observing the meat handling and temperature control in these environments. Further studies are also needed to investigate consumer knowledge in detail as well as their food safety behaviors when handling food in their households.

FUNDING SOURCE

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil (CAPES; Finance Code 001) and the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS; PQG 21/2551-0002061-0). Declarations of interest: none.

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