

# Occurrence of Total and Thermotolerant Coliforms in fried pastries sold in bars in downtown Curitiba-PR, Brazil

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## Abstract

Food sold in bars, which sometimes do not have adequate sanitary conditions for producing, handling and selling them, end up being contaminated by microorganisms that may be harmful to health, causing infections and food poisoning. The main objective of the study is to identify the presence of total and fecal coliforms in fried pastels sold in bars in downtown Curitiba-PR, Brazil. The study had quantitative approach and included 20 randomly chosen establishments. Microbiological analyzes were performed using the Petrifilm method for counting colony-forming units and identification of microorganisms. There were total coliforms in 17 samples, and the sample with the highest contamination presented 41 colony-forming units. None of the 20 samples showed fecal coliform, which makes ground-beef pastels in accordance with the standards established by Resolution RDC 12 / 2001. Based on the results, the high concentration of total coliforms suggests greater caution in good manufacturing practices and handling to avoid spoilage and disease spread by food.

**Key words:** Food Handling. Food Microbiology. Foodborne Diseases. Coliform Group.

## Introduction

Lack of time and haste make that everything is done fast, including eating, and this ultimately results in the search for practical, easily accessible and low-cost food – the kind of food sold in cafeterias, bars, fairs and by street vendors in big urban centers.<sup>1,2</sup> The big problem of these foods is the unknown origin or the lack of information on the conditions under which these foods were prepared, such as fried and baked salty snacks, pastels (typical Brazilian fast food, consisting of deep fried pastry filled with meat, cheese, shrimp or vegetables), pies, cakes, sandwiches, among others, often prepared at the points of sale.<sup>3,4</sup>

There are various stages in the production of processed foods, wherein foods are exposed to contamination by diverse microorganisms, resulting from improper handling, contact with equipment and utensils not properly cleaned, or from the environment. Each of these stages can enable survival and growth of microorganisms that can lead to infections or food contaminations<sup>5</sup> by fungi, bacteria, viruses, pathogenic parasites and microbial toxins. Bacteria account for nearly 90% of the cases, and those more commonly found are *Escherichia coli*, *Listeria monocytogenes*, *Salmonella spp.*, *Staphylococcus aureus* and *Yersinia enterocolitica*.<sup>6</sup>

Survey of coliforms in foods is used as an indicator of the hygienic and sanitary conditions of the environment where foods are produced and of the individual who handles them. The presence of total and thermotolerant coliforms in foods indicates that there has been direct or indirect contact with fecal matter. Such survey is made according to the standards set forth in the Resolution RDC no. 12, of January 02, 2001.<sup>7,8</sup>

The group of total coliforms consists of gram-negative rod-shaped bacteria, non-sporulating aerobes or facultative anaerobes, capable of fermenting lactose that produces gas in 24 to 48 hours under a temperature of 35°C. The group of thermotolerant coliforms has the same definition as of the total coliforms, but they are restricted to bacteria capable of fermenting lactose that produces gas in 24 hours at 44.5-45.5°C. Bacteria belonging to these groups are predominantly of the family *Enterobacteriaceae*, bacteria of the genus *Escherichia spp.*, *Enterobacter spp.*, *Citrobacter spp.* and *Klebsiella spp.*, which are found in feces, vegetation and soil, except for *Escherichia coli*, only present in the intestinal tract of humans and homeothermic animals.<sup>9-11</sup>

Meat is an important source of proteins, which helps provide the human nutritional needs and micronutrients and is one of the major sources of iron, B vitamins, especially B12, and zinc. However, meat is also an excellent medium for proliferation of microorganisms, particularly

ground meat, commonly used to make sandwiches, pastel, pastries and rolls filling mixtures, among other dishes. Ground meat offers higher risk of contamination because it requires a larger contact surface, more handling, and has high water activity and suitable pH for the growth and multiplication of bacteria of food interest due to their ability to cause diseases.<sup>12</sup>

Foodborne diseases (FBD) are acquired by ingestion of water or foods contaminated by chemical or biological agents. They are very common, but many cases are not reported because they are confused with other pathologies, such as colds or indigestion. Main symptoms include vomits, diarrhea, stomach ache, nausea and fever. From 2001 to 2011, according to data from the Secretary of Health Surveillance, 8,663 FBD outbreaks were reported, which affected 163,425 Brazilians, of whom 112 died. FBDs may cause complications to the individual and are responsible for a high number of hospitalizations every year, with irreversible conditions.<sup>13-15</sup>

The present work aims to evaluate the microbiological quality of pastels filled with ground beef and sold in bars in downtown Curitiba-PR, Brazil, by determining the presence of total and thermotolerant coliforms.

## Methodology

Twenty samples of ground-beef pastels, each one weighing approximately 100 grams, were collected from establishments in the central region of Curitiba-PR. Samples were collected from May to June, 2014. The inclusion criteria were commercial establishments (bars) that usually sold ground-beef pastel, having a permit granted by the Curitiba City Administration, and valid sanitary license granted by the Health Surveillance agency. As exclusion criteria, it was considered: day-old ground-beef pastel; ground-beef pastel that burst during frying; and ground-beef pastel whose organoleptic properties have been changed at the time they were bought, such as the form and consistency.

The samples were then stored in an ice-filled isothermal box to maintain the same microbiological conditions they had when they were bought. Then, they were immediately transported to the Microbiology Laboratory of the University Autonomous Center of Brazil for microbiological analyses. After determination, the results of the microbiological analyses were related to the RDC no. 12/2001 of food microbiology for food quality control and safety.

All material used to process the samples was sterile and the entire operation was carried out near a Bunsen burner with half flame in a laminar flow cabinet. The microbiological analyses were performed using the Petrifilm® (3M Company) method – standard method of the Association of

Official Analytical Chemists (AOAC). The Petrifilm® method changes the gel color for the count of the colony-forming units (CFU) in plates, comprised of two sterile re-hydratable films impregnated with the culture medium that has violet red bile agar (VRBA) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity (5-bromo-4chloro-3indolil- $\beta$ -D-glucuronide) and a tetrazole indicator.<sup>16,17</sup>

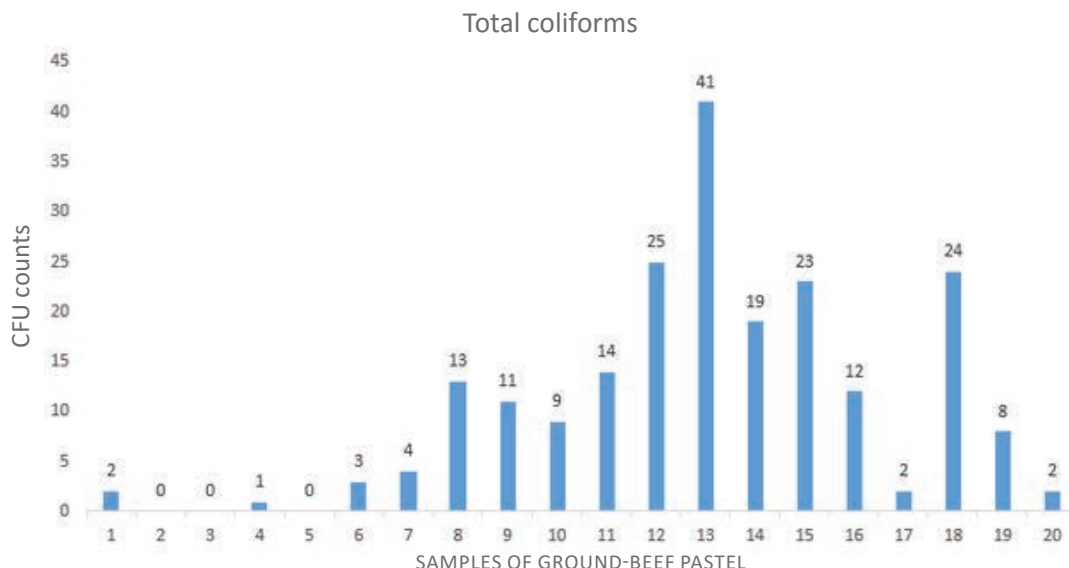
In laboratory, 25 grams of each ground-beef pastel were weighed and added to 225 ml of sterile peptone water, which were liquefied and homogenized for ten minutes, resulting in the sample. Following this process, three serial dilutions ( $10^1$ - $10^3$ ) were performed with the sample, composed of 9 ml of peptone water and 1 ml of the sample. With a pipette, 1 ml of the third dilution was inoculated onto the lower film of the Petrifilm® plate and covered again with the top film; then, after the gel solidification, the plates were incubated at 35°C for 48 hours for the colonies growth.<sup>16,17</sup>

For determination of the presence of total and thermotolerant coliforms in the samples, counts of CFUs were performed, where colonies of total coliforms that grew on the Petrifilm® film produced acid, making the pH indicator change the color of the gel to dark red and production of gas that was trapped around the red colonies, indicating the presence of total coliforms. For determination of thermotolerant coliforms (*Escherichia coli*), blue or red-blueish colonies occurred associated with gas bubbles.

Glucuronidase produced by *Escherichia coli* reacts with the color agent on the plate, forming a blue precipitate around the colony. The colonies that grew at the edge of the foam on the plate were not considered and counted, because they are not under the selective influence of the medium.<sup>16,17</sup> The results were recorded and analyzed in simple descriptive form through a databank built with the aid of Microsoft EXCEL®, and then tabulated and presented in the form of graphs.

## Results

It is very important to perform microbiological analysis, as of this study, for fried foods to be consumed on the spot, such as pastel filled with ground meat. It is possible to assess the quality of the product that is being sold, ensuring to consumers that the products are suitable for consumption.<sup>18</sup> Figure 1 shows the CFUs counts of total coliforms present in the samples.



**Figure 1.** Determination of the presence of total coliforms in fried pastels filled with ground beef sold in bars in downtown Curitiba-PR, Brazil. May to July 2014.

## Discussion

Current legislation does not provide microbiological parameters for total coliforms. The samples were analyzed for determination of the microbial count combined with the assessment of the hygienic-sanitary conditions of ground-beef pastels, knowing that these parameters reflect the quality of the food, environment hygiene, and the handlers' care in handling foods.<sup>18-20</sup> The presence of bacteria of the coliform group, especially total coliforms, is associated with improper handling and processing practices, procedures and sanitation in food production.<sup>20</sup> However, meat products with bacterial concentration around 10 CFUs/g already have their organoleptic properties compromised and so may pose health risk to consumers.<sup>10</sup>

In this study, only in three samples were not found bacteria of this group. The highest count was found in sample number 13, where 41 CFUs were detected. None of the 20 samples analyzed were contaminated with thermotolerant coliforms, which leaves the fried ground-beef pastels in conformity with the standards of the Resolution RDC no. 12, of January 02, 2001. It should be considered, however, that the product under analysis was sold after being cooked at temperatures above 60°C.<sup>17</sup>

These findings are similar to the study conducted by Brito<sup>21</sup> in Juazeiro do Norte-CE, where coliforms counts in the samples of hamburgers and hot dogs sold by street vendors in this town were in conformity with normal law standards. It is also possible to compare the results obtained by Pierozan et al.<sup>22</sup> in a study carried out at the Municipal Lake in Toledo-PR, which assessed the hygienic-sanitary quality of hot dogs sold on the streets. None of the six samples analyzed indicated the presence of thermotolerant coliforms, but in the group of total coliforms, growth of this group of microorganisms was detected in five samples.

However, a study carried out by Bezerra et al.<sup>23</sup> in Cuiabá-MT showed that 11.4% of the samples of sandwiches sold on the streets did not comply with the Brazilian regulations. The presence of these coliforms in the food determines the hygienic-sanitary quality either in handling or in preparation and whether the food is suitable for consumption.<sup>18</sup>

Contamination of this kind of food may be of various sources, such as storage areas, transport, meat grinders, handlers, containers and utensils. According to Normative Instruction no. 83, of November 21, 2003, issued by the Ministry of Agriculture, Livestock and Supply,<sup>24</sup> cooled ground meat should be kept at a temperature of 0°-4°C, and frozen ground meat at a maximum temperature of -18°C during storage. But, because of the high microbial count present in the samples analyzed in this study, it can be assumed that failures in some parts of these processes may have occurred.

Meat grinding is one of the key factors that favor contamination and multiplication of microorganisms. Because meat is a product largely consumed and for having factors that enable the development and proliferation of food spoilage and pathogenic microorganisms, strict hygiene and sanitary control procedures should be followed in all stages of foods processing and handling.<sup>25-27</sup>

The resolution RDC no. 216, of September 15, 2004, which sets forth Technical Regulation for the Best Practices in Food Services,<sup>28</sup> corroborates the present study regarding proper cleaning and sanitation of the facilities, equipment, furniture and utensils, in order to prevent propagation of microorganisms. The foods preparation sector must be cleaned as many times as needed and immediately after completion of the work. The handlers' hygiene must be ensured by means of written records and by regular monitoring of their personal hygiene and proper foods handling in order to prevent FBDs. Heat treatment of cooking must be considered, as well as the time-temperature relationship, to ensure hygienic-sanitary quality and food safety.<sup>28</sup>

## Conclusion

The findings indicate that the microbiological quality of ground meat-filled pastels are in conformity with the recommendations defined by the Health Surveillance, when compared to the RDC no. 12/2001. However, the high levels of total coliforms found indicate that the product may pose risk to consumers. Although there are no reference values for total coliforms, their presence in the food may suggest that it was prepared and handled in improper conditions for consumption, which diminishes the foods shelf-life.

From the point of view of the National Health Surveillance Agency (ANVISA), society should have access to safe foods, of good quality, according to established standards, not only regarding the nutritional values but the hygiene conditions that provide food safety and good health to consumers.

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