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Short communication

Removal of final wash in chicken slaughter process does not affect microbiological quality of carcasses

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ARTICLE INFO

Keywords:
Escherichia coli
Indicator microorganism
Slaughterhouse
Water

ABSTRACT

Water is used in several stages of the technological process of chicken slaughter. Since it is a finite natural resource, the objective of this study was to evaluate the effect of reducing the water flow in the final wash on the superficial microbial contamination of chicken carcasses in a slaughterhouse. The experimental design was completely randomized ($3 \times 10 \times 3$), and comprised the evaluation of three treatments, i.e., absence of water (T1), flow of 1 L per carcass (T2), and flow of 1.5 L per carcass (T3), with 10 repetitions performed in triplicates. A total of 240 samples were obtained at three points in the slaughter line: before (A, $n = 90$) and after (B, $n = 60$) the final washing of the carcasses and after cooling (C, $n = 90$). Analyses of counts of aerobic mesophilic microorganisms, enterobacteria, total coliforms, and *Escherichia coli* were performed. The final wash reduced the microbiological contamination of the carcasses but suffered direct interference from the pre-cooling system ($P > 0.05$), which canceled the reduction. Hence, the final wash step may be removed without affecting the microbiological quality of the carcasses, making it possible to reduce water consumption and effluent generation.

1. Introduction

The technology for obtaining and processing chicken meat in slaughterhouses involves several stages, numerous installations, and various equipment that, together with handling and the binomial time and temperature, affect the sanitary aspects of the products (Brasil, 1998; Brasil, 2019a; Soares et al., 2018; Viana et al., 2020). During slaughter, the microbiological quality of the products and process are determined by quantifying indicator microorganisms such as aerobic mesophiles, total coliforms, thermo-tolerant coliforms, enterobacteria, and *Escherichia coli*, to infer the occurrence of handling, processing, production, and storage failures (Jarvis et al., 2016; Matias, Pinto, Cossi, & Nero, 2010; Rodrigues et al., 2008).

In chicken meat slaughtering and processing industries, water is a tool of great importance for ensuring hygienic quality and attention to parameters of quality and safety programs. Stages such as scalding, evisceration, plucking, and pre-cooling consume large amounts of water, and are therefore considered critical because this resource is finite, and has a high cost and great environmental impact due to the generation of effluents (Barana, Botelho, Wiecheteck, Doll, & Simões, 2014; Mekonnen & Hoekstra, 2012). Hence, the sustainable use of water and pursuit of cleaner production make it necessary to plan the processing of chicken meat, with the aim to increase the efficiency of the technological steps, and consequently, reduce the environmental impact, consumption of available resources, and costs (Lopes Silva, Delai, Castro, & Ometto, 2013; Seidavi, Zaker-Esteghamati, & Scanes, 2019; UNESCO, 2001).

Regardless of the size of the slaughterhouse, the average water consumption is 30 L per chicken slaughtered, considering all stages of the process, and in the final washing system before pre-cooling in a chiller, a volume of 1.5 L per carcass is recommended (Brasil, 1998; 2019a). Considering the volume of chicken slaughtered in the first quarter of 2019 (Brasil, 2019b), approximately 2.2 billion liters of water would have been spent only in the final wash shower.

Considering the necessity to reduce the use of water in slaughter, studies have been carried out to evaluate the consequences of

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BAC TRAT 2A® biological modulator performed well in the control of *Salmonella* sp. in production environment and digestive tract of broilers

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HIGHLIGHTS

- BAC TRAT 2A® demonstrated efficacy in the control of *Salmonella* sp.
- Serovars of *S. enterica* subsp. *enterica* (O:4,5) and (O:4,5:k-), single-phase were the most prevalent.
- The evaluated isolates showed multidrug resistance.

ARTICLE INFO

Keywords:
Biological modulator
Poultry industry
Health challenge
Salmonellosis

ABSTRACT

Biological methods such as the use of competitive and beneficial microorganisms that hinder microbial survival have been gaining prominence in the poultry industry, with a view to reducing contamination by pathogens of importance in the sector, such as *Salmonella* sp. The objectives of this study were to evaluate the effect of the biological modulator BAC TRAT 2A® on the reduction of *Salmonella* sp. in the poultry bedding and in the gastrointestinal tract of broilers under real housing conditions subject to natural infection by the pathogen, as well as to evaluate the genotypic and phenotypic profile of the isolates obtained. The experiment was carried out between March 2018 and August 2019, in the state of Paraná. The study consisted of two sheds, the bed used in shed 1 being the negative control (C) and the bed in shed 2 being the treatment (T), being treated with the biological modulator BAC TRAT 2A®. The performance of BAC TRAT 2A® was positive for reducing the health challenge involving the presence of *Salmonella* sp. during poultry farming. Both in the environment and in the birds' caecum there was a decrease in the pathogen load, mainly in the final stages of the breeding cycle, possibly caused by the competitive exclusion in the aviary litter and biological modulation of the birds' microbiota in contact with the product.

1. Introduction

Brazil stands out in the world poultry scenario for being the largest producer and the second largest exporter of chicken meat in the world, being responsible for 14 % of world production in 2018 (USDA, 2019). To be in this position, it is necessary for the country to maintain the

health status of the national squad and to control pathogens such as *Salmonella*, the main cause of bacterial diarrhea in humans, with a worldwide estimate of 153 million cases and 57 thousand deaths annually (CDC, 2020).

In the production of broilers there are several sources of contamination of birds by *Salmonella* in the field and one of them is bedding,

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Pure and mixed biofilms formation of *Listeria monocytogenes* and *Salmonella* Typhimurium on polypropylene surfaces

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ARTICLE INFO

Keywords:
Biofilms
Food industry
Gene expression
Persistence

ABSTRACT

This study evaluated the capacity of *Listeria monocytogenes* and *Salmonella* Typhimurium to form pure and mixed biofilms on polypropylene surface and analyzed the expression of genes related to biofilm formation within 24 h and 120 h. The planktonic and sessile growth of *L. monocytogenes* and *S. Typhimurium* were related to incubation temperature. There was no statistical difference between the total population not only in pure *L. monocytogenes* and pure *S. Typhimurium* biofilms, but also in mixed biofilms at 12 °C and 37 °C. Pure and mixed biofilms were able to maintain viable cells for 3000 h at 10 °C with counts greater than when incubated for 120 h, with a greater population increase in mixed biofilms. Regarding the 24 h and 120 h biofilms of *L. monocytogenes* in both pure and mixed conditions at 12 °C and 37 °C, we were able to find expression of *prfA*, *agrB*, *agrC* and *agrD* genes. Concerning *S. Typhimurium* biofilms, we found expression of *agfA*, *adrA* and *csgD* genes in all situations evaluated, except for mixed *S. Typhimurium* biofilm at 12 °C for 24 h. This article documents the ability of *L. monocytogenes* and *S. Typhimurium* to form mixed biofilms and suggests that these two microorganisms may act synergistically.

1. Introduction

Listeria monocytogenes and *Salmonella* spp. are microorganisms that can be carried by food and cause illnesses to consumers (Brasil, Unidade de Vigilância, & de Transmissão, 2018; CDC, 2019). These microorganisms have several mechanisms to endure adverse conditions, such as nutrient deprivation, low temperatures, pH variation, high salt concentration, resistance to antimicrobials and sanitizers, and capacity to form biofilms (Kadam et al., 2013; Lamas et al., 2018; Lee, Hébrard, & Bernardi, 2017; Viana et al., 2019).

Several studies have reported the occurrence and persistence of *L. monocytogenes* and *Salmonella* spp. in food processing environments and facilities (Carvalho et al., 2019; Nowak et al., 2017; Sereno et al., 2019; Viana et al., 2019), and their presence in these niches may be related to their ability to adhere, colonize, and form biofilms. Biofilms

are complex ecosystems normally consisted of more than one microbial species immersed in an array of extracellular polymers, depending on the interaction amidst microbial cells, fixation surface, and environmental conditions (Costerton, 1999; Ferreira, Wiedmann, Teixeira, & Stasiewicz, 2014). Such structures provide protection, adaptability, and resistance of microorganisms involved in this matrix, becoming a source of contamination among environments and products (Kadam et al., 2013; Lee et al., 2017; Oliveira, Brugnara, Alves, & Piccoli, 2010).

However, most *in vitro* studies are about monospecies biofilms, which do not simulate natural conditions (Kadam et al., 2013; Lee et al., 2017; Wang, Dong, Wang, Xu, & Zhou, 2016). Studies on multispecies biofilms are able to search for characteristics as synergism, antagonism, and competition, which are factors that can interfere with adhesion, maintenance, and resistance of biofilms (Fagerlund, Merette, Heir, Briand, & Langsrud, 2017; Grandi, Pinto, & Destro, 2018; Rieu,

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Characterization of microbial ecology, *Listeria monocytogenes*, and *Salmonella* sp. on equipment and utensil surfaces in Brazilian poultry, pork, and dairy industries

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ARTICLE INFO

Keywords:
Accompanying microbiota
Biofilms
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Microbial diversity
Pathogen prevalence

ABSTRACT

This study aimed to evaluate the level of counting by indicator microorganisms, identify the microbial ecology, detect *Listeria monocytogenes* and *Salmonella* sp., and determine the presence of virulence genes and biofilm formation. A total of 480 samples were collected from the surfaces of the equipment and utensils using sterile swabs for the detection of *L. monocytogenes* and *Salmonella* sp. and counting mesophilic aerobes, Enterobacteriaceae, *Escherichia coli*, and *Pseudomonas* sp. The microbial ecology was evaluated by sequencing the 16S rRNA gene. Genes for virulence and biofilm formation were analyzed and adhesion capacity was evaluated for *L. monocytogenes* and *Salmonella* sp. The mesophilic aerobe count was the highest in the dairy processing facility, followed by the pork and poultry slaughterhouses. *L. monocytogenes* was detected in all facilities, with the highest detection in the pork slaughterhouse, followed by the poultry and dairy facilities. *Salmonella* sp. was only detected in the dairy. Isolates of *L. monocytogenes* and *Salmonella* sp. showed poor adhesion to polystyrene surfaces, virulence genes, and biofilm formation. The frequent contaminants in the slaughterhouses were *Pseudomonas*, *Acinetobacter*, and *Aeromonas* in poultry, *Acinetobacter*, *Pseudomonas*, and *Brevibacterium* in pork, and *Pseudomonas*, *Kocuria*, and *Staphylococcus* in dairy. Our results provide useful information to understand the microbiological risks associated with contamination.

1. Introduction

The close contact that occurs in industries between products of animal origin, equipment, and utensils during the food production process serves as a constant source of microbial food contamination (Mues et al., 2019; De Filippis, Valentino, Alvarez, Ordóñez, Cotter, & Ercolini, 2021). Once contaminated, these surfaces can retain deteriorating and pathogenic microorganisms (Vidal et al., 2017; Sereno et al., 2019; Song, Wang, & Xu, 2021; Agostinho Davanzo, Dos Santos, Castro, Palma, Pribul, Dallago, & Santana, 2021).

To remove dirt and reduce contamination to acceptable levels, Sanitation Standard Operating Procedure (SSOP), which includes cleaning and disinfecting, is routinely applied to surfaces that come into contact with food (Brasil, 2003; Moretto & Langsrud, 2017). However, failures in these procedures can influence the survival of microorganisms and the quality and safety of the final food products (Saini et al., 2011; Agüeria, Libonatti, & Civit, 2021).

The efficiency of SSOPs is usually monitored by counting indicator microorganisms or using other techniques, such as bioluminescence. These techniques either directly measure viable microorganisms or

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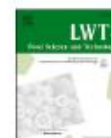
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Preoperational cleaning processes interfere with microbial ecology and presence of *Listeria monocytogenes* and *Salmonella* spp. on food conveyor belts of a poultry slaughterhouse in Brazil

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ARTICLE INFO

Keywords:
Cleaning
Conveyor belt surfaces
Food industry
Microbiota
Pathogen occurrence

ABSTRACT

This study aimed to evaluate the preoperational cleaning process of a poultry slaughterhouse regarding microbial ecology, microorganisms indicators of hygienic-sanitary conditions, *Listeria monocytogenes* and *Salmonella* spp. Three hundred and twenty samples were collected from the surfaces of meat cut conveyor belts before (n = 160) and after (n = 160) the preoperational cleaning process to count mesophilic aerobes, Enterobacteriaceae, and *Pseudomonas* spp., in addition to the detection of *L. monocytogenes* and *Salmonella* spp., and identification of microbial ecology by sequencing the 16S rRNA gene. The hygiene process reduced the counts of all the indicator microorganisms (87% a 91%) and eliminated *Salmonella* spp. Despite this significant decrease, *L. monocytogenes* persisted on some of the conveyor belts. Microorganisms associated with obtaining and handling meat cuts, including *Anaerobacillus*, *Anoxybacillus*, *Enterococcus*, and *Pseudomonas*, were used to characterize the microbial ecology before cleaning. A disturbance in microbial ecology was identified post-cleaning, in which microorganisms with greater adaptation and tolerance to industrial conditions, such as *Pseudomonas*, *Acinetobacter*, and *Aeromonas*, were more prevalent. Our results revealed that the cleaning and sanitization procedures were effective in reducing the levels of indicator microorganisms and *Salmonella* spp. The applied hygiene process also shaped the composition of the existing microbial ecology.

1. Introduction

During the slaughtering and processing of chicken meat, several pieces of equipment and utensils can serve as potential sources of both pathogenic and deteriorating microorganisms for food, affecting the sanitary and organoleptic quality of the products. (Agostinho Davanzo et al., 2021; Saenz-García, Castañeda-Serrano, Mercado Silva, Alvarado, & Nava, 2020; Samapundo et al., 2019; Viana et al., 2020). Contamination of these surfaces may be associated with the autochthonous microbiota found in birds as well as with technological and hygienic sanitation while obtaining meat cuts (Golden, Rothrock, & Mishra, 2021; Marmion, Ferone, Whyte, & Scannell, 2021). To reduce the

chances of contamination, industries implement cleaning and sanitization procedures in their facilities, which are conducted after the production processes in order to remove organic matter, reduce the microbial load to acceptable levels, and eliminate pathogenic microorganisms (Marette & Langsrud, 2017).

In Brazil, the food industry relies on the detection of *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* and the total bacterial count to evaluate the effectiveness of preoperational hygiene processes (Agostinho Davanzo et al., 2021; Schafer et al., 2017; Viana et al., 2020). Mesophilic aerobes and enterobacteria are good quality indicators of such cleaning processes, as they are sensitive to microbial control methods (EC, 2001; Saimi et al., 2011). *L. monocytogenes* and *Salmonella* spp. are among the main pathogenic foodborne bacteria worldwide

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Inhibitory effects of piperine and black pepper essential oil on multispecies biofilm formation by *Listeria monocytogenes*, *Salmonella Typhimurium*, and *Pseudomonas aeruginosa*

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ARTICLE INFO

Keywords:
Antibiofilm
Bioactive compounds
Gene expression
Inhibition mechanism

ABSTRACT

This study aimed to evaluate the antimicrobial and antibiofilm activities of black pepper essential oil (BPEO) and piperine and the effect of piperine on gene expression in a multispecies biofilm composed of *Listeria monocytogenes*, *Salmonella Typhimurium*, and *Pseudomonas aeruginosa* on a polypropylene surface. The minimal inhibitory concentrations of BPEO and piperine were 100 and 25 mg/mL, respectively, against this consortium of microorganisms. Sessile cell counts were 5.35–7.35 log CFU/cm² and varied over time. The total population eradicated in the biofilm ranged from 78.9% (5.88 log CFU/cm²) to 99.8% (4.16 log CFU/cm²). Evaluation of biofilm-related gene expression showed upregulation of the *L. monocytogenes* genes *agrC* (24 and 72 h), *agrD* (72 h), and *prfA* (72 h) and downregulation of all evaluated *S. Typhimurium* and *P. aeruginosa* genes (24 and 72 h) in the untreated control biofilm. The addition of piperine resulted in upregulation of the *L. monocytogenes* genes *agrB* (24 and 72 h), *agrC* (72 h), *agrD* (24 and 72 h), and *prfA* (24 h); the *S. Typhimurium* genes *agrA* (24 and 72 h), *agrA* (24 and 72 h), and *csgD* (72 h); and all evaluated *P. aeruginosa* genes (24 and 72 h). Piperine more effectively controlled the multispecies biofilm.

1. Introduction

In the food industry, persistence of microorganisms on abiotic surfaces has been reported. Interactions among these microorganisms can alter their survival and persistence on industrial surfaces (Fagerlund, Langsrud, & Moreira, 2020; Iniguez-Moreno, Gutiérrez-Lomeli, & Avila-Novoa, 2019; Sereno et al., 2019). The interactions in biofilms can be mutually beneficial, increasing microorganism survival due to improved resistance to environmental stress and increasing their individual persistence in these environments (Bridier et al., 2015; Pang, Chen, & Yuk, 2019). Adherent bacterial cells in a biofilm and free cells differ in terms of gene expression and metabolism (Guzmán-Soto et al., 2021). Understanding the underlying mechanisms can improve the detection and control of microbial biofilms in food processing

environments.

Listeria monocytogenes and *Salmonella Typhimurium* are important pathogens in food handling environments (CDC, 2019; Sereno et al., 2019; Scobie et al., 2019). The cooperation and interaction between pathogenic and non-pathogenic microorganisms have been evaluated in vitro. There are studies reporting the formation of mixed biofilms by *L. monocytogenes* and *Salmonella* spp. (Govaert, Smet, Wabits, & Van Impe, 2019; Tadielo et al., 2022) and the interaction of these pathogens together with *Lactobacillus* sp. (Jara et al., 2020) and *Pseudomonas aeruginosa* (Yamakawa, Tomita, & Sawai, 2018) on food handling surfaces.

P. aeruginosa is an opportunistic microorganism with high food deterioration capacity at refrigeration temperatures that can directly affect the shelf life of food products (Raposo, Pérez, De Faria, Ferrás, & Carrascosa, 2017). The ability of *P. aeruginosa* to form biofilms on

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Detection of adenovirus, rotavirus, and hepatitis E virus in meat cuts marketed in Uruguaiana, Rio Grande do Sul, Brazil

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Keywords:
Contamination
Enteric virus
Food
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ABSTRACT

The aim of this study was to investigate the presence of adenovirus (AdV), rotavirus (RV), and hepatitis E virus (HEV) in beef, pork, and chicken meat cuts in retail trade in the city of Uruguaiana, RS, Brazil. A total of 131 meat products were collected (beef, $n = 35$; chicken, $n = 47$; pork, $n = 29$) from 18 commercial establishments (supermarkets, $n = 7$; butchers, $n = 7$; markets/grocery stores, $n = 4$). All samples were evaluated for AdV, RV, and HEV. The genomes of RV and AdV were identified in 29% ($n = 38$) and 5.34% ($n = 7$) of the samples, respectively. HEV was not identified in any of the samples. Chicken cuts had a higher frequency of AdV and RV isolates compared to beef and pork ($P < 0.05$). Among the categories of commercial establishments evaluated, all revealed at least one positive sample for AdV and RV; however, supermarkets showed a higher frequency of RV than others ($P < 0.05$). The genetic material of AdV and RV was identified simultaneously in 2.29% ($n = 3$) of samples from supermarkets ($n = 2$) and grocery stores ($n = 1$). This is the first report on detection of enteric viruses in meat cuts in the western region of the state of Rio Grande do Sul, Brazil, and the presence of AdV and RV in these products may indicate flaws during the process of handling these foods, especially in places where commercialization provides important public health issues.

1. Introduction

Foodborne diseases are classified as a global public health issue, directly affecting the economic situation of developed and underdeveloped countries [1,2]. Currently, official data show an increase in cases of gastroenteritis and more serious illnesses related to the consumption of contaminated food. Among the reports on outbreaks, bacterial pathogens have been identified as the main etiological agents [3–5].

However, despite underreporting and underdiagnosis, foodborne diseases caused by viruses are a common cause of outbreaks and

gastroenteritis in humans and animals [6–8]. Between 2009 and 2018, 6809 outbreaks were reported in Brazil, involving 120,584 individuals, causing 99 deaths. The viral etiologic agents associated with these outbreaks included 3.9% norovirus (NoV), 3.1% rotavirus (RV), and 1.2% hepatitis A virus (HAV) [3]. Over the same period, these etiologic agents were responsible for 18,156 outbreaks in the US, involving 620,853 individuals, resulting in 765 deaths [4]. The main foods associated with the transmission of human enteric viruses are raw fish, meat, fruits, and vegetables grown or handled in contaminated environments [9–12].

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Effect of ginger essential oil and 6-gingerol on a multispecies biofilm of *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Pseudomonas aeruginosa*

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Abstract

The objective of this study was to evaluate the potential antimicrobial and antibiofilm effect of ginger essential oil (GEO) and 6-gingerol on a multispecies biofilm formed by *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Pseudomonas aeruginosa* on a polypropylene surface. The minimum inhibitory concentration concentrations obtained for GEO were 100 and 50 mg/mL and for 6-gingerol 1.25 mg/mL. Sessile cell counts ranged within 5.35–7.35 log CFU/cm² in the control biofilm, with the highest sessile growth at 72 h. GEO treatments acted on the total population regardless of concentration at 1 and 48 h. *L. monocytogenes* behaved similarly to the total population, showing GEO action at 1 h and keeping the same pattern at 48, 72, and 96 h. Better action on *S. Typhimurium* was obtained at times of 1, 72, and 96 h. *P. aeruginosa* showed logarithmic reduction only when treated with GEO 50 mg at 24 h. As for 6-gingerol, in general, there was no significant action ($p > 0.05$) on the evaluated sessile cells. GEO showed antimicrobial activity against *L. monocytogenes*, *S. Typhimurium*, and *P. aeruginosa*, acting as an inhibitor of biofilm formation. As for 6-gingerol, it was considered a possible antimicrobial agent but without efficacy during biofilm formation.

Keywords Antibiofilm · Antibacterial · Control methods · Essential oils

Introduction

Listeria monocytogenes, *Salmonella* Typhimurium, and *Pseudomonas aeruginosa* have the ability to adhere and form biofilm on the surfaces of equipment and utensils used in food processing [1–3]. Microorganisms secrete an extracellular matrix composed of polysaccharides, proteins, and nucleic acids during biofilm formation, which originates an

ecosystem that provides cell protection and maintenance in hostile environments [4, 5]. This barrier help increase tolerance to biocides and antibiotics at usual concentrations, resulting in persistent bacterial contamination in the production environment, with a potential risk to public health [1].

The inappropriate use of commercial biocides has been responsible for negative effects on the environment, residual accumulation in the production system, reduced efficacy, and development of co-resistance in pathogenic microorganisms [6–8]. Thus, in recent years, the scientific community has shown greater interest in studying the use of natural compounds such as essential plant oils and their active principles in the industrial context [9, 10] owing to its bactericidal and biodegradable characteristics [11, 12], with great environmental gain and low toxicity.

Ginger essential oils (GEO) (*Zingiber officinale*) are liquid, oily, and aromatic substances extracted from roots that have anti-inflammatory [13], antioxidant [14], antimicrobial [10], and antifungal properties [15]. These natural compounds act on the cell membrane integrity of microorganisms, interfering in protein and microbiological marker

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3) Produção Científica – Artigo publicado em periódicos – A3

3.1

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Research Paper

Residues of Veterinary Drugs in Animal Products Commercialized in the Border Region of Brazil, Argentina, and Uruguay

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ABSTRACT

The traffic in international animal products can become a public health hazard when legal import sanitary procedures are not followed. In Brazil, due to its extensive border area, the importation of animal products is a common practice in many areas, especially in Rio Grande do Sul, a state that borders Argentina and Uruguay. The objective of this study was to evaluate the presence of residues of veterinary drugs (antibiotics and antiparasitics) in animal products consumed in Rio Grande do Sul. The presence of residues of veterinary antibiotics and antiparasitics was assessed in 189 meat (beef, pork, and chicken), processed dairy, and meat product samples bought in Argentina ($n = 90$) and Uruguay ($n = 99$). Residues of these veterinary drugs were detected in 50 (26.45%) of the samples; 28 samples (14.81%) had antibiotic residues, and 22 samples (11.64%) had antiparasitic residues. Of the 50 positive samples, 40% (15 from Argentina and 5 from Uruguay) had residues above the maximum residue limits (MRLs). Of these 20 samples, 12 had antiparasitic residues above the MRLs (11 beef samples had ivermectin and 1 pork sample had ivermectin and doramectin) and 8 had antibiotic residues above the MRLs (2 pork and 2 sausage samples had doxycycline, 2 cheese samples had doxycycline and chlortetracycline, 1 poultry meat sample had chloramphenicol, and 1 cheese sample had monensin). Because of the potential toxic effects on humans and the potential for pathogens to develop antibiotic resistance, the presence of these residues above the MRLs is a potential risk to public health. The negative impact of consumption of imported animal products can be reduced by implementation of an effective surveillance system and educational campaigns for the general population.

HIGHLIGHTS

- Residues of veterinary drugs were detected in 26.45% of animal product samples.
- Antibiotic residues above the MRLs were detected in 4.23% of samples.
- Antiparasitic residues above the MRLs were detected in 6.34% of samples.
- Action by the Brazilian health surveillance agencies is needed to protect public health.

Key words: Animal products; Border; Chemical residues; Chloramphenicol; Ivermectin

International movement of animal products may pose a risk to public and animal health (33). Meat and milk and their products may contain chemical and biological hazards, particularly where production for sale does not comply with sanitary rules to assure safety. In this context, international border regions have been identified as places where animal products trafficked among countries can endanger public health (27, 31, 36, 38).

Rio Grande do Sul is a southern Brazilian state that has an extensive border with Argentina and Uruguay, and despite the efforts of border health surveillance agencies, the transit of animal products and animals among these three countries occurs clandestinely. Many of the products sold across these borders are produced without proper sanitary control of the raw material, potentially compromising product safety and exposing consumers to numerous hazards (37). One type of potential public health hazard is residues of veterinary drugs, which are widely used in animal production to prevent and cure diseases (5). As in

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Identification of *Salmonella* spp., *Listeria monocytogenes*, and indicator microorganisms in commercialized raw meats and fresh sausages from Uruguaiana, Rio Grande do Sul, Brazil

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ABSTRACT: This study evaluated the sanitary hygienic quality and the presence of pathogenic microorganisms in raw meats and fresh sausages marketed in the city of Uruguaiana, Rio Grande do Sul (RS), Brazil. We analyzed 238 samples of fresh sausages, beef, pork, and chicken from 18 commercial establishments (butchers, supermarkets, and groceries). Samples were subjected to enumerate hygiene indicator microorganisms (mesophilic aerobes and enterobacteria) and detection of *Salmonella* spp. and *Listeria monocytogenes*. The mean counts of mesophilic aerobes and enterobacteria were 5.09 and 3.54 log CFU/g, respectively. Beef samples presented the highest frequency of *Salmonella* spp. (7.93%) and fresh sausages present the highest frequency of *L. monocytogenes* (19.04%). Among the analyzed samples, 43.70% did not comply with the microbiological parameters established by the Brazilian Ministry of Health. The presence of *Salmonella* spp. and *L. monocytogenes* in different samples and commercial establishments demonstrate the failures of good manufacturing practices in industrial environmental and retail points and the need to train food handlers to reduce the exposure of consumers to potential risks.

Key words: microbial contamination, pathogens, retail trade, sanitary hygienic quality.

Identificação de *Salmonella* spp., *Listeria monocytogenes* e micro-organismos indicadores em carnes cruas e embutidos frescos comercializados na cidade de Uruguaiana, Rio Grande do Sul, Brasil

RESUMO: Este estudo teve como objetivo avaliar a qualidade higiênica sanitária e a presença de microrganismos patogênicos em carnes in natura e linguiças frescas comercializadas na cidade de Uruguaiana, Rio Grande do Sul (RS), Brasil. Foram analisadas 238 amostras de linguiças, carne bovina, suína e de frango de 18 estabelecimentos comerciais (açougues, supermercados e mercearias). As amostras foram submetidas à enumeração de microrganismos indicadores de higiene (aeróbios mesófilos e enterobactérias) e detecção de *Salmonella* spp. e *Listeria monocytogenes*. As contagens médias de aeróbios mesófilos e enterobactérias foram 5,09 e 3,54 log UFC/g, respectivamente. *Salmonella* spp. esteve presente mais frequentemente em amostras de carne bovina (4,91%), enquanto *L. monocytogenes* foi mais frequente em linguiças frescas (19,04%). Das amostras analisadas, 43,70% não atenderam aos parâmetros microbiológicos estabelecidos pelo Ministério da Saúde. A presença de *Salmonella* spp. e *L. monocytogenes* em diferentes amostras e estabelecimentos comerciais demonstra falhas de boas práticas de fabricação na indústria e pontos de venda, além da necessidade de treinar manipuladores de alimentos para reduzir a exposição dos consumidores a riscos.

Palavras-chave: comércio varejista, contaminação microbiana, patógenos, qualidade higiênico-sanitária.

INTRODUCTION

The high consumption of meat and meat products is a trend in developing countries like Brazil and has led to increased concerns from health authorities regarding food quality and safety (LOPES et al., 2017; ABUJAMRA et al., 2017; FANALLI et al., 2018). Raw meats and fresh sausages are characterized

by high water content, and the presence of nutrients and pH that provide a favorable environment for multiplication of microorganisms (LAWRIE, 2005). Failures in good manufacturing practices at any stage of the processing can result in contamination by pathogenic microorganisms, demanding rigorous control along the production chain (JARVIS et al., 2016; DEMAÏTRE et al., 2020).

Food handling in the domestic environment: an online questionnaire study with respondents from 24 of 26 Brazilian states

Manipulação de alimentos no ambiente doméstico: um estudo realizado com emprego de questionário online respondido por habitantes de 24 dos 26 estados brasileiros

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ABSTRACT

Using an online questionnaire, this study evaluated the profile of a Brazilian population's food handling practices in the home environment. The questionnaire, containing questions about domestic behavior in terms of hygiene and food handling, was built and available through social media sites. Information about the participants' profiles, their food pre-preparation, food preparation, and food post-preparation practices, and the occurrence of foodborne diseases (FBDs) was included in the questionnaire. A total of 701 responses were obtained. The interviewees included 78.31% female participants and 21.68% male participants, with an average age of 31.2 years. Nearly all (94.3%) had a complete or incomplete higher education. In the pre-preparation stage, the participants evaluated the shelf life (97.28%) and storage temperature (44.79%) of the products while purchasing them. Regarding food handling practices, only a few participants washed the food packages before storing them (31.95%) or removed hand jewelry or other adornments when washing food (61.48%). Most participants washed their hands (91.58%) and washed vegetables (99.28%). But a group of interviewees reported washing raw meat (27.81%) before preparing it. Cutting surfaces such as plastic (50.36%) and glass (49.36%) tops were the most prevalent in the study. Most respondents did not know how long they had been using their cutting boards (67.62%) and mentioned using the same surface to handle both raw and ready-to-eat products (84.17%). As for the preparation, most interviewees declared they did not check the food temperature during preparation (86.31%), ignoring the ideal cooking temperature (88.26%). Regarding the occurrence of FBDs, 79.17% of the interviewees reported having suspicious clinical signs associated with contaminated foods and 65.59% did not seek medical help. Thus, the participants demonstrated ignorance about adequate practices for food safety in the home environment, highlighting the need to conduct health education programs within the Brazilian population.

Keywords: Food safety. Good handling practices. Food consumption. Foodborne diseases.

RESUMO

Este estudo teve como objetivo avaliar o perfil das práticas de manipulação de alimentos no ambiente domiciliar no Brasil utilizando um questionário online. Um questionário contendo perguntas sobre comportamento doméstico em nível de higiene e manipulação de alimentos foi construído e disponibilizado por redes sociais. O questionário continha informações sobre o perfil dos participantes, suas práticas de pré-preparo, preparo e pós-preparo de alimentos e a ocorrência de doenças transmitidas por alimentos (DTA). Obteve-se 701 respostas, os entrevistados foram 78,31% do sexo feminino e 21,68% do sexo masculino, com média de idade de 31,2 anos. A maioria (94,3%) possuía ensino superior completo ou incompleto. Na etapa de pré-preparo, os participantes avaliam o prazo de validade (97,28%) e a temperatura de armazenamento (44,79%) dos produtos no momento da compra. Em relação às práticas de manipulação dos alimentos, apenas alguns participantes lavavam as embalagens dos alimentos antes de armazená-los (31,95%) ou retiravam adornos ao lavar os alimentos (61,48%). A maioria dos participantes lavam as mãos (91,58%) e os vegetais (99,28%); entretanto, um grupo de entrevistados relatou lavar carne crua (27,81%) antes de prepará-la. Superfícies de corte como tábuas de plástico (50,36%) e de vidro (49,36%) foram os mais prevalentes no estudo. A maioria dos entrevistados não sabe há

SOBREVIVÊNCIA DE *SALMONELLA ENTERICA* ÀS CONDIÇÕES GÁSTRICAS SIMULADAS *IN VITRO*: PAPEL DOS FATORES DE ADESÃO E RESISTÊNCIA ANTIBACTERIANA

Survival of Salmonella enterica under in vitro simulated gastric conditions: role of adhesion factors and antibacterial resistance

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Resumo: O estudo tem como objetivo verificar a capacidade de adesão e formação de biofilme de isolados de *Salmonella* spp. produtores de enzimas beta-lactamases de espectro estendido (ESBL), bem como a resistência de isolados produtores ou não de ESBL, frente às condições gástricas e intestinais simuladas *in vitro*. Foram utilizados 18 isolados de *Salmonella* spp. obtidos de cortes de frangos congelados produzidos no Estado do Paraná, Brasil. Os isolados produtores de ESBL foram avaliados de forma fenotípica e genotípica quanto a capacidade de adesão e formação de biofilme em lâminas de polipropileno. Além disso, todos os isolados foram submetidos a avaliação *in vitro* de resistência às condições gástricas e intestinais e após as simulações, realizadas contagens das células sobreviventes. Com relação à adesão e formação de biofilme, 13 isolados apresentaram essa capacidade. Quanto a tolerância às condições gástricas e intestinais, a quantificação de *Salmonella* spp. diminuiu ($p < 0,05$) conforme a passagem do trato gastrointestinal. Em relação à comparação de isolados produtores e não produtores de ESBL, não houve diferença ($p > 0,05$) na taxa de sobrevivência total. Os resultados demonstram que, apesar da diminuição da contagem de microrganismos conforme a passagem simulada pelos sucos gástricos e intestinais, a taxa de sobrevivência média total dos isolados de *Salmonella* spp. foi alta, o que permite inferir que os isolados possuem a capacidade de sobreviver às condições gastrointestinais simuladas, e ainda sim permaneceram em número capaz de causar infecção.

Palavras-chave: Microrganismos resistentes. Beta-lactamases. Biofilme. Tolerância gastrointestinal. Cortes carnes. Frango de corte.

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6) Produção Científica – Artigo publicado em periódicos – B2

6.1

SCIENTIFIC ARTICLE

Presence of *Listeria monocytogenes* and *Salmonella* spp. in lamb meat commercialized in Uruguiana, Rio Grande do Sul, Brazil

Presença de *Listeria monocytogenes* e *Salmonella* spp. em carne de cordeiro comercializada em Uruguiana, Rio Grande do Sul, Brasil

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Abstract

The aim of this study was to evaluate the hygienic-sanitary quality of lamb meat sold in the city of Uruguiana, Rio Grande do Sul (RS) by counting the indicator microorganisms and detecting pathogens such as *Salmonella* spp. and *Listeria monocytogenes*. Thirty-nine lamb meat samples were collected from 10 commercial establishments in Uruguiana. The samples were subjected to counts of aerobic mesophilic microorganisms and enterobacteria, and to the detection of *Salmonella* spp. and *L. monocytogenes*, all following standard methods. The average counts of mesophilic microorganisms and enterobacteria were 6.08 log CFU/g (minimum 4.07 and max 6.87) and 4.73 log CFU/g (minimum 0 and max 5.88), respectively. For pathogens, *L. monocytogenes* was isolated from five samples (12.82%), with three samples in the same location. Only two samples (5.13%) were positive for *Salmonella* spp. The results demonstrated unsatisfactory hygienic-sanitary conditions because high counts of pathogens such as *Salmonella* spp. and *L. monocytogenes*. The counts of enterobacteria showed poor hygiene conditions during the various stages of production. The results also indicated fecal contamination, as *Salmonella* spp. and *L. monocytogenes* are present in the intestinal tract of both humans and animals. The high count of mesophilic microorganisms obtained could be owing to contaminated raw material or unsatisfactory processing, including unsanitary conditions and the inappropriate use of binomial time/temperature during storage.

Keywords: contamination, Lamb meat, *Listeria monocytogenes*, *Salmonella* spp.

Resumo

O objetivo foi avaliar a qualidade higiênico-sanitária da carne de cordeiro comercializada na cidade de Uruguiana, Rio Grande do Sul (RS) através da contagem dos microrganismos indicadores e detecção de patógenos como *Salmonella* spp. e *Listeria monocytogenes*. Trinta e nove amostras de carne de cordeiro foram coletadas em 10 estabelecimentos comerciais em Uruguiana. As amostras foram submetidas a contagens de microrganismos aeróbios mesófilos e enterobactérias e à detecção de *Salmonella* spp. e *L. monocytogenes*, todas seguindo metodologias padrão. As contagens médias de microrganismos mesófilos e enterobactérias foram 6,08 log UFC/g (mínimo 4,07 e máximo 6,87) e 4,73 log UFC/g (mínimo 0 e máximo 5,88), respectivamente. Para os patógenos, *L. monocytogenes* foi isolado de cinco amostras (12,82%), com três amostras no mesmo local. Apenas duas (5,13%) foram positivas para *Salmonella* spp. Os resultados demonstraram condições higiênico-sanitárias insatisfatórias, baseado nas altas contagens de patógenos como *Salmonella* spp. e *L. monocytogenes*. As contagens de enterobactérias indicaram más condições de higiene durante as várias etapas de produção. Os resultados também indicaram contaminação fecal, uma vez que *Salmonella* spp. e *L. monocytogenes* estão presentes no trato intestinal de humanos e animais. A alta contagem de microrganismos mesófilos obtidos pode ser devida a matéria-prima contaminada ou processamento insatisfatório, incluindo condições insalubres e uso inadequado do binômio tempo/temperatura durante o armazenamento.

Palavras-chave: contaminação, carne de cordeiro, *Listeria monocytogenes*, *Salmonella* spp.



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Activity of rosemary (*Rosmarinus officinalis* - Family: Lamiaceae) essential oil compared to peracetic acid in *Listeria monocytogenes* biofilms

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ABSTRACT. The objective of this study was to evaluate the activity of *Rosmarinus officinalis* essential oil (EO) compared to peracetic acid (PA) regarding formation and elimination of *Listeria monocytogenes* biofilms on polystyrene surface. The minimum inhibitory concentration (MIC) was determined according to standard protocol. Isolates were inoculated according to MIC standards polystyrene plate wells, which were then incubated at 37°C/96 hours for evaluation of biofilm formation. Regarding the evaluation of biofilm elimination, the biofilms were treated under MIC for 10 minutes. The MIC obtained were 2.0 and 3.0 mg mL⁻¹ for EO and 0.015% for PA. Therefore, the results showed a reduction in the formation of biofilm with the presence of EO and PA, EO being more efficient ($p < 0.05$). Both compounds had a good capacity of eliminating biofilms, however the EO reduced the biofilm formation when compared to PA, highlighting its potential as an antibacterial agent and antibiofilm.

Keywords: control methods; industrial surface; rosemary; sanitization.

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Introduction

Listeria monocytogenes is an important pathogen that can cause listeriosis, an infection that in its severe form may result in a mortality rate of 20 to 30%, mainly affecting risk groups (Li et al., 2020). Most listeriosis outbreaks are associated with Ready-To-Eat foods, in which the risk of contamination is considered to be higher as cooking would not be required while processing these products (Smith et al., 2019; Gray et al., 2021). *Listeria monocytogenes* has long-term persistence in processing equipment and facilities in the food industry (Serenio et al., 2019). This behavior is related to their high tolerance to stress conditions such as refrigeration temperatures, high salt concentrations and their ability to form biofilms on several surfaces (Ziech et al., 2016; Kocot & Olszewska, 2017; Papaioannou, Giaouris, Berillis, & Bozaris, 2018). Biofilms are also able to facilitate the persistence of *L. monocytogenes* for long periods in food processing plants (Gray et al., 2021) and play an important role in the survival of the pathogen in industrial environments (Kocot & Olszewska, 2017).

The biofilm formation is a major problem for the food industry, as it can cause serious technological issues that lead to uselessness of equipment and contamination of products that reach final consumers, being an important public health problem (Brasileiro et al., 2016; Smith et al., 2019).

With respect to biofilms arrangement, sessile cells have a higher resistance to antimicrobial agents than planktonic cells, due to their structure and presence of extracellular polymeric substances (EPS), sublethal concentrations, metabolic heterogeneity, activation of adaptive responses to stress, and horizontal gene transfer (Olszewska, Zhao, & Doyle, 2016; Campana, Ciandrini, & Baffone, 2018).

To reduce the likelihood of developing microbial resistance and expand the range of antimicrobial activity in the food industry, new approaches to biofilm prevention and control have been explored (Olszewska et al., 2016; Pang, Wong, Chung, & Yuk, 2019), new approaches on their use in the food industry have been