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Hygienic-sanitary aspect of artisan and industrial ice creams: analysis of genetic variability and of antimicrobial resistance in *Escherichia coli* isolates

Aspecto higiênico-sanitário de sorvetes artesanais e industriais: análise da variabilidade genética e da resistência a antimicrobianos em *Escherichia coli* isoladas

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Abstract

Objective: The aim of the present study was to evaluate the hygienic-sanitary aspect of artisan and industrial ice creams and to analyze genetic variability and antimicrobial resistance in *Escherichia coli* isolates. *Methods*: Twenty-seven ice cream samples were purchased, detecting total coliforms at 30-35 °C by fermentation in multiple tubes. The positive tubes were inoculated in EC broth and in SS agar for the detection of *Escherichia coli* and *Salmonella spp*, respectively. To antimicrobial tests, the disk diffusion technique was used, discriminating levels of resistance. Genetic variability was diagnosed by the RAPD-PCR and ERIC-PCR techniques through the degree of polymorphism determined by the band pattern. *Results and Discussion*: From the microbiological samples, there was positivity for *E. coli* (48%) and *Salmonella spp* (22%). The antimicrobial resistance tests in *E. coli* diagnosed resistance to 85% of the obtained samples, and of these, 34% were multiresistant. A high degree of polymorphism was found in the RAPD-PCR and ERIC-PCR assays, demonstrating the great genetic variability among the bacteria. *Conclusion*: The samples presented unsatisfactory conditions, offering risks to consumers' health. The antibiotic sensitivity test expressed resistance and multiresistance, while the RAPD-PCR and ERIC-PCR assays indicated great genetic variability among the bacteria, considering multiple sources of contamination. Therefore, measures to control contamination prevention are considered important.

Keywords: Feeding. Enterobacteria. Mutation. Antibacterial. Ice cream.

Resumo

Objetivo: O presente trabalho teve como objetivo avaliar o aspecto higiênico-sanitário de sorvetes artesanais e industriais e analisar a variabilidade genética e a resistência a antimicrobianos em isolados de *Escherichia coli*. *Métodos*: Foram compradas 27 amostras de sorvetes, detectando coliformes totais a 30-35 °C por fermentação em tubos múltiplos. Os tubos com positividade foram inoculados em caldo EC e em ágar SS para detecção de *Escherichia coli* e *Salmonella spp*, respectivamente. Para testes antimicrobianos, utilizou-se técnica de difusão em placas, discriminando níveis de resistência. A variabilidade genética foi diagnosticada pelas técnicas PCR-RAPD e ERIC-PCR através do grau polimórfico determinado pelo padrão de bandas. *Resultados e Discussão*: A partir das amostras microbiológicas, houve positividade para *E. coli* (48%) e *Salmonella spp* (22%). Os testes de resistência a antimicrobianos em *E. coli* diagnosticaram resistência para 85% das amostras obtidas, sendo que destas, 34% apresentaram-se multirresistentes. Encontrou-se um alto grau de polimorfismo nos testes PCR-RAPD e ERIC-PCR, demonstrando a grande variabilidade gênica entre as bactérias. *Conclusão*: As amostras apresentaram condições insatisfatórias, oferecendo riscos à saúde dos consumidores. O teste antibiograma expressou resistência e multirresistência, enquanto os testes PCR-RAPD e ERIC-PCR indicaram grande variabilidade genética entre as bactérias, considerando múltiplas fontes de contaminação. Portanto, medidas para controlar a prevenção da contaminação são tidas como importantes.

Palavras-chave: Alimentação. Enterobactérias. Mutação. Antibacteriano. Sorvete.

Introduction

Ice cream is an edible iced food obtained by the emulsion of fats and proteins, with or without the addition of other ingredients and substances, subjected to freezing.[1] Ice creams may be classified as: creamy, popsicles and special iced products and must be preserved under appropriate temperature during transportation and storage, to maintain product quality.[2,3]

Ice creams may have dairy products and/or eggs in their composition, in which case they should be subjected to heat treatment, as described in Resolution SAA - 03 of January 10, 2008, since they may cause outbreaks of foodborne illnesses (FBI), since they present a potential risk of biological contamination.[2-4,5-7]

Outbreaks of FBI may be caused by bacteria of the *enterobacteriaceae* family, such as *Salmonella spp* and *Escherichia coli*, gram-negative bacteria that are potential biological risks. These microorganisms are responsible for severe food poisoning and outbreaks in several countries. In addition, they may cause potentially lethal manifestations to the individual, and immunocompromised and debilitated people, children, elderly, and pregnant women are highly susceptible to them.[3, 8-11]

Among the most common bacteria isolates in clinical samples, enterobacteria are the main responsible for localized or systemic hospital, intestinal and extraintestinal infections, as well as the involvement of the urinary tract, lungs, central nervous system, skin, mucous membranes and meninges.[12] Because they naturally inhabit the human intestine, the presence of these microorganisms in foods indicates fecal contamination, revealing the deficiency of hygienic-sanitary conditions.[8,12-15]

After the discovery of antibiotics, the treatment of infectious diseases with a high degree of morbidity and mortality had a great therapeutic advance. However, the prolonged or indiscriminate use of these drugs has favored the increase of bacterial resistance and the advances of pharmaceutical industries are not fast enough to discover new drugs. This interferes in the effective treatment and control of infections by these agents.[5,16]

Bacteria have the ability to adapt to the environment, which is associated with genomic structure, allowing the exchange of genes among them, thus originating an allelic diversity determined as genetic variability.[17] From laboratory assays such as Random Amplification of Polymorphic DNA - Polymerase Chain Reaction (RAPD-PCR) and Enterobacterial Repetitive Intergenic Consensus - Polymerase Chain Reaction (ERIC-PCR), which seek to establish genetic parameters that allow the grouping of isolates, it is possible to detect means of reproduction, migration levels, evolution and dispersal of a species, as well as their ability to adapt to environmental changes.[18,19]

Based on the aforementioned, the objective was to evaluate the hygienic-sanitary aspect of artisan and industrial ice creams and to analyze the genetic variability and antimicrobial resistance in *Escherichia coli* isolates.

Material and Methods

Twenty-seven samples of artisan and industrial ice creams were purchased at random: 22 of the creamy type and five of the popsicle type. The ice creams were obtained between March and September 2016 at commercial establishments in Araçatuba, Promissão, Piacatu, Rinópolis and Gabriel Monteiro, in the State of São Paulo.

Obtaining the samples: The creamy ice creams were obtained in establishments such as malls, ice cream parlors and self-service snack bars, and the popsicle were taken directly from freezers of

convenience stores. The utensils and containers offered by the establishment were used, depicting the conditions with which the consumer receives the product. The ice creams were transported in isothermal boxes to the laboratory of microbiology and molecular biology of the Centro Universitário Católico UniSalesiano Auxilium (Catholic Salesian Auxilium University Centre), Araçatuba-SP, where the analyzes were carried out.

Research with total coliforms (30-35 °C), thermotolerant (45 °C) and *E. coli*: It was performed by the fermentation technique in multiple tubes.[20] From the sample in the liquid state, 1 mL was pipetted in tubes containing Peptone water 1% (ACUMEDIA), thus obtaining the concentrated sample; after homogenization, 1 mL of it was transferred to another tube, obtaining the dilution 10^{-1}, and from it, a 1 mL aliquot was used to obtain the dilution 10^{-2}. Aliquots of 1 mL of the concentrated sample and its dilutions 10^{-1} and 10^{-2} were serially pipetted from three threaded tubes containing 9 mL of the culture medium Brilliant Green Bile Broth 2% (BGBB) (KASVI) with inverted Durham Tubes for detecting coliforms at 35 °C. Positivity was confirmed by gas capture inside the Durham tubes released during bacterial fermentation.

From the positive tubes, 1 mL of the Escherichia coli Broth (EC) (KASVI) sample was collected to detect coliforms at 45 °C, following the same fermentation technique in multiple tubes. The tubes were placed in a bacteriological oven at 35 °C ±2 (BGBB) and water bath at 45 °C ±2 (EC) for 48 hours.

Known *E. coli* and *Salmonella spp* isolates were inoculated together with the samples as positive control, and the negative control was achieved by inoculation of the culture medium without sampling. Non-standard tests were remade.

The Most Likely Number (MLN) per milliliter of each ice cream sample was quantified in accordance with the standards established by the RDC's Resolution No. 12 of January 2, 2001, which provides for maximum acceptable food standards of 10^2/g for coliforms at 35 °C, and 5.0x10 for coliforms at 45 °C.[21]

Detection of *Salmonella spp*: The BGBB medium was used to select only gram-negative bacteria. Therefore, only the tubes that showed positivity were transferred into Petri dishes containing *Salmonella-Shigella* agar (SS) (HIMEDIA) medium. The dishes were placed in a bacteriological oven at 37 ºC ±2 for 24 hours. Black colonies indicated positivity.[22]

Isolation and storage of *E. coli*: The positive samples in the EC broth were transferred into solid culture medium Eosin methylene blue (EMB) (KASVI). The dishes were maintained in a bacteriological oven at 37 °C ±2 for 24 hours. Metallic green colonies indicated positivity.

Antimicrobial resistance profile: The samples that showed positivity in the EC broth were inoculated on MacConkey agar (KASVI) and maintained in a bacteriological oven at 37 ºC ±2 for 18-24 hours. Isolated rosy colonies, indicative of *E. coli*, were transferred into tubes containing

Lyptone Soya Broth (HIMEDIA) to adjust the inoculum pattern using a turbidimeter at 0.5 of the McFarland scale (1.5 x 10^8 CFU/mL). Using the disk diffusion technique, the samples were seeded in Mueller Hinton agar medium (KASVI), where DME Sensi-Discs of the following antibiotics were placed: Cephalothin (30 µg), Imipenem (10 µg), Gentamicin (120 µg), Ciprofloxacin (05 mcg), Nalidixic acid (30 µg), Chloramphenicol (30 µg) and Nitrofurantoin (300 µg).[14,23,24] The dishes were stored at 37 °C ± 2 for 24 hours. The reading was performed according to the manufacturer's recommendations (DME), measuring the diameter in millimeters of the obtained halos and classifying the sample as resistant, intermediate and sensitive to each antibiotic used.

Analysis of the genetic variability of *E. coli* isolates: Two isolated colonies were removed from MacConkey agar and suspended in 200 µl of ultrapure water.[25] From this suspension, 2 µl were withdrawn for the RAPD-PCR and ERIC-PCR reactions in order to analyze genetic variability.

For a final volume reaction of 25 µl, 2 µl of cell suspension were used as template DNA, 2.5 mM of each dNTP, 1.75 mM $MgCl_2$, 1X Taq DNA polymerase buffer, 1U Taq Polymerase, 400 ng/µl of OPA12 primer (5' CAATCGCCGT 3') or Ecoli-2 (5'AGAAGCGATG 3') for the RAPD assay and 400 ng/µl of each of oligonucleotides ERIC1R (5' TGTAGGCTCCTGGGATTCAC 3') and ERIC2R (5'AAGTAAGTGACTGGGGTGAGCG 3') for the ERIC-PCR technique.[19]

The amplification reaction for RAPD was started with denaturation at 94 °C for three consecutive minutes of 45 cycles of 1 minute at 94 °C, 1 minute at 30 °C and 2 minutes at 72 °C, ending with an extension of 72 °C for 5 minutes. For the ERIC-PCR technique, the reaction had an initial denaturation cycle at 94 °C for 7 minutes, followed by 30 denaturation cycles at 94 °C for 1 minute and 30 seconds, annealing at 51 °C for 1 minute, extension at 72 °C for 1 minute and 30 seconds and a final extension cycle at 72 °C for 15 minutes. The amplification reactions were carried out in an "Amplitherm thermal cycler". The amplification products were separated on agarose gel (1.5%) using a 100 bp ladder molecular weight marker as reference. The fragments were viewed in an ultraviolet light transilluminator.[26]

The bands visible on the agarose gel were converted into binary matrix, establishing 1 for present bands and 0 for absent bands. The degree of similarity (Jaccard) was determined by the simple matching coefficient, relating the presence (1) and absence (0) of band, and the respective dendrograms were constructed with the aid of the software Past 2.17, version 2013.

Results and Discussion

The presence of total coliforms was observed in 25 samples (93%), of which 20 were creamy ice cream and five were popsicle. Of these samples, 16 (61%) had thermotolerant coliforms. In general, 44% and 7% of the samples presented non-standard counts for coliforms at 35 °C and 45 °C, respectively (Table 1).

Table 1. Microbiological aspects of ice cream samples from Araçatuba-SP and region in 2016.

Microorganism	Unit	Federal standard*	Count	No. of samples	%
Total coliforms (35 °C)	NMP/g	10^2/g	$<10^1$	10	37
			11^1 -10^2	5	19
			11^2 - $>11^2$	12	44
Thermotolerant coliforms (45 °C)	NMP/g	5.0x10	0	10	37
			1 to 49	15	56
			50 to >110	2	7
Salmonella spp	In 25 g	Negative	Positive	6	22
			Negative	21	78

* Microbiological standards according to RDC No. 12 of January 2, 2001 and Ordinance No. 451 of September 19, 1997.

Because these are food samples, the high index of such bacteria may lead the consumer to an infectious condition.[27] Contamination may be due to a poor hygienic-sanitary condition throughout the ice-cream manufacturing process, as well as during thermal treatment, especially in small artisan trades, where handlers are rarely made aware through an efficient quality policy on the risk of contamination.[21,28] Presuming that *E. coli* is one of the main causes of diarrhea, and that, according to the Organização Mundial da Saúde (World Health Organization - WHO), almost 2 million children die every year in the world due to this dysfunction, of which 200,000 alone are in Brazil, its presence in ice creams generates an alarming concern about the public health situation.[29,30]

Similar results were found in Araraquara-SP, where, of the 24 unpasteurized ice cream samples, 66.6% were not in accordance with the standards established by the Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal (RIISPOA - Regulation of Industrial and Sanitary Inspection of Products of Animal Origin).[31,32] In Arapongas-PR, 21 samples of analyzed ice cream (industrial and artisan) were contaminated by coliforms at 30-35 ºC.[6]

Regarding the qualitative tests for *Salmonella spp*, this was found in six (22%) of the samples (Table 1). In addition, fly larvae were observed in one of the culture dishes, indicating a possible contamination with eggs in the ice cream collector utensil, since it was inserted in an unprotected reservoir with water containing ice cream remains from previous washes.

It is estimated that *E. coli* and *Salmonella spp* have diversified from the same ancestor, this explains the similarity in the symptomatology of the diseases caused by these bacteria. About 100 million years ago, mutations and gene selections have naturally occurred, giving rise to the current distinct microorganisms.[30,33]

Positive results for *Salmonella spp* were found in Fortaleza-CE, where 75% of the ice cream samples were in disagreement with ANVISA's Resolution No. 12, dated January 2, 2001, which provides for the absence of bacteria in any food product.[21,34] Strains of *Salmonella spp* may be destroyed during pasteurization, however, food may be recontaminated by materials, equipment and poorly washed hands of handlers.[35]

In the United States, the Centro de Comunicação de Doenças (Centers for Disease Control and Prevention - CDC) estimates that 95% of *Salmonella spp* contamination is from food sources. The Rede de Vigilância Ativa de doenças de origem alimentar (Foodborne Diseases Active Surveillance Network - FoodNet) believes that out of 1.4 million annual cases of salmonellosis, 1.3 million are due to the consumption of food contaminated with *Salmonella spp*. This type of contamination classifies the product as potentially capable of causing foodborne illness (FBI), denominating it as inappropriate for human consumption.[30,36,37]

Between 1993 and 1997, in the countryside of the State of São Paulo, 23 outbreaks of *Salmonella* were analyzed, in which 95.7% of the cases were associated with the ingestion of foods containing eggs, 87% of which were linked to the ingestion of foods based on raw eggs, as in the case of some emulsifiers used in ice cream.[38]

Contamination by *Salmonella spp* may not have occurred due to imprudence of the ice cream manufacturer, but rather by the raw material purchased from the external environment, especially the *in natura* egg. To ensure that the product does not become contaminated, the ideal would be that manufacturers make sure of the quality of the products offered by their suppliers.

Regarding the antimicrobial resistance test, 27 (85%) *E. coli* samples showed resistance to at least one of the antimicrobial agents, three (9%) presented intermediate resistance and only 2 (6%) were sensitive. An infection caused by a sensitive organism may be treated with the recommended dose of the drug, on the other hand, when caused by an agent that demonstrates intermediate resistance, the treatment should be administered at maximum dose if possible, and in case of resistant bacteria, the microorganism will not be inhibited by the antimicrobial agent.[39,40]

The most efficient antibiotics were Chloramphenicol and Gentamicin, inhibiting 100% of bacterial growth, followed by Ciprofloxacin (94%), Nalidixic Acid (78%), Imipenem (79%), Nitrofurantoin (37%) and Cephalothin (31%), as seen in Figure 1.

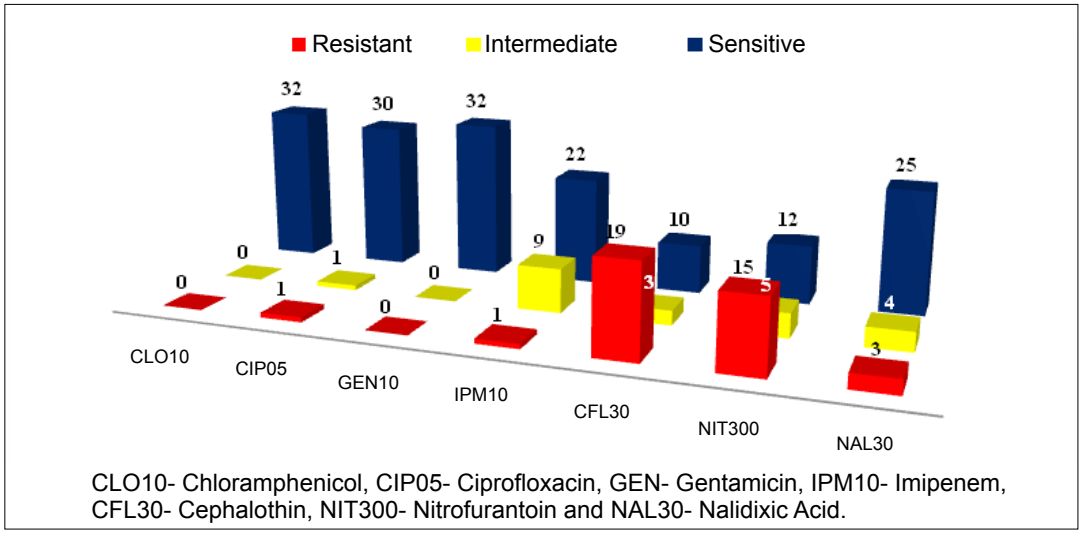


Figure 1. Efficiency of antimicrobials in *Escherichia coli* isolated from ice cream samples from Araçatuba-SP and region, 2016.

Furthermore, 11 samples (34%) presented multiresistance to antimicrobials, a disturbing factor, since these microorganisms appear with several factors, including abusive and inappropriate use of antibiotics by the population.[41,42]

The analyzed Gram-negative bacilli were from samples that showed positivity at any concentration and triplicate repetition. Bacteria of the same sample origin presented different resistance profiles, according to the examples of Table 2.

Table 2. Antimicrobial resistance profile of bacteria of same sample origin isolated from ice cream samples from Araçatuba-SP and region, 2016.

Sample	Concentration	IPM10	CFL30	NIT300	NAL30
2	10	S	R	I	S
2	10	S	R	S	R
2	10^{-1}	S	R	R	S
13	10	S	R	R	R
13	10^{-2}	S	R	S	S
14	10	S	I	S	S
14	10^{-2}	I	R	S	S
19	10	I	R	R	S
19	10	S	R	R	S
19	10^{-2}	S	S	R	I
23	10	I	S	I	R
23	10	I	R	R	I
23	10^{-1}	S	S	S	S
23	10^{-2}	R	R	I	S
23	10^{-2}	I	R	S	S
24	10^{-1}	S	R	R	S
24	10^{-1}	S	S	R	I
24	10^{-2}	I	R	S	S

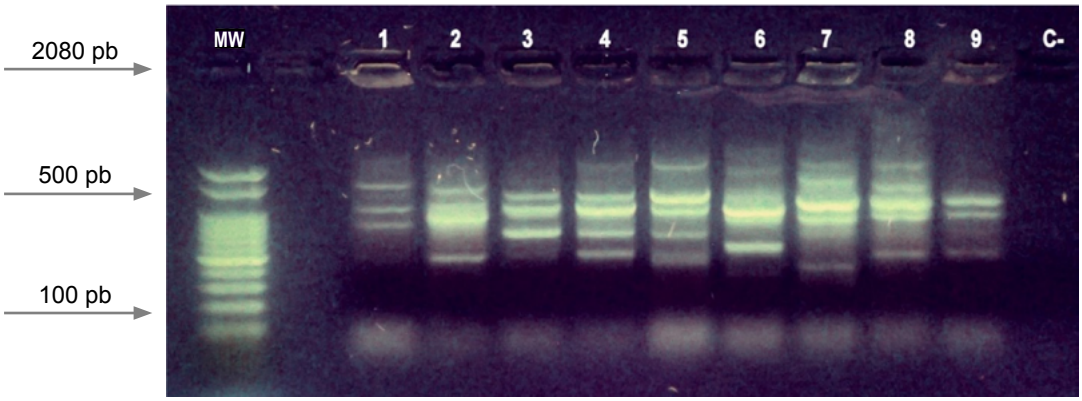
Genetic mutation develops when the microorganism acquires resistance to an antimicrobial or adapts to a different medium. This mutation is stable and is transmitted to successive generations. Resistance to antibiotics is already a global problem, as the higher risk infections are caused by "superbugs", which are uncontrollable to the drugs often used.[43]

Resistant microorganisms have resistance phenotype marked by a minimum inhibitory concentration (MIC) for a certain type of antibiotic.[44] The bacterium may be resistant to an antimicrobial just because it does not have the structure on which it exerts its activity. The acquired

resistance alludes to the development of the resistance structure obtained by mutation of existing genes or of materials from other microorganisms (transformation, transduction and conjugation) by mutation of the acquired genetic material.[45]

Divergent results were obtained by analyzing the antimicrobial resistance of *E. coli* isolated from suckling pig feces, which showed high levels of resistance, such as 97% for Chloramphenicol and 86% for Gentamicin.[5] A 2001 study showed the prevalence of *E. coli O157: H7* resistance in Cephalothin and Chloramphenicol.[46]

The genetic variability test demonstrated a high degree of polymorphism from the two primers tested in RAPD-PCR and the amplification revealed a total of 20 bands, all of which were polymorphic, indicating the wide and considerable gene variability among the isolated bacteria (Figure 2). Nine bands were obtained with the Ecoli2 primer with a size between 400 bp and 2,080 bp and a Jaccard similarity coefficient of 0.85. A strong similarity is considered for correlations above 0.80. The use of OPA12 primer revealed the presence of 11 distinct bands, also ranging between 400 bp and 2,080 bp and a correlation coefficient of 0.87. The dendrograms referring to these results were obtained through the PAST software and are shown in Figure 3A.



* MW-Molecular weight. Pools 1 to 5 refer to sample 23 and pools 6 to 9 indicate sample 24.

Figure 2. Genetic divergence of the same origin and presence of *Escherichia coli* conservative bands in ice cream samples from Araçatuba-SP and region, 2016.

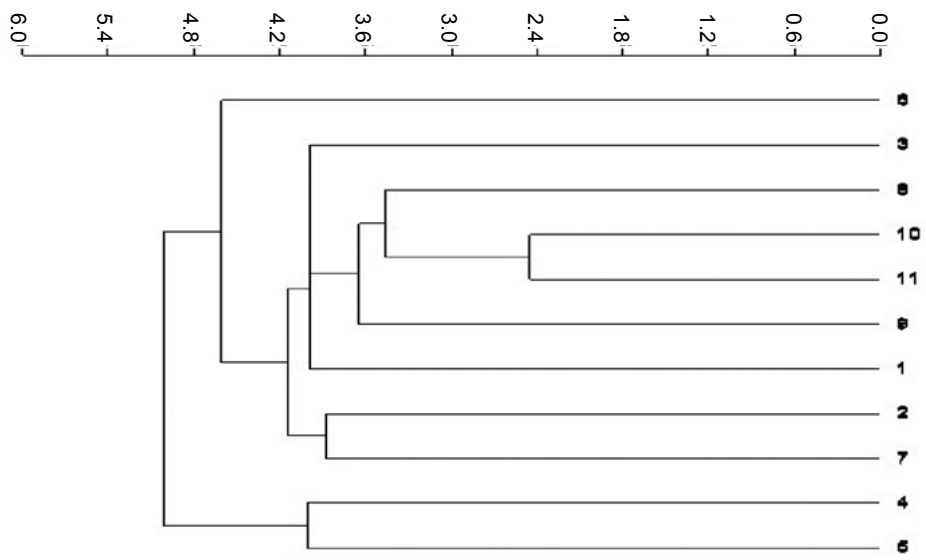


Figure 3A. RAPD assay using Ecoli1 and OPA12 primers, respectively.

Different bands were also obtained by studying *E. coli* samples isolated from the water of the Lagoa dos Patos-RS, the bands patterns provided a great genetic variability, with 5 to 15 fragments from 150 bp to 1,800 bp.[47]

For the ERIC-PCR assay, 16 bands were obtained which, when compared directly with the 100 bp molecular weight ladder, had fragments ranging from 100 bp to 1,500 bp and a correlation coefficient of 0.78 (Figure 3B), the samples being distributed into four groups. The genetic variability present in a bacterial species may be demonstrated by the study of DNA fragments of different sizes (ERIC-PCR), generated from an amplification of repetitive sequences existing in its genome, through the use of specific oligonucleotides by PCR.[48]

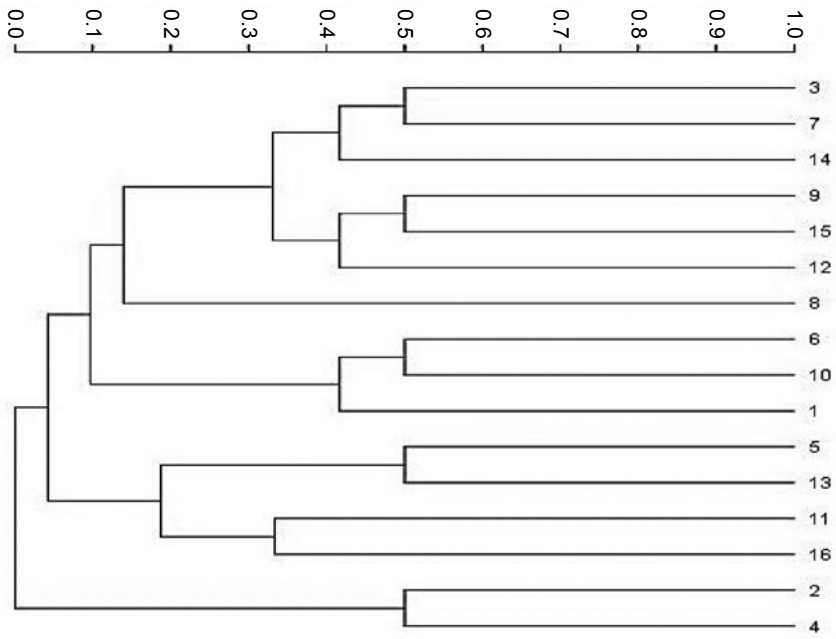


Figure 3B. ERIC-PCR assay.

The use of the ERIC-PCR technique was also effective, demonstrating the genetic variability and characterization of *Leishmania chagasi* samples, obtaining reliable, sensitive and specific results, being able to be used in diagnostic and differentiation situations, in addition the results are easily obtained.[19]

Figures 3 (A and B) represent, respectively, dendrograms referring to the RAPD-PCR and ERIC-PCR genetic variability test of *E. coli* isolated from ice cream samples from Araçatuba-SP and region, 2016.

The conservation of bands, common in the RAPD-PCR assay in similar species, appears only in some samples with the same origin (Figure 2), differentiating only the concentrations of the media in which they were isolated, however, when compared to the rest of the samples, the persistence was absent. The gene profile found in all samples was extremely diverse.

An occurrence to be also considered is the gene difference among bacteria isolated from culture medium in differentiated concentrations, however, they all come from the same sample, assuming a heterogeneous strains contamination.

Conclusion

The samples showed a high contamination index by coliforms at 30-35 °C and coliforms at 45 °C, and positivity to *Salmonella spp*, making the food a threat to the consumer's health. The antimicrobial susceptibility test of *E. coli* isolated from ice cream showed resistance and multiresistance, in addition to differentiated results for bacteria from the same sample. A discordant aspect in a same sample was also found in RAPD-PCR, which detected a polymorphism index, indicating great genetic variability among bacteria, considering different sources of contamination.

Contributors

Andrade, A. P. and Luche, D. E. D. participated in all stages of the study, from the conception of the study to the final version. Matos, D. J. participated in the guidance of this study, aiming at the microbiological part. Cervelatti, E. P. participated in the co-guidance, aiming at the molecular genetics part of the study.

Conflict of interest: The authors declare that there are no conflicts of interest.

References

1. Brasil. Agência Nacional de Vigilância Sanitária. Resolução n. 266 de 22 de set. de 2005. Regulamento técnico para fixação de identidade e qualidade de gelados comestíveis e, preparados para gelados comestíveis, pós para o preparo e bases para gelados comestíveis. Diário Oficial da União 23 set. 2005.
2. Brasil. Agência Nacional de Vigilância Sanitária. Portaria n. 379, de 26 de abr. de 1999. Aprova o regulamento técnico referente a gelados comestíveis, preparados, pós para o preparo e bases para gelados comestíveis. Diário Oficial da União 29 abr.1999.
3. Alves ARF. Doenças alimentares de origem bacteriana [dissertação]. [Porto]: Universidade Fernando Pessoa, Faculdade Ciências da Saúde, 2012.
4. São Paulo. Secretaria de Agricultura e Abastecimento. Resolução SAA-03 de 10 de jan. de 2008. Que baixa normas técnicas sobre as condições higiênico-sanitárias mínimas necessárias para aprovação, funcionamento e reaparelhamento dos estabelecimentos destinados a leite e produtos lácteos. Diário Oficial 11 jan. 2008; 118(7):31
5. Baccaro MR, Moreno, AM, Corrêa A, Ferreira AJP, Calderano FF. Resistência antimicrobiana de amostra de Escherichia coli isoladas em fezes de leitões com diarreia. Arq Inst Biol. 2002; 69(2):15-18.
6. Pazianotti L, Bosso AA, Cardoso S, Rezende Costa M, Sivieri K. Características microbiológicas e físico-químicas de sorvetes artesanais e industriais comercializados na região de Arapongas-PR. Rev Inst Latic Cândido Tostes 2010; 65(377):15-20.

7. Padilha GR. Boas práticas de fabricação em indústria de gelados comestíveis como pré-requisito para implantação do sistema APPCC [Trabalho de Conclusão de Curso]. [Bento Gonçalves]: Instituto Federal do Rio Grande; 2011.

8. Koneman E. As enterobacteriaceae In: Koneman E. Diagnóstico microbiológico. 6ª ed. Rio de Janeiro: Guanabara; 2008. p. 208-210.

9. Mikilita IS, Cândido LMB. Fabricação de sorvetes: perigos significativos e pontos críticos de controle. Brasil Alimentos 2004; 26:34-37.

10. Mikilita, IS. Avaliação do estágio de adoção das boas práticas de fabricação pelas indústrias de sorvete da região metropolitana de Curitiba-PR: proposição de um plano de análise de perigos e pontos críticos de controle [dissertação] [Curitiba]: Programa de Pós-Graduação em Tecnologia de Alimentos, Setor de Tecnologia, Universidade Federal do Paraná; 2002.

11. World Health Organization. Food safety and foodborne illness. Reviewed March 2007. Fact sheet no 237 [Internet]. [accessed 29 April 2017]. Available at: https://foodhygiene2010.files.wordpress.com/2010/06/who-food_safety_fact-sheet.pdf

12. Martinez MB, Taddei CR. Enterobacteriaceae. In: Alterthum F. Microbiologia. 9ª ed. São Paulo: Atheneu; 2015. 293-301

13. Jakabi M, Franco BDGM. Frequência de isolamento de cepas de E. coli patogênica em alimentos de origem animal. Ciênc Tecnol Alim. 1991; 11:170-181.

14. Hobbs BC, Roberts D. Toxinfecções e controle higiênico-sanitário de alimentos. São Paulo: Varela; 1999. 376 p.

15. Ribeiro MG, Pinto JPNA, Silva EOTR. Escherichia coli O157:H7 de hambúrguer, leite e outros gêneros alimentícios à colite hemorrágica e síndrome urêmico-hemolítica. Hig Aliment. 1999; 13(66/67):88-99.

16. Mota RA, Silva KPC, Freitas MFL, Porto WJN, Silva LBG. Utilização indiscriminada de antimicrobianos e sua contribuição a multirresistência bacteriana. Braz J Vet Res Anim Sci. 2005; 42(6)465-470.

17. Martho GR, Amabis JM. Biologia das populações. v. 3. Rio de Janeiro: Moderna; 1997.

18. Solé-Cava AM. Biodiversidade molecular e genética da conservação: Biologia Molecular e Evolução. Ribeirão Preto: Holos; 2001.

19. Leal CRB. Uso da ERIC-PCR para caracterização de amostras de Leishmania Chagasi [Tese]. [Campo Grande]: Universidade Federal do Mato Grosso do Sul; 2010.

20. Boreli K, Brito NJN, Santos ECG, Silva GA. Avaliação de coliformes totais e termotolerantes em bebedouros de escolas públicas e ginásios de esportes em um município do Norte de Mato Grosso. REBES Rev. Bras. Educ. e Saúde 2014; 5(1):96-9.

21. Brasil. Agência Nacional de Vigilância Sanitária. Resolução nº 12, de 2 de janeiro de 2001. Aprova o regulamento técnico sobre padrões microbiológicos para alimentos. Diário Oficial da União 10 jan. 2001.

22. Silva N, Junqueira VCA, Silveira NFA. Manual de métodos de análise microbiológica de alimentos. São Paulo: Varela; 2007. 105 p.

23. Bauer AW, Kirby VMM, Sherris JC, Turck M. Antibiotics susceptibility testing by a stantardized single disk method. Am J Clin Pathol. 1965; 45(4):493-496.

24. Clinical and Laboratory Standards Institut. Performace sandards for antimicrobial disk susceptibility tests. 8ª ed. Pennsylvania: CLSI; 2003.

25. Paradela A, Barroso S. Disseminação de genes de resistência em estirpes clínicas de Escherichia coli [Dissertação]: [Aveiro]: Universidade de Aveiro; 2008.

26. Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in eubactéria and appplication to fingerprinting of bacterial genomes. Nucleic Acids Res. 1991; 19(24):6823-6831.

27. Trigo VC. Manual prático de higiene e sanidade das unidades de alimentação e nutrição. São Paulo: Varela; 1999.

28. Paiva YF, Silva EV, Araújo AS, Azevedo PTM, Sousa TCA. Condições higiênico-sanitárias de sorvetes do tipo italiano (soft), comercializados em Pombal, Paraíba. Revista Verde de Agroecologia e Desenvolvimento Sustentável 2016; 11(3):75-79.

29. Trabulsi LR, Keller R, Tardelli Gomes TA. Typical and atypical enteropathogenic, Escherichia coli. Emerg Infect Dis. 2002; 8(5):508-513.

30. Whittam TS. Genetic variation and evolutionary processes in natural populations of Escherichia coli. Escherichia coli and Salmonella spp. In: Neidhardt FC, editor. Cellular and molecular biology. Washington (DC): ASM Press; 1996. p. 2708.

31. Falcão DP. Exame microbiológico de sorvetes não pasteurizados. Rev. Saúde pública 1983; 17:2-8.

32. Brasil. Ministério da Agricultura. Decreto nº 30691, de 29 março 1952. RIISPOA - Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal. Brasília: Ministério da Agricultura, 1952. Diário Oficial da União 07 jul. 1952.

33. Pupo GM, Karaolis DK, Lan R, Reeves PR. Evolutionary relationships among pathogenic and nonpathogenic Escherichia coli strains inferred from multilocus enzyme electrophoresis and mdh sequence studies. Infect Immun. 1997; 65(7):2685-2692.

34. Queiroz HGS, Sampaio Neta NA, Pinto RS, Rodrigues MCP, Costa JMC. Avaliação da qualidade físico-química e microbiológica de sorvetes do tipo tapioca. Revista Ciência Agronômica 2009; 40(1):60-65.

35. Bryan FL, Teufel P, Riaz S, Roohp S, Qadar F, Malik ZUR. Hazards and critical control points of street-vending operations in a mountain resort town in Pakistan. Journal of Food Protection 1992; 55(9):701-707.

36. Hoffmann FL, Penna ALB, Coelho AR, Mansor AP, Vinturim TM. Qualidade higiênico-sanitária de sorvetes comercializados na cidade de São José do Rio Preto-SP-Brasil. Hig Aliment. 2000; 14(76):62-68.

37. Warke R, Kamat A, Kamat M, Thomas P. Incidence of pathogenic psychotrophs in ice cream sold in some retail outlets in Mumbai, India. Food Control 2000; 11(2):77-83.

38. Perese JTM, Almeida IAZC, Lima SI, Marques ECA, Rodrigues AS, Fernandes DSG, et al. Surtos de enfermidades transmitidas por alimentos causados por Salmonella enteridis. Rev Saúde Pública1998; 32(5):477-483.

39. Ribeiro Furtini LL, Abreu LR. Utilização de APPCC na indústria de alimentos. Ciênc Agrotec. 2006; 30(2):358-363.

40. Forsythe SJ. Microbiologia da segurança dos alimentos. 2ª ed. Porto Alegre: Artmed; 2013.

41. Ribeiro MC, Stelato MM. Microbiologia prática: aplicações de aprendizagem de microbiologia básica. 2ª ed. São Paulo: Atheneu; 2011.

42. McGeer A. News in antimicrobial resistance: documenting the progress of pathogens. Infect Control Hosp Epidemiol. 2004; 25(2):97-8.

43. Gaite FB, Sáenz AJ, Vidal MV, Castellana Perelló D. Nuevas opciones terapêuticas para el tratamiento de las bactérias multirresistentes en Unidades de Cuidados Intensivos. Rev Esp Quimioter. 2008; 21(1):9-13.

44. Correia CE. Infecções urinárias e susceptibilidade de uropatógenos aos antimicrobianos [Dissertação]. Aveiro: Universidade de Aveiro, Departamento de Biologia; 2009.

45. Tavares JC. Microbiologia e farmacologia simplificada. Rio de Janeiro: Revinter; 2002.

46. Schroeder Carl M, Zhao C, Debroy C, Torcolini J, Zhao S, Branco DG, et al. Antimicrobial resistance of Escherichia coli O157 isolated from humans, cattle, swine, and food. Appl Environ Microbiol. 2002; 68(2):576-581.

47. Canal N. Caracterização de resistência a antimicrobianos e diversidade genética em Escherichia coli isolada de amostras de água da Lagoa dos Patos, RS [Dissertação]. [Porto Alegre]: Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde; 2010.

48. Saiki RK, Gelfand DH, Stoffel S. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1998; 239(4839):487-491.

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