BRIEF COMMUNICATION
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Antimicrobial resistance of *Escherichia coli* isolated from Minas Frescal Cheese in the city of Rio de Janeiro - Phenotypic and genotypic profile

Resistência antimicrobiana em cepas de *Escherichia coli* isoladas de queijo Minas Frescal no município do Rio de Janeiro - Perfil fenotípico e genotípico

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ABSTRACT

Introduction: Antimicrobial resistance is a public health problem. Foods may be carriers of antimicrobial resistant bacteria and resistance genes for humans. **Objective:** To isolate, identify, and evaluate the antimicrobial susceptibility and identification of resistance genes and integrons in *Escherichia coli* strains isolated from Minas Frescal cheese. **Method:** The presence of *E. coli* in thirty samples of Minas Frescal cheese was evaluated through the 3M™ Petrifilm plates. Thirty *E. coli* isolates were evaluated through the Disk Diffusion Susceptibility Test for 17 antimicrobial agents. Through PCR and Multiplex PCR the isolates were examined for the presence of 24 antimicrobial resistance genes. Genes and variable regions of class 1, 2 and 3 integrons were also evaluated. **Results:** Phenotypic resistance to one or more classes of antimicrobial agents were found in 50% of *E. coli* isolates. One hundred percent of the isolates showed PCR amplification for the bla_{TEM} gene and there was also amplification for bla_{SHV} and *tet*B genes. In addition, 46.6% of the phenotypically resistant isolates amplified to one or more classes of integrons. This is one of the first studies to identify these genes in Minas Frescal cheese in Brazil. **Conclusions:** Cheese can be a source of multiresistant bacteria and those can disseminate their resistance genes to other bacteria being in the food and human gastrointestinal tract, emphasizing the importance of Good Manufacturing Practices and greater supervision on products on sale.

KEYWORDS: Antimicrobial Resistance; *E. coli*; Integron; Minas Frescal Cheese

RESUMO

Introdução: A resistência antimicrobiana é um problema de saúde pública. Os alimentos podem ser veículos de bactérias resistentes a antimicrobianos e genes de resistência para os seres humanos. **Objetivo:** Isolamento, identificação, avaliação da susceptibilidade antimicrobiana e identificação de genes de resistência e integrons em *Escherichia coli* isoladas de queijo Minas Frescal. **Método:** Avaliação da presença de *E. coli* em trinta amostras de queijo Minas Frescal por meio de Placas Petrifilm 3M™. Trinta isolados de *E. coli* foram avaliados através do teste de sensibilidade por disco difusão para 17 agentes antimicrobianos. Isolados fenotipicamente resistentes foram examinados por meio de PCR e Multiplex PCR para 24 genes de resistência. Genes e regiões variáveis dos integrons de classe 1, 2 e 3 também foram avaliados. **Resultados:** Em 50% dos isolados verificou-se resistência fenotípica a uma ou mais classes de antimicrobianos. Cem por cento dos isolados apresentaram o gene bla_{TEM} e houve amplificação dos genes bla_{SHV} e *tet*B. Além disso, 46,6% dos isolados fenotipicamente resistentes amplificaram para uma ou mais classes de integrons. Este é um dos primeiros estudos a identificar esses genes em queijo Minas Frescal no Brasil. **Conclusões:** O queijo pode ser uma fonte de bactérias multirresistentes e estas podem disseminar seus genes de resistência a bactérias presentes no alimento e no trato gastrointestinal humano, demonstrando a importância das Boas Práticas de Fabricação e a necessidade de maior fiscalização dos produtos colocados à venda.

PALAVRAS-CHAVE: Resistencia Antimicrobiana; *E. coli*; Integron; Queijo Minas Frescal

INTRODUCTION

Food and its production may be vehicles for antimicrobial resistant bacteria and resistance genes toward humans and this fact is directly related to public health[1].

Antimicrobial resistant bacteria can spread antimicrobial resistance through horizontal gene transfer, a process that transfers resistance genes between bacteria[2]. The acquisition and transfer of genes resistant to antimicrobial agents, associated with the selection caused by the intensive use of these substances in livestock, highlights the importance and need to evaluate the presence of antibiotic resistant bacteria in food[3].

Interventions that limit the use of antibiotics in animal feed appear to be associated with a reduction in the development of antimicrobial resistance in humans and, therefore, further research is necessary to explore this complex association[4].

Integrons play an important role in the acquisition and spread of antibiotic resistance genes[5] and in multidrug resistance in Gram-negative bacteria because they capture and incorporate gene cassettes from the environment[6]. Thus, the detection and characterization of integrons containing antibiotic resistance genes are key steps to evaluate the potential of a given environment to represent a reservoir of antibiotic resistance[7].

Antibiotic resistant bacteria and resistance genes can be transferred directly to humans via food chain[8]. Thus, it is important to know the phenotype and genes involved in antibiotic resistance of *Escherichia coli* strains that colonize food products, since these may act as reservoirs of antimicrobial resistance genes[9].

It is also important to highlight that this is one of the first studies to evaluate the presence of these bacteria, their resistance genes and integrons in this food matrix, the Minas Frescal cheese.

METHODS

E. coli isolation

We evaluated 30 samples of Minas Frescal cheese from ten different brands, purchased in supermarkets in the city of Rio de Janeiro, Brazil.

For *E. coli* isolation, we used *E. coli* counting 3M™ Petrifilm™ plates and obtained several isolates. Then, we selected 30 *E. coli* isolates, each one from a different sample of Minas Frescal cheese. We checked and confirmed that all the isolates were strains of *E. coli* through polymerase chain reaction (PCR) according to Bej et al.[10].

Kirby-Bauer disk diffusion sensitivity test

All 30 isolates of *E. coli* underwent the antimicrobial sensitivity test for 17 antimicrobial agents using the disk diffusion method in Mueller-Hinton agar, according to the criteria of the Clinical and Laboratory Standards Institute (CLSI)[11]. The antibiotics used in this test and their concentrations on the disks were: ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), ampicillin/sulbactam (10/10 µg), tetracycline (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), imipenem (10 µg), ertapenem (10 µg), gentamicin (10 µg), amikacin (30 µg), streptomycin (10 µg), sulfazotrim (30 µg) and chloramphenicol (30 µg).

Polymerase chain reaction

We tested all the isolates that presented phenotypic resistance to any of the 17 antimicrobial agents used in the sensitivity test for the presence of 24 antimicrobial resistance genes[12,13,14,15,16,17,18,19] (Table 1).

Integron detection

We looked for the *intI* 1, *intI* 2, *intI* 3 genes (which encode for integrases classes 1, 2 and 3, respectively) and for the variable regions of the class 1 and 2 integrons by PCR (Table 2) in all the *E. coli* isolates according to Ryu et al.[20].

RESULTS

E. coli isolation in Minas Frescal Cheese

In a 1:10 dilution, most of the samples of Minas Frescal cheese (96.6%) presented a countless number of *E. coli* isolates. This fact demonstrates the level of contamination by this microorganism in this food. To obtain standard isolates of *E. coli*, that is, blue colonies with gas formation, we had to dilute the samples at 1:1,000 or 1:10,000.

Antimicrobial sensitivity test

Of the 30 isolates of *E. coli*, 15 (50%) were resistant to one or more antimicrobial classes (Table 3).

Resistance encoding genes

All 15 isolates of resistant *E. coli* tested positive for one or more resistance genes. All isolates (100%) amplified to the bla_{TEM} gene, and the isolate N also amplified to the bla_{SHV} gene and isolate C, for the *tet*B gene. This is one of the first studies to isolate these genes in Minas Frescal cheese.

Integrons

Of the phenotypically resistant isolates, 46.6% amplified to one or more classes of integrons. Isolate C amplified to class 2 and 3 integrons and also to the variable class 2 integron. Isolate H amplified to the class 3 integron and isolate M tested positive for class 1 and variable class 1 integron. Four other isolates (E, F, K, L) amplified only to the variable class 1 integron.

Table 1. Genes, sizes and references that were used for genetic identification of antimicrobial resistance.

Gene	Size (bp)	Reference
CTX	593	Al-Agamy et al., 2014[12]
Multiplex: TEM/SHV/OXA	800/713/564	Dallene et al., 2010[13]
Tet A/ Tet B/ Tet C/ Tet D/ Tet E	372/288/379/436/442	Srinivasan, 2008[14]
Tet G	884	Ng, 2001[15]
MOX	520	Székely et al., 2013[16]
CIT	462	
DHA	405	
EBC (MIR)	302	
ACC	346	
FOX	190	
Multiplex: GES/ PER/ VEB	398/520/648	Al-Agamy et al., 2014[12]
OXA48	438	Karuniawati et al., 2011[17]
NDM	621	Karuniawati et al., 2011[17]
KPC	635	Azimi et al., 2013[18]
IMP	188	Zeighami et al., 2015[19]
VIM	390	Zeighami et al., 2015[19]

bp: base pairs

Table 2. Components used for integron identification

Gene	Primer	DNA Sequence (5'-3")	Size (bp)	Annealing Temperature (°C)=
intI1	int1-L	CTGCGTTCGGTCAAGGTTCT	882	68
	int1-F	GGAATGGCCGAGCAGATCCT		
intI2	int2.F	CACGGATATGCGACAAAAAGGT	746	59
	int2.R	GTAGCAAACGAGTGACGAAATG		
intI3	int3.F	GCCTCCGGCAGCGACTTTCAG	939	62
	int3.R	ACGGATCTGCCAAACCTGACT		
IntI1 variable region	5'CS	GCCATCCAAGCAGCAAG	Variable	60
	3'CS	AAGCAGACTTGACCTGA		
IntI2 variable region	hep74	CGGACGGCATGCACGATTTGTA	Variable	60
	hep51	GATGCCATCGCAAGTACGAG		

bp: base pairs

DISCUSSION

According to Amos et al.[2], class 1 integrons are vehicles for adaptive genes because of their ability to capture and integrate mobile cassettes into a variable region where they are expressed under a common promoter. These gene sequences can provide several phenotypes, including resistance to a wide range of antimicrobial classes.

Karuniawati, Saharman, Lestari[17] described class 1 integron as the most frequent resistance gene carrier integron, and the production of the ß-lactamase enzyme is one of the most common resistance mechanisms. More recent studies on extended-spectrum ß-lactamase (ESBL) genes have shown that they are located in integrons. Therefore, ESBL genes can be located in integrons and easily transferred to various bacteria[21].

Mohamed, Hassan, Ahmed[22] evaluated class 1, 2, and 3 integrons and carbapenem resistance in Gram-negative bacteria and detected class 1 integron in most carbapenem resistant isolates. That is similar to what we found in this study, since of the three carbapenem (imipenem and ertapenem) resistant isolates (K, M, N), only one (N) did not amplify to that integron. The other two isolates (K and M) amplified to class 1 and/or variable class 1 integrons.

De Paula et al.[23] analyzed class 1, 2 and 3 integrons in Minas Frescal cheese samples and noticed that the presence of class 1

Table 3. Antimicrobial susceptibility test results according to the resistant *E. coli* isolates.

Isolate	Antibiotics									
A	Ampicillin									
B	Tetracylcine									
C	Ampicillin	Streptomycin	Amoxicillin/ clavulanic acid	Tetracylcine	Cefoxitin					
D	Ampicillin	Amoxicillin/ clavulanic acid	Cefoxitin							
E	Ampicillin	Streptomycin								
F	Ampicillin	Cefoxitin								
G	Ceftriaxone	Nalidixic acid	Cefepime	Ceftadizime						
H	Ceftriaxone	Nalidixic acid								
I	Streptomycin	Nalidixic acid								
J	Nalidixic Acid									
K	Ampicillin	Imipenem								
L	Nalidixic Acid									
M	Streptomycin	Amoxicillin/ clavulanic acid	Ampicillin	Ampicillin / sulbactam	Cefoxitin	Ceftriaxone	Sulfazotrim	Nalidixic acid	Imipenem	Ertapenem
N	Amikacin	Streptomycin	Ampicillin	Tetracylcine	Cefoxitin	Ceftriaxone	Imipenem	Ertapenem	Cefepime	Ceftadizime
O	Ampicillin	Nalidixic acid								

and 2 integrons was 77% and 97% respectively. Class 3 integrons were not detected in any of the samples. These results are different from what we found in this study, in which we detected class 3 integron in 13.3% of the *E. coli* isolates from Minas Frescal cheese and class 1 and variable class 1 in 33.3% of the samples.

Regarding resistance to quinolone class, several mechanisms seem to contribute to this resistance. Q*nr* genes mediated by plasmids are a common mechanism of resistance to quinolone[24]. A study suggests that the relation between the *qnr* gene and ESBL is associated with genes encoding ESBL[21]. High levels of quinolone resistance in clinical strains of *E. coli* and other ESBL-producing bacteria were reported in several studies[25].

The studies described above corroborate our results, since the resistance to quinolone, represented by nalidyxic acid here, was always associated with the bla_{TEM} gene and, in two of the three isolates, we detected the integron and confirmed that the genes encoding for the ESBL may be located there.

The bla_{TEM} and bla_{SHV} genes are related to resistance to antimicrobial agents of the cephalosporin class. The SHV-type ESBL, together with the TEM-type, stood out during the 1980s as a cause of cephalosporin resistance among enterobacteriaceae[26], as occurs, for example, with isolate N, that contains the two genes (bla_{TEM} and bla_{SHV}) and is resistant to second, third and fourth generations cephalosporins (cefoxitin, ceftriaxone, ceftazidime, cefepime).

Traditionally, ESBL-producing isolates, mainly bla_{TEM} and bla_{SHV} producers, show co-resistance to aminoglycosides, tetracyclines and sulfonamides[27]. This evidence corroborates the fact that isolate N was also resistant to aminoglycosides (amikacin, streptomycin) and tetracycline.

The study of Juma et al.[28] evaluated the bla_{TEM} and bla_{SHV} genes in isolates of *E. coli* and found that bla_{TEM} was more common than bla_{SHV}. In this study, the presence of both genes (bla_{TEM} and bla_{SHV}) was confirmed in only one (6.6%) of the *E. coli* isolates (isolate M). The bla_{TEM} gene was found in all the other isolates (100%). A similar result was also found in the study of Freitas et al.[29].

Isolate C was resistant to ampicillin, streptomycin, tetracycline, cefoxitin and amoxicillin/clavulanic acid. Regarding the genotype, the following genes were found: bla_{TEM}, *tet*B and *Intl* 2, 3 and 2 variable. The resistance to tetracycline can, in this case, be confirmed by the existence of the *tet*B gene. Tetracyclines are the most widely used antimicrobials in animal therapy[30].

CONCLUSIONS

This study found *E. coli* in 100% of the commercial brand samples of Minas Frescal cheese. This fact is enough to show bacterial contamination from fecal origin in this type of food.

Half (50%) of the *E. coli* isolates were resistant to one or more antimicrobial classes and we were able to confirm by PCR the presence of the genes that justified the resistance phenotype presented by most of them. In addition, 46.6% of the phenotypically resistant isolates amplified to one or more integron classes, which corroborates the resistance phenotype presented by some isolates and demonstrates its importance in the spread of resistance genes among bacteria.

Since this is one of the first studies about Minas Frescal cheese in Brazil to detect the bla_{TEM}, bla_{SHV}, *tet*B and *intI* 1, 2 and 3 genes, we expect it to contribute to the generation of data that improve the characterization of antibiotic resistant *E. coli* isolates and their spread through this type of food.

In the area of health surveillance, the studies about antimicrobials in food-producing livestock are very important, since this is the main way through which bacteria from animal GI tract acquire resistance. Furthermore, studies about Good Manufacturing Practices in the production of this type of food are also important.

We also expect that this study can work as a foundation for further studies and public health policies so that food will not become a reservoir or transmitter of multiresistant bacteria to people.

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Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.