INVESTIGATION OF DIARRHEAGENIC *ESCHERICHIA COLI* IN CHEESES

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Introduction

Escherichia coli (*E. coli*) is a gram-negative, facultatively anaerobic bacterium present in the intestinal microbiota of humans and warm-blooded animals, contributing to the health of the host. Although in many cases it is harmless, certain strains can acquire virulence factors, leading to the development of diseases in humans and animals[1]. Diarrheagenic *Escherichia coli* (DEC) are associated with outbreaks of waterborne and foodborne diseases, especially in developing regions[2]. DEC contamination can occur from food in the field to home preparation, including the processing, marketing and distribution stages[3]. According to the virulence mechanisms and the signs and symptoms of the diseases caused, pathogenic strains of *E. coli* are classified into six pathotypes: classical enteropathogenic *E. coli* (EPEC), atypical enteropathogenic *E. coli* (ATEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), Shiga toxin-producing *E. coli* (STEC), and enteroaggregative *E. coli* (EAEC)[4]. Milk is one of the most consumed foods by the human population, however, due to its intrinsic characteristics, it is an ideal substrate for the development of microorganisms. The quality and safety of various dairy products are directly influenced by the quality of the raw milk used as raw material[2]. The production of various types of cheese must follow good manufacturing practices, control the potability of the water, and ensure care during marketing, transportation, and registration of the product[4]. Therefore, the objective of this study is to research the genotypic markers of the categories of DEC in polymicrobial suspensions obtained directly from cheese samples.

Material and Methods

Thermal DNA extraction was performed on 46 polymicrobial suspensions of Minas Frescal cheese (n=15), Minas Padrão cheese (n=12) and Minas Artesanal cheese (n=19) obtained in a previous study, purchased at the local market and with different suppliers, subsequently subjected to the Polymerase Chain Reaction (PCR) technique, according to the protocol exposed in the literature[5] by Muller (2007), to identify specific genes from the different DEC categories, such as stx1 and stx2 (STEC), escV and bfpB (EPEC). The analysis was performed by agarose gel electrophoresis and visualization under ultraviolet light.

Results and Discussion

Among the 46 samples of Minas cheese, only one sample of Minas Artesanal cheese tested positive for the *stx* gene, characteristic of STEC, as shown in Table 1 below, indicating a potential risk to public health. Minas cheese is widely available in the country and is highly accepted by consumers. Its production involves a relatively simple technology, which makes its production important for the national economy. However, foods such as cheese can harbor several pathogenic microorganisms, including E. coli[2].

Table 1. Number of samples of Minas Artesanal cheese (QMA), Minas padrão cheese (QMP) and Minas Frescal cheese (QMF) positive for the *escV, bfp* and *stx* genes.

Amostras	Genes		
	EPEC		STEC
	escV	*bfp*	*stx*
QMA (n=19)	0	0	1
QMP (n=12)	0	0	0
QMF (n=15)	0	0	0
TOTAL (n=46)	0	0	1

Shiga toxin-producing *Escherichia coli* (STEC) is a major foodborne pathogen and has been implicated in several disease outbreaks. STEC infection may be asymptomatic or present with symptoms such as mild diarrhea, and may progress to more severe forms, such as severe hemorrhagic colitis and hemolytic uremic syndrome (HUS)[5]. The presence of STEC in dairy products suggests that milk may be one of the main sources of *E. coli* infection, either due to inadequate pasteurization during the production process or through transmission by cattle, which are natural reservoirs of pathogenic *E. coli* strains. These results are a warning sign for health authorities, since Minas cheese is a ready-to-eat food and, therefore, should not pose a risk to the health of the population[2].

Conclusion

The presence of virulence genes of diarrheagenic *E. coli* in ready-to-eat foods such as cheese reinforces the importance of strict sanitary control measures during the production of these products. The multiplex PCR technique proved to be efficient for the simultaneous detection of different virulence genes, reducing costs and analysis time, contributing to epidemiological surveillance and food safety.

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