TITLE: VIRULENCE FACTORS, PHYLOGENETIC CLASSIFICATION AND BIOFILM PRODUCTION IN *ESCHERICHIA COLI* ISOLATED FROM MILK OF BOVINE CLINICAL MASTITIS

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ABSTRACT:

Bovine mastitis is responsible for large losses in the dairy industry because it is caused by several bacteria, such as *Escherichia coli*. Although *E. coli* is not responsible for most cases of mastitis, the relevance is due to its ability to cause severe and acute conditions of this disease. In addition, a possible zoonotic character should be researched. This study aims to characterize 213 strains of *E. coli* isolated from dairy cows with clinical mastitis, from dairy farms in the interior of Sao Paulo and Minas Gerais, regarding molecular classification, virulence genes and biofilm production. Ten dairy herds were sampled, and inclusion criteria were mastitis control programs with data storage, mean milk yield > 20 kg, minimum of 200 lactating cows and use of mechanical milking. Strains were isolated and identified according to the National Mastitis Council. Multiplex PCR tests were performed in order to find the phylogenetic group for each sample. Biofilm production in polystyrene plates were performed by seeding the isolates in Brain-Heart Infusion broth (BHI) and incubated at 37°C/24 h. Then, each growth was diluted until McFarland 0.5 standard using BHI + 0,5% glucose . The samples were seeded in quadruplicate in 96-well plates and incubated at 37°C/24 hours again. The plates were washed to remove unbound cells, stained with violet crystal 1% and optical density values were obtained on an ELISA reader. To this moment, out of 141 analyzed strains, 75 (53.2%) were assigned to the phylogenetic group B1, 54 (38.3%) to A, seven (4.9%) to E, two to B2 (1.4%), one (0.7%) to C, one to D and one was classified as belonging to a unknown group. Considering the biofilm production, 59 (41,8%) were classified as non-producers, 69 (48.9%) were weak producers, 11 (7.8%) were moderate and two (1%) isolates showed a strong biofilm production. Only five isolates presented one (*escN* or *aaiA*) out of the 12 researched characteristic diarrheogenic *E. Coli* (DEC) genes. Regarding the 23 searched extraintestinal pathogenic *E. Coli* (ExPEC) genes, the most commom profiles were a variant of a *fimH*, *ecpA*, *traT* and *ompT* combination. The only statistical relation found between virulence factors and phylogenetic groups was a higher prevalence of the gene *ompT* on group B1, absence of *kpsMT II* on group B1 and *astA* on A. However, relation between biofilm production and virulence factors or phylogenetic group were not observed.

Keywords: biofilm, *Escherichia coli,* mastitis, PCR