

TITLE: ANTIMICROBIAL RESISTANCE GENES AND BIOFILM FORMATION IN MULTIDRUG-RESISTANT *Escherichia coli* ISOLATED FROM BOVINE MASTITIS

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ABSTRACT:

Bovine mastitis is an inflammation in the mammary glands of the cow due to bacteria infection, standing out as one of the main diseases that affect dairy herd. *Escherichia coli* is one of the main species responsible for mastitis infection and the dairy cows affected are usually treated with several antibiotics, which could lead to drug resistance in those bacterias. Given that bovine mastitis caused by bacteria resistant to antibiotics and able to form biofilms are more difficult to eradicate, this study aimed to investigate the antimicrobial resistance and biofilm formation in *E. coli* isolated from bovine mastitis cases. *E. coli* were collected from bovine mastitis cases on 11 dairy farms of Southeast Brazil. Antimicrobial susceptibility was assessed by the disk diffusion method. The multidrug-resistant (MDR) *E. coli*, resistant to ≥ 3 antimicrobial classes were tested for biofilm formation using crystal violet method with a 96-well polystyrene plate. Antimicrobial resistance genes (*ampC*, *bla*_{CTXM}, *bla*_{SHV}, *bla*_{TEM}, *florA*, *gyrA*, *parC*, *sul1* and *tetA*) and virulence factors related to biofilm formation (*ag43*, *csgA*, *crl*, *fimH* and *kpsII*) were tested by PCR for MDR strains. Fisher's exact test was performed to biofilm formation and detection of antimicrobial resistance genes. Of 90 strains isolated, only five were MDR (resistant to ampicillin, cefoperazone, ciprofloxacin, chloramphenicol, enrofloxacin, sulfamethoxazole-trimethoprim, and tetracycline), interestingly strains from the same dairy farm. All MDR samples formed biofilm on the polystyrene plate; three of them formed weak biofilm, one formed moderate biofilm and one formed strong biofilm. Most frequent resistance genes were *ampC* (3), *bla*_{CTXM} (1), *florA* (2), *gyrA* (2), *parC* (3) and *tetA* (3). The *fimH* and *crl* genes were detected in all of the MDR *E. coli*, and *csgA* (4) gene was not found in moderate biofilm strain. None strain showed *ag43*, *bla*_{SHV}, *bla*_{TEM}, *kpsII* and *sul1* genes. There was no statistical relevance between biofilm formation (weak, moderate or strong) and antimicrobial resistance, but the one strong biofilm strain showed genes *ampC*, *florA*, *gyrA*, *parC*, and *tetA*. We found MDR *E. coli* strains able to form biofilm, which

brings an alert to the persistence of those bacteria in the milking environment as a source of infection to animals.

Keywords: intra-mammary inflammation, dairy farm, antibiotic resistance

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