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# Analysis Of Biofilm Formation By Escherichia Coli Strains Isolated From Calves Feces

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***Abstract:***
***Background****: Dysbiosis in the gastrointestinal tract of ruminants can lead to significant health issues, including reduced microbial diversity and overgrowth of pathogenic bacteria. Escherichia coli is a common commensal bacterium in ruminants, with some pathogenic strains causing serious health problems, including diarrhea and systemic infections. The ability of E. coli to form biofilms complicates infection management, as biofilms provide resistance to antibiotics and hinder the immune response.*
***Materials and Methods****: The study assessed the biofilm-forming capacity of E. coli strains isolated from calves feces using Congo Red Agar (CRA) culture medium. Bacterial strains were inoculated onto CRA plates and incubated at 35 ± 2°C for 24 and 48 hours, with results interpreted based on colony morphology and coloration. The tests were performed in triplicate to ensure reliability, with positive and negative controls included for validation.*
***Results****: Out of ten E. coli strains isolated, five demonstrated the ability to form biofilms, indicated by black colonies with a dry, crystalline appearance on CRA. The study found that 50% of the isolates from diarrheic cattle were biofilm producers, which is higher than previously reported averages for bovine-derived strains.*
***Conclusion:*** *The significant biofilm-forming capacity observed in E. coli strains from diarrheic cattle highlights the importance of this phenotype in bacterial persistence and dissemination in agricultural environments. There is a need for enhanced surveillance and characterization of these strains to understand the mechanisms influencing biofilm formation and to improve microbiological control in food safety and public health.*
***Key Word****: Biofilm, E. coli, Dysbiosis, Congo Red Agar, Calves.*

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## I. Introduction

Dysbiosis in the gastrointestinal tract of ruminants can trigger significant health issues. This imbalance occurs when the gut microbiota becomes disorganized, often due to dietary changes, stress, or antibiotic use[1]. Such alterations can reduce microbial diversity and promote the overgrowth of pathogenic bacteria. This disruption not only affects the digestive process but also compromises the animals' immune response, making them more susceptible to infections and diseases, which negatively impacts herd productivity and welfare[2,3].

Among the many microorganisms that inhabit ruminants, *Escherichia coli* is a common commensal bacterium found in the intestines[4,5]. While many of its strains are harmless, some pathogenic variants can lead to serious clinical conditions, ranging from diarrhea to systemic infections[6]. The presence of these strains can adversely affect weight gain and feed conversion in animals, as well as reduce milk production, resulting in significant economic losses[7,8].

The ability of *E. coli* to form biofilms presents an additional challenge in managing these infections. Biofilms are structures composed of bacterial communities that adhere to surfaces and are encased in a protective matrix[9,10]. This configuration provides greater resistance to antibiotics and complicates the host's immune response. In ruminants, biofilms can establish themselves in the gastrointestinal tract, contributing to chronic infections and the perpetuation of dysbiosis[11]. The presence of these structures complicates treatment and prolongs recovery time. Therefore, understanding the mechanisms of biofilm formation by *E. coli* is essential for developing effective control strategies aimed at improving animal health and productivity[12,13].

The application of phenotypic tests has emerged as a relevant strategy for identifying the biofilm-forming capacity of bacterial isolates[14]. Among the available methods, the use of Congo Red Agar (CRA) culture medium stands out, allowing for the visual screening of biofilm-producing strains based on specific morphological characteristics of the colonies. This methodology is advantageous due to its accessibility, low cost, and effectiveness in the preliminary detection of potentially virulent strains[15].

This study aims to assess the biofilm-forming capacity of *E. coli* strains isolated from fecal samples of calves, using the phenotypic method based on CRA medium. By evaluating the frequency of biofilm-forming strains in herds, the goal is to enhance understanding of their potential association with the pathogenesis of bovine mastitis. The data obtained may provide valuable insights for adopting more effective control strategies, aimed at reducing productivity losses and promoting animal health.

## II. Material And Methods

**Sampling**

We sampled dairy calves raised in collective and/or mixed housing systems. The animals received milk twice a day, along with supplemental feed, concentrates, and roughage, with ad libitum access to water. Both male and female calves exhibiting clinical signs of diarrhea at the time of collection were included in the study to investigate potential pathogenic strains associated with enteric conditions. Collections were conducted under controlled conditions, with physical restraint of the animals to ensure the safety of all involved and to standardize the procedure. The perianal region was sanitized using a 10% PVPI (povidone-iodine) solution to eliminate external contaminants that could interfere with microbiological results.

**Isolation and Biochemical Identification**

The samples were inoculated onto MacConkey agar plates and incubated at 37 °C for 24 hours. Colonies with cultural characteristics similar to *E. coli* were identified and characterized as *E. coli* for biochemical testing. The results indicated positive indole reaction, negative Voges-Proskauer (VP) test, positive Methyl Red (MR) test, and negative results for $H_2S$ and citrate. Confirmation of the identification was performed through inoculation in Rugai medium. A total of 10 strains identified phenotypically as *E. coli* were used in the study.

**Evaluation of Biofilm Production on Congo Red Agar**

Biofilm formation was analyzed using Congo Red Agar (CRA) culture medium, prepared according to a methodology adapted from Tavares et al.[15]. The medium composition included BHI broth (37 g/L), agar (10 g/L), sucrose (50 g/L), and Congo red (0.8 g/L). Bacterial strains were inoculated onto plates containing CRA and incubated under aerobic conditions at $35 \pm 2°C$, with evaluations conducted after 24 and 48 hours of incubation. The interpretation of results was based on the morphological characteristics and coloration of the colonies. Black colonies with a dry, crystalline appearance were considered indicative of biofilm formation, while those with a reddish or pink coloration were classified as non-producers. All assays were performed in triplicate to ensure the reproducibility and reliability of the obtained data.

## III. Result

The isolation and preliminary characterization of *E. coli* strains were performed on MacConkey agar, where typical morphological and colorimetric characteristics of the colonies were observed. The *E. coli* colonies exhibited a red or dark pink coloration, indicative of lactose fermentation, with sizes ranging from medium to large, an opaque surface, and an umbilicated appearance (raised in the center).

Complementary phenotypic identification was conducted on modified Rugai medium with lysine. In this medium, *E. coli* displayed a biochemical profile consistent with the species, including glucose fermentation, typically without visible gas production, and positive activity for lysine decarboxylation, evidenced by a color change in the medium from purple to yellow. Additionally, indole production was observed, indicated by a reddish coloration in the cotton plug at the top of the tube.

Out of the ten *E. coli* strains isolated from fecal samples of cattle, five demonstrated the ability to form biofilms (Figure no 1 and Table no 1). The assays were conducted in triplicate, with no variations observed among the repetitions. The validation of the tests was performed using positive controls (*S. aureus* ATCC

25923) and negative controls (*E. coli* ATCC 8739), as well as a sterile culture medium without microbial inoculation.

**Figure no 1** - Morphological Appearance of *E. coli* Strains on RCA Medium

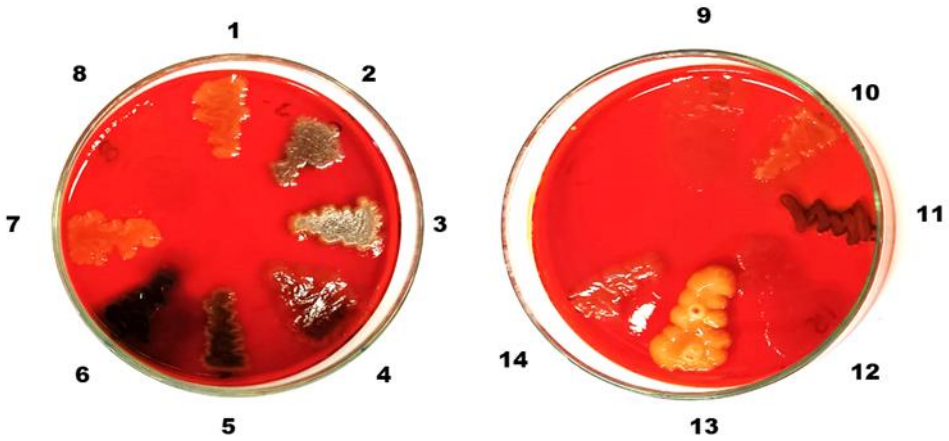


**Table no 1:** Table 1 - Results of biofilm production analysis using CRA medium

| nº | Strain | Plate 1 | Plate 2 | Plate 3 |
|---|---|---|---|---|
| 01 | *E. coli* - FFX | Negative | Negative | Negative |
| 02 | *E. coli* - F2E | Positive | Positive | Positive |
| 03 | *E. coli* - F2G | Positive | Positive | Positive |
| 04 | *E. coli* - F2D1 | Positive | Positive | Positive |
| 05 | *E. coli* - ICA7 | Positive | Positive | Positive |
| 06 | *E. coli* - ICA5 | Positive | Positive | Positive |
| 07 | *E. coli* - F1C | Negative | Negative | Negative |
| 08 | *E. coli* - F1E | Negative | Negative | Negative |
| 09 | *E. coli* - F1F | Negative | Negative | Negative |
| 10 | *E. coli* - F1G | Negative | Negative | Negative |
| 11 | *S. aureus* - ATCC 25923 | Positive | Positive | Positive |
| 12 | *E. coli* - ATCC 8739 | Negative | Negative | Negative |
| 13 | *Klebsiella sp.* | Negative | Negative | Negative |
| 14 | *Salmonella sp.* | Negative | Negative | Negative |

One strain of *Klebsiella* sp. and one strain of *Salmonella sp*. were used as references to observe the morphological patterns of the colonies and to detect potential contaminations, aiding in the phenotypic characterization of the isolates on RCA. The *E. coli* colonies exhibited distinctly uniform morphological characteristics compared to the other inoculated Enterobacteriaceae strains.

## IV. Discussion

The formation of biofilms by *E. coli* has been widely recognized as an important mechanism for survival and environmental persistence, particularly in clinical and industrial contexts. However, there is a notable lack of studies specifically addressing the biofilm-forming capacity of strains isolated from the feces of ruminants, such as cattle. This gap limits the possibilities for direct comparative analysis and often necessitates the use of data from human, environmental, or industrial surface strains.

The adaptive evolution of *E. coli*, driven by intense selective pressure over time, has resulted in the development of various resistance strategies, including biofilm formation. This complex structure, primarily composed of exopolysaccharides (EPS), provides protection against antimicrobials and the host's immune response, complicating the treatment of infections and bacterial eradication. Studies indicate that biofilm production is related to specific regulatory mechanisms, such as adaptive mutations and the overexpression of membrane channels, particularly in pathogenic strains[16].

In the livestock context, toxin-producing strains, such as Shiga toxin-producing *E. coli* (STEC), are often associated with biofilm formation, contributing to their spread through fecal contamination of carcasses and food. Adherence to the intestinal epithelium and subsequent detachment of biofilm-embedded cells facilitate cross-contamination during slaughter and processing[6]. However, the literature shows that the ability to form biofilms varies significantly among animal-derived strains. For instance, Stanford et al.[17] reported that only 7.1% of cattle isolates formed biofilms, in contrast to 87.3% of isolates from equipment surfaces, highlighting the influence of ecological niche on the expression of this phenotypic characteristic.

Moreover, *E. coli* is a key species in the study of biofilm formation due to its abundance as a facultative anaerobe in the gastrointestinal tract and the wealth of genetic tools available for experimental manipulation. Biofilm formation is a complex and highly regulated process involving extracellular appendages, such as fimbriae, flagella, and adhesion proteins, whose expression is dependent on specific environmental conditions[18]. Factors such as temperature, nutrient availability, pH, and surface type directly influence this capability[19].

In the present study, 50% of the isolates from diarrheic feces of cattle demonstrated the ability to form biofilms, as assessed by the CRA method. This result stands out as being above the average reported for bovine-derived strains in some previous studies, which may be related to the pathological state of the animals at the time of collection. Diarrhea may be associated with the presence of more virulent or adapted strains, which often exhibit greater adherence and biofilm-forming capacity as part of their pathogenic arsenal.

Additionally, the CRA method has proven to be an effective tool for the phenotypic screening of biofilm producers, with positivity rates (49%) similar to those reported in studies with uropathogenic strains, suggesting that its application in studies with bovine strains may yield reliable and comparable data[20,21]. Identifying biofilm-forming strains in this context is crucial, as such characteristics may enhance persistence in rural environments, facilitate transmission among animals, and increase the risk of contamination of animal-derived food products.

## V. Conclusion

In conclusion, the biofilm-forming capacity observed in 50% of the *E. coli* strains isolated from the feces of diarrheic cattle highlights the significance of this phenotype in bacterial persistence and dissemination within the agricultural environment. This finding underscores the need to intensify efforts in the surveillance and phenotypic and genotypic characterization of these strains to better understand the mechanisms involved and the environmental factors that influence their expression. In particular, there is a critical need for studies focused on intensive production systems, where microbiological control is essential to ensure food safety, prevent zoonotic infections, and promote public health.

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