

**TITLE:** ANTIMICROBIAL-RESISTANT *Escherichia coli* IN ANAEROBIC DIGESTION TREATMENT OF CATTLE MANURE

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**ABSTRACT:**

To avoid direct discharge of manure into soil and aquatic ecosystems anaerobic digestion is pointed out as a sustainable alternative resulting in production of biogas and biofertilizer. However, effluent digesters are known to contain pathogenic enteric microorganisms that may be a risk to human health, as *Escherichia coli*. The purpose of this work was to evaluate the microbiological safety of the anaerobic digestion effluents by analysing the antimicrobial susceptibility of *E. coli* isolated from influent and effluents from pilot scale anaerobic biodigesters operated at room temperature. In total, 168 *E. coli* strains from the biodigesters were isolated, referred to as the influent (n = 23) and effluent (n = 145), by selective isolation of enterobacteria. Biochemical identification was performed using commercial kits. Antimicrobial susceptibilities were determined by agar-dilution method, whereas the level of antibiotic resistance was determined by the calculation of the multiple antibiotic resistance index (MAR). Out of 23 isolated from influent, 21.7% presented a MAR  $\geq 0.2$ , ranging between 0.2 to 0.3 and showed resistance to ampicillin (13.04%), ampicillin-sulbactam (8.7%), gentamycin (8.7%), and sulfamethoxazole/trimethoprim (4.35%). High susceptibility rates (100%) were observed for levofloxacin, amikacin and chloramphenicol. With regards to *E. coli* isolated from effluent samples, 11.3% presented a MAR  $\geq 0.2$ , ranging between 0.2 to 0.3 and showed resistance to ampicillin (22.0%), ampicillin-sulbactam (10.0%), gentamycin (8.7%), and chloramphenicol (3.0%). High susceptibility rates (100.0%) were observed for levofloxacin, amikacin and sulfamethoxazole/trimethoprim. In this work, an important proportion of strains recovered from the influent samples exhibited resistant to different antibiotics, and practically the same pattern of resistance was detected in effluent samples. Frequency of MAR  $> 0.2$  in the influent samples may reflect a microbial adaptive response to the empirical use of antimicrobials as prophylactics or therapeutics in cattle farm. Overall, our findings suggested discussion on the use of antimicrobials in animal farms and surrounding environments. Further prospective studies are needed to better discuss the extent of the antimicrobial resistance phenomena and maintenance of these resistant strains in the environment.

**Keywords:** Minimal inhibitory concentration, dairy cattle manure, microbiological safety.

**Development Agency:** Fundação de Amparo à Pesquisa e Inovação do Espírito Santo – FAPES