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Characterization of Phenotypic Resistance of Bacterial Isolates and Comparison with Metagenomic Data in Poultry Slaughterhouse Effluent: An Initial Analysis to Contribute to One Health

Evelyn Cristine da Silva[1,2], Emanoelli Aparecida Rodrigues dos Santos[2], Patrícia Regina Lopes Melo[2], Leonardo Ereno Tadielo[2], Gabriella Rodrigues Cazolda[2], Karina Pires Gutierrez[1], Wanderson Sirley Reis Teixeira[2], Juliano Gonçalves Pereira[2], João Pessoa Araújo Junior[1], Fábio Sossai Possebon[1,2]

[1.] UNIVERSIDADE ESTADUAL PAULISTA, INSTITUTO DE BIOTECNOLOGIA, BOTUCATU/SP, BRASIL
[2.] UNIVERSIDADE ESTADUAL PAULISTA, FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA, BOTUCATU/SP, BRASIL

Phenotypic methods identify and treat antibiotic-resistant diseases. Genotypic methods, like metagenomic sequencing, are increasingly used to detect resistance genes in clinical and environmental samples, providing detailed genetic profiles. This study aimed to perform the phenotypic and genotypic characterization antimicrobial resistance of the microbiota of effluent sample from a poultry slaughterhouse. The samples were collected from a conventional poultry slaughterhouse located in the state of São Paulo. Four samples were used for bacterial isolation and antibiotic susceptibility testing, with a portion allocated for total DNA extraction. Specific microbiological analyses were conducted to detect *Salmonella*, *Escherichia coli*, *Enterococcus* sp., and *Pseudomonas* sp. The isolates were cultured in Trypticase Soy Broth (TSB) and incubated at 37°C for 18-24 hours. Disk diffusion and interpretation of the zones of inhibition followed the Clinical and Laboratory Standards Institute (CLSI) guidelines (2020) to classify the isolates as resistant, intermediate, or susceptible. Previously confirmed isolates underwent antibiotic resistance testing across different classes using the disk diffusion method. Antibiotic selection for testing adhered to CLSI (2020) guidelines. For metagenomic analysis, DNA was purified using the AllPrep® PowerViral® DNA/RNA Kit. DNA library preparation was performed using the Illumina CoviSeq Kit with modifications. Sequencing followed the Illumina preparation guide (NextSeq System - Denature and Dilute Libraries Guide) and sequenced on the NextSeq platform (Illumina, San Diego, CA). For resistome analysis, we utilized the ARGS_OAP pipeline, which identifies and characterizes antibiotic resistance genes in metagenomic data. As a result of the microbiological analyses of the effluents, 4 strains of *E. coli*, 4 strains of *Enterococcus* sp., and 2 strains of *Pseudomonas* spp. were isolated. *E. coli* isolates were resistant to Chloramphenicol, Tetracycline, and Ciprofloxacin. The remaining antibiotics were sensitive. For *Enterococcus*, the strains were resistant to Ampicillin, Penicillin, Clindamycin, Vancomycin, Tetracycline, Ciprofloxacin, and Norfloxacin, with one of them also resistant to Chloramphenicol. The *Pseudomonas* isolates analyzed showed resistance to Nalidixic Acid, Chloramphenicol, and Amoxicillin. In the metagenomic data analysis, resistance genes (ARGs) to 23 classes of antimicrobials were identified. ARGs stand out for macrolide-lincosamide-streptogramin (MLS), tetracyclines, beta-lactams, polymyxins, florfenicol, bacitracin, and multidrug resistance. Analysys by ARGS_oap showed that the abundance of ARGs in the sample, normalized by the number of 16S rRNA genes, revealed that the most prevalent antimicrobial classes in the effluent were MLS (0.751), followed by tetracyclines (0.476) and beta-lactams (0.354). Additionally, significant presences of Multidrug resistance (0.227), Polymyxins (0.204) and

Bacitracin (0,200) were also identified. The results from the antimicrobial susceptibility testing and metagenomic data present an analytical challenge due to the distinct methodologies employed. Traditional antimicrobial susceptibility testing directly measures bacterial resistance to specific drugs, while metagenomics detects potential pathogens and analyzes host-microbiome interactions. Combining these methods provides a comprehensive view of the resistome.

Agradecimentos: