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Occurrence and diversity of gram-negative bacteria isolated in poultry meat marketed in Ungheni, Republic of Moldova

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Abstract: The contamination of poultry meat with Gram-negative bacteria constitutes a significant food safety concern due to the potential involvement of these microorganisms in foodborne diseases and the dissemination of antimicrobial resistance determinants. The present study aimed to evaluate the occurrence and diversity of Gram-negative bacteria isolated from poultry meat products marketed in Ungheni, Republic of Moldova, and to determine their potential public health significance. A total of 17 poultry-derived samples, including minced meat, neck skin, and meat cuts, were subjected to bacteriological analysis. Isolation of bacterial strains was performed using conventional microbiological techniques, while species-level identification was achieved by MALDI-TOF (Bruker). A wide diversity of Gram-negative bacteria was identified among the analyzed samples. *Escherichia coli* was the most frequently isolated species (88.2% of samples), followed by *Proteus mirabilis*, *Hafnia alvei*, *Enterobacter hormaechei*, *Citrobacter freundii*, *Aeromonas veronii*, and *Morganella morganii*. Other opportunistic and potentially pathogenic bacteria identified included *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Raoultella ornithinolytica*, *Aeromonas hydrophila*, and *Providencia rustigianii*. Minced meat samples exhibited the greatest diversity of Gram-negative bacterial species, whereas neck skin samples were characterized by a high prevalence of *Escherichia coli* and *Proteus* sp. The predominance and heterogeneity of bacterial isolates observed in minced meat may be associated with increased surface exposure and extensive handling during processing, favoring microbial contamination and proliferation. These findings underscore the necessity for continuous microbiological monitoring, the implementation of stringent sanitary and hygienic control measures, and the strengthening of food safety management systems throughout poultry production and commercialization in order to minimize public health risks associated with contaminated poultry meat in the Republic of Moldova.

Keywords: poultry meat; Gram-negative bacteria; food safety; Republic of Moldova

1. Introduction

Global antimicrobial resistance (AMR) has emerged as a major threat to public health and the effective control of bacterial infections worldwide. Recognized among the top global health risks, AMR was associated with approximately 1.27 million deaths in 2019, with projections suggesting that AMR-related mortality could exceed 10 million deaths annually by 2050 if effective interventions are not implemented [Krishnaprasad et al., 2025]. Resistant microorganisms can enter the food chain through contaminated food products, increasing the risk of difficult-to-treat foodborne infections and facilitating the dissemination of antimicrobial resistance genes across human, animal, and environmental interfaces [Elbehiry et al., 2025].

Poultry-associated microbial communities are of particular concern due to their significant contribution to foodborne diseases globally. Environmental contamination during poultry production and processing represents a critical source of microbial transmission and provides important opportunities for surveillance and quality control. The growing global population, increased international travel, and expanding trade of imported food products further intensify the challenges associated with controlling foodborne pathogens and antimicrobial-resistant bacteria [Rouger et al., 2017]. Vulnerable populations, particularly immunocompromised individuals, are at greater risk of severe foodborne infections. Consequently, the development of comprehensive surveillance systems and improved monitoring protocols throughout food production

and distribution processes is essential for minimizing contamination risks and mitigating the spread of antimicrobial resistance within the food chain.

The microbial quality and safety of meat and meat products are influenced by multiple factors, including animal management before slaughter, slaughterhouse hygiene, temperature control, packaging systems, preservation methods, transportation, and consumer handling practices. Inadequate sanitation during slaughtering and processing can lead to microbial contamination that spreads throughout the food chain, affecting both formal and informal markets [Hossain et al., 2025, Popa et al., 2025]. These contamination pathways have become increasingly concerning in the context of global antimicrobial resistance (AMR), which requires a comprehensive One Health approach integrating surveillance, research, and public health interventions across human, animal, and environmental sectors.

Food-borne diseases are commonly associated with pathogenic microorganisms such as *Escherichia coli*, *Salmonella spp.*, *Campylobacter spp.*, and toxin-producing species [Crespi et al., 2025]. These pathogens are responsible for a wide range of illnesses in humans, including gastroenteritis, septicemia, meningitis, urinary tract infections, toxic shock syndrome, food poisoning, and listeriosis. Poultry meat, one of the most widely consumed and economically accessible protein sources worldwide, is particularly vulnerable to microbial contamination during production and processing [Abebe et al., 2020, Roth et al., 2019]. Monitoring microbial populations and their shifts throughout poultry production is therefore essential for evaluating sanitation efficiency, identifying contamination sources, and controlling foodborne pathogens.

The extensive use of antibiotics in poultry production since the 1950s has significantly contributed to the emergence and dissemination of antimicrobial-resistant bacteria. Antibiotics have been widely used not only for therapeutic purposes but also as growth promoters and prophylactic agents to enhance animal productivity by modulating gut microbiota and immune responses. However, this practice has accelerated the development of multidrug-resistant (MDR) microorganisms in food-producing animals [Elbehiry et al., 2025]. Studies have reported resistance among meat-associated pathogens to multiple antibiotic classes, including resistance in *E. coli*, *Salmonella sp.*, and *Campylobacter sp.*, and methicillin-resistant *Staphylococcus aureus* (MRSA). The presence of mobile resistance genes shared among animal-associated bacteria further facilitates horizontal and vertical gene transfer, increasing the risk of resistant pathogens entering the human food chain through contaminated meat products [Alem et al., 2025].

Poultry production environments, particularly concentrated animal feeding operations (CAFOs), strongly influence the composition of the gastrointestinal microbiota of chickens. Factors such as feed composition, housing conditions, and environmental exposure shape intestinal microbial communities, gene expression, and host immunity. The repeated inclusion of antibiotics in feed has altered commensal microbiota and contributed to the persistence of resistant bacterial populations. Recognizing the growing threat posed by AMR, the World Health Organization identified antimicrobial resistance as a major global health concern and recommended reducing antibiotic use in animal production systems in favor of alternative strategies.

Despite ongoing efforts by Republic of Moldova to harmonize its food safety framework with European Union requirements, several structural and operational challenges continue to hinder the effective implementation of modern sanitary standards. Key concerns include the inconsistent application of hygiene measures throughout the food production and distribution chain, insufficient monitoring and surveillance systems, and limited laboratory infrastructure for routine microbiological analysis and rapid pathogen detection. According to the European Commission, these limitations remain significant obstacles to achieving a fully integrated and reliable food safety system (European Commission, 2025). In particular, informal marketplaces and small-scale distribution networks represent critical points of vulnerability, where traceability mechanisms and sanitary oversight are often fragmented, inadequately regulated, or difficult to enforce.

The present study aimed to evaluate the occurrence and diversity of Gram-negative bacteria in poultry meat samples collected from Ungheni. By identifying the predominant bacterial groups and their distribution among sample types, the research provides insight into possible contamination sources and related public health risks, while supporting improved microbiological surveillance and safer food handling practices.

2. Materials and Methods

During September 2025 - October 2025, a microbiological screening focused on Gram-negative bacterial microbiota was conducted on a total of 17 poultry meat samples. The samples were collected from commercial retail units located in Ungheni, Republic of Moldova, and transported to the ROVETEMERG laboratory (IULS) under refrigerated conditions in order

to preserve sample integrity and minimize alterations in the microbial population prior to analysis. Although the study was based primarily on qualitative microbiological screening, a standardized sample preparation protocol was applied to ensure objective and reproducible analytical conditions. For this purpose, 10 g of each meat sample were aseptically homogenized in 90 mL of sterile liquid culture medium.

The isolation of *Enterobacteriaceae* was initiated through a pre-enrichment stage using peptone water, followed by incubation at 37°C under aerobic conditions for 24 hours. This step was intended to promote the recovery and growth of stressed or low-abundance bacterial cells potentially present in the samples. Following pre-enrichment, aliquots were inoculated onto selective and differential culture media, including MacConkey agar (Oxoid), Eosin Methylene Blue agar (EMB, Levine formulation, Oxoid, UK), and Xylose Lysine Deoxycholate agar (XLD, Oxoid, UK). These media were selected to facilitate the isolation and preliminary differentiation of Gram-negative enteric bacteria based on their biochemical and metabolic characteristics.

For the detection and isolation of *Campylobacter* species, samples were sub-cultured on *Campylobacter*-Karmali Agar (Oxoid, UK) and Butzler Agar (Oxoid, UK), selective medium specifically designed for the recovery of thermotolerant *Campylobacter jejuni* and *Campylobacter coli* strains. The inoculated plates were incubated under microaerophilic conditions at 42°C, which favor the growth of *Campylobacter* spp. while inhibiting competing microorganisms. Distinct colony morphotypes obtained from all selective media were subsequently subcultured on Columbia Agar (Oxoid, UK) to obtain pure bacterial cultures suitable for further identification.

Identification of the isolated microorganisms was performed using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) with the Bruker Daltonics system (Bremen, Germany). Sample preparation and spectral acquisition were carried out according to the manufacturer's recommended protocols. The generated protein spectra were analyzed using MALDI BioTyper 1.1 software and compared against the reference spectra contained in the MALDI Biotyper database. Only isolates that achieved identification scores of ≥ 2.0 were considered reliably identified at the species level and were subsequently included in the final microbial screening analysis. This approach ensured a high degree of accuracy and reproducibility in the characterization of the Gram-negative bacterial microbiota associated with the analyzed meat products.

Chromogenic selective media: Brilliance™ ESBL and Brilliance™ CRE agar (Thermo-Scientific), were used for evaluation of the antimicrobial resistance phenotypes of the gram-negative bacteria isolates. These media were specifically selected for the detection and differentiation of extended-spectrum β -lactamase (ESBL)-producing bacteria and carbapenem-resistant *Enterobacterales* (CRE), respectively.

3. Results

The microbiological screening of the 17 analyzed poultry meat samples revealed a high diversity of Gram-negative bacterial species, indicating substantial microbial contamination throughout the examined poultry and meat products. The predominance of *Enterobacteriaceae* and other opportunistic Gram-negative bacteria suggests possible deficiencies in hygiene practices during slaughtering, processing, handling, storage, or commercialization (Figure 1).

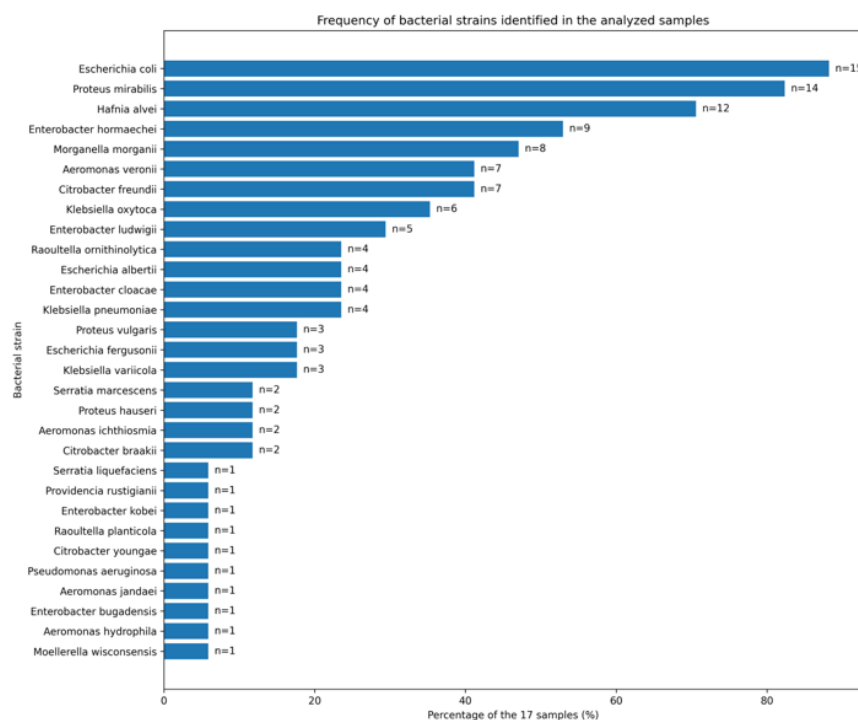


Figure 1. Bacterial strains identified in analysed poultry meat samples.

High prevalence of *E. coli* (88.2%) and *P. mirabilis* (82.4%) suggests widespread fecal contamination or process-hygiene deficiencies during slaughtering, handling, or processing. High abundance of spoilage organisms like *Hafnia alvei* (70.6%) and *Morganella morganii* (47.1%) are known psychrotrophic bacteria responsible for meat spoilage and biogenic amine production (such as histamine), posing potential chemical food safety risks (Table 1).

Table 1. Overall prevalence of the most frequently isolated bacteria.

Bacterial species	Total isolates	Prevalence (%)	95% Confidence Interval (CI)
<i>Escherichia coli</i>	15	88.20%	[72.9% – 99.9%]
<i>Proteus mirabilis</i>	14	82.40%	[64.3% – 99.8%]
<i>Hafnia alvei</i>	12	70.60%	[48.9% – 92.3%]
<i>Enterobacter hormaechei</i>	9	52.90%	[29.2% – 76.6%]
<i>Morganella morganii</i>	8	47.10%	[23.4% – 70.8%]
<i>Citrobacter freundii</i>	7	41.20%	[17.8% – 64.6%]
<i>Aeromonas veronii</i>	7	41.20%	[17.8% – 64.6%]
<i>Klebsiella oxytoca</i>	6	35.30%	[12.6% – 58.0%]
<i>Enterobacter ludwigii</i>	5	29.40%	[7.8% – 51.0%]
<i>Klebsiella pneumoniae</i>	4	23.50%	[3.4% – 43.6%]
<i>Raoultella ornithinolytica</i>	4	23.50%	[3.4% – 43.6%]
<i>Enterobacter cloacae</i>	4	23.50%	[3.4% – 43.6%]
<i>Escherichia alberti</i>	4	23.50%	[3.4% – 43.6%]

The phenotypic screening performed on Brilliance™ CRE and Brilliance™ ESBL chromogenic media showed a high prevalence of antimicrobial resistance-associated phenotypes among the analyzed Gram-negative bacterial isolates. The results indicated that a substantial proportion of isolates demonstrated growth on selective screening media designed for the detection of carbapenem-resistant *Enterobacterales* (CRE) and extended-spectrum β -lactamase (ESBL)-producing

microorganisms, highlighting the potential dissemination of clinically significant resistance determinants within meat-associated bacterial populations (Figure 2.).

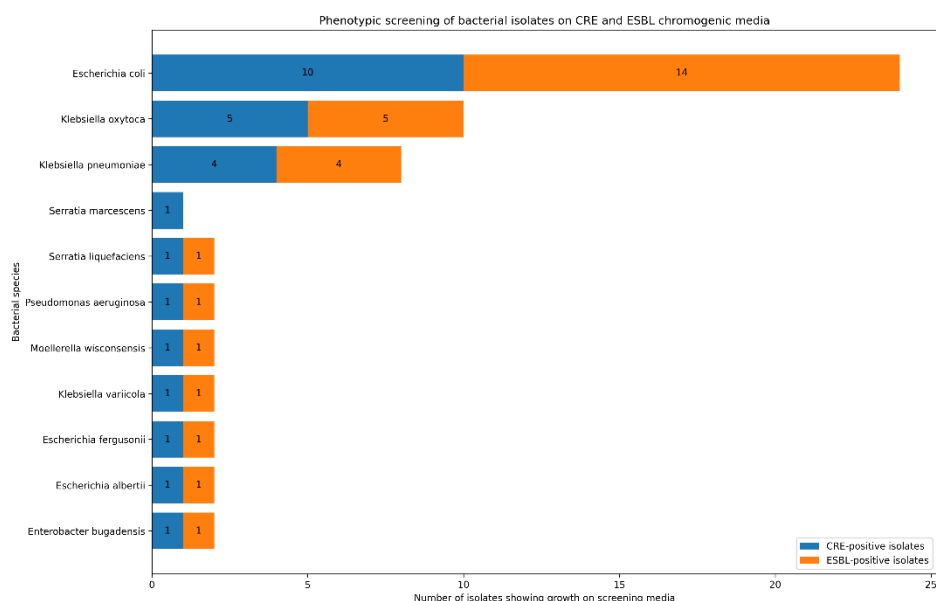


Figure 2. Bacterial isolates demonstrating growth on selective media for carbapenem-resistant *Enterobacterales* (CRE) and extended-spectrum β -lactamase (ESBL)-producing bacteria.

Among all tested isolates, *Escherichia coli* was the predominant species exhibiting positive growth on both selective media. Of the 15 isolates identified, 10 (66.7%) showed growth on Brilliance™ CRE medium, whereas 14 (93.3%) exhibited an ESBL-associated phenotype on Brilliance™ ESBL medium (Figure 3).

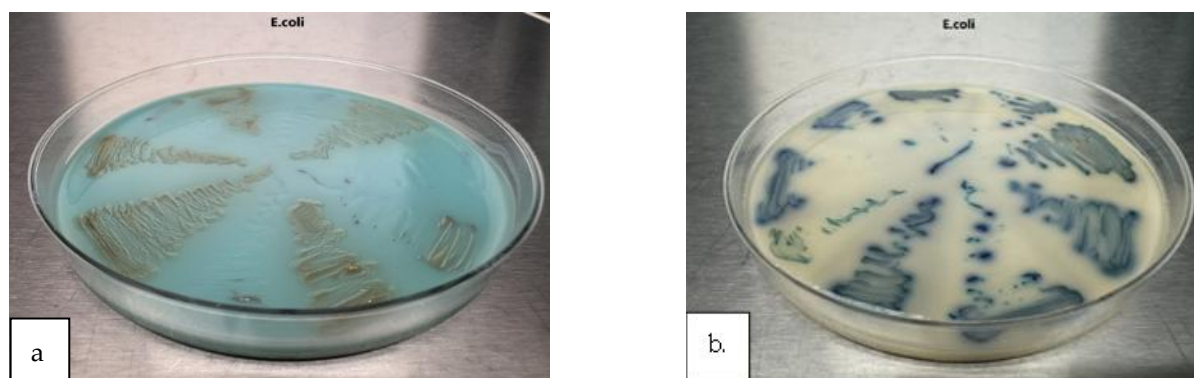


Figure 3. Phenotypic screening of *Escherichia coli* isolates on Brilliance™ CRE (a) and Brilliance™ ESBL (b) chromogenic media.

4. Discussion

The increasing prevalence of antimicrobial-resistant foodborne pathogens represents a critical public health and socioeconomic challenge. Resistant bacteria and resistance genes originating from meat production systems can spread throughout the food supply chain, contributing to hospital-acquired, community-associated, and zoonotic infections. Despite growing awareness of AMR, substantial gaps remain in understanding the prevalence, diversity, and transmission dynamics of resistant pathogens and resistance genes in meat and meat products, particularly across large geographic regions. Comprehensive surveillance and advanced molecular approaches are therefore essential for improving food safety, monitoring resistance dissemination, and supporting evidence-based interventions aimed at reducing AMR risks associated with meat production and consumption.

Among the identified Gram-negative microorganisms, *Escherichia coli* was the most frequently detected species, being present in 88.2% of the samples. Its widespread occurrence is considered an important indicator of fecal contamination and inadequate sanitary conditions during meat processing. The second most prevalent species was *Proteus mirabilis* (82.4%), followed by *Hafnia alvei* (70.6%), demonstrating that environmental and intestinal-associated bacteria are commonly distributed across the analyzed products.

A relatively high prevalence of *Enterobacter hormaechei* (52.9%), *Morganella morganii* (47.1%), *Aeromonas veronii* (41.2%), and *Citrobacter freundii* (41.2%) was also observed. These bacterial species are recognized as opportunistic pathogens and may pose public health concerns, particularly in immunocompromised individuals [Forsythe et al., 2015, Osei et al., 2021]. Their presence may additionally reflect insufficient temperature control or cross-contamination during processing and retail distribution.

Species belonging to the genera *Klebsiella*, *Enterobacter*, *Raoultella*, and *Aeromonas* were detected in multiple samples, indicating a complex bacterial microbiota associated with poultry meat products [Thorns et al., 2000]. The detection of *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Pseudomonas aeruginosa* is especially important due to their known association with antimicrobial resistance and hospital-acquired infections [Ho et al., 2025]. Several less frequently isolated bacteria, including *Providencia rustigianii*, *Moellerella wisconsensis*, *Aeromonas hydrophila*, and *Enterobacter bugadensis*, were identified only sporadically; however, their occurrence highlights the broad microbial diversity present in the analyzed samples.

These findings are of particular concern, as *E. coli* is widely recognized as both an indicator of fecal contamination and an important reservoir of antimicrobial resistance genes [Khanom et al., 2025, Much et al., 2019]. The elevated prevalence of ESBL-positive *E. coli* suggests the possible circulation of β -lactam resistance mechanisms within the food chain, potentially facilitating the transfer of resistance determinants to other bacterial species [Montoro-Dasi et al., 2021].

Species belonging to the genus *Klebsiella* also demonstrated significant resistance-associated growth patterns. *Klebsiella oxytoca* showed positive growth in five isolates on both CRE and ESBL media, while *Klebsiella pneumoniae* exhibited four positive isolates for each phenotype. The detection of resistant *Klebsiella* species is particularly important due to their well-documented involvement in nosocomial infections and multidrug resistance dissemination. Their presence in meat samples may indicate contamination originating from animal production systems, environmental sources, or inadequate sanitary practices during processing and commercialization.

Additional bacterial species, including *Escherichia albertii*, *Escherichia fergusonii*, *Enterobacter bugadensis*, *Moellerella wisconsensis*, *Pseudomonas aeruginosa*, and *Serratia liquefaciens*, also demonstrated the ability to grow on both selective media, although at lower frequencies. These findings emphasize the broad diversity of resistant Gram-negative microorganisms associated with the analyzed poultry meat products. Notably, *Pseudomonas aeruginosa* is recognized for its intrinsic resistance to multiple antimicrobial classes and its capacity to survive under diverse environmental conditions, further increasing its public health relevance [La Rosa et al., 2025, Botelho et al., 2019].

Serratia marcescens also demonstrated growth on CRE medium but not on ESBL-selective medium, suggesting the possible presence of alternative carbapenem resistance mechanisms independent of ESBL production. This observation highlights the complexity of antimicrobial resistance phenotypes among environmental and food-associated bacteria and underlines the importance of complementary molecular investigations for accurate characterization of resistance determinants.

While these findings provide valuable baseline data, the relatively small sample size constitutes a key limitation of the present study. Because the sampling was restricted in scope, it may not fully capture the broader microbiological profile of poultry meat distributed across diverse retail channels, geographic regions, or production systems throughout the Republic of Moldova. Future longitudinal studies incorporating a larger, stratified sampling framework are warranted to account for seasonal variations and regional supply chain differences.

Nevertheless, the current data clearly demonstrate that the analyzed meat products harbor a heterogeneous and potentially pathogenic Gram-negative microbiota. These findings emphasize the need to strengthen national microbiological monitoring systems and elevate hygiene standards throughout the poultry supply chain. Implementing strict cold-chain management and rigorous sanitary controls will be paramount to mitigating contamination risks and safeguarding public health and food safety within the Republic of Moldova.

5. Conclusions

The present study revealed a high prevalence and diversity of Gram-negative bacteria in the analyzed poultry meat samples, with *Escherichia coli*, *Proteus mirabilis*, and *Hafnia alvei* being the predominant species. The frequent detection of Enterobacteriaceae and other opportunistic pathogens indicates possible fecal contamination and inadequate hygienic conditions during meat processing and commercialization.

Phenotypic screening on Brilliance™ CRE and Brilliance™ ESBL media demonstrated the widespread presence of antimicrobial resistance-associated phenotypes, particularly among *Escherichia coli*, *Klebsiella oxytoca*, and *Klebsiella pneumoniae* isolates. The high occurrence of ESBL- and CRE-positive strains suggests that meat products may act as reservoirs and transmission pathways for antimicrobial-resistant bacteria. Overall, these findings highlight the importance of continuous microbiological surveillance, improved sanitary control, and stricter food safety measures to reduce contamination risks and limit the dissemination of antimicrobial resistance within the meat chain in the Republic of Moldova.

6. Patents

Author Contributions: Conceptualization, A.A.E. and S.N.; methodology, R.C.M.; software, A.D.C.; validation, A.D.C., R.C.M.; formal analysis, C.V., B.M.; investigation, C.V., A.D.C.; resources, R.C.M.; data curation, A.D.C.; writing—original draft preparation, C.V., S.N.; writing—review and editing, A.A.E.; visualization, C.R.M.; supervision, A.D.C.; project administration, A.A.E.; funding acquisition, A.A.E. All authors have read and agreed to the published version of the manuscript.” Please turn to the [CRediT taxonomy](#) for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

Funding: This research was funded by Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI), grant number 26ROMD/20.05.2024, code PN-IV-P8-8.3-ROMD2023-0053.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

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