PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE IN GRAM-NEGATIVE BACTERIA ACROSS HOSPITAL AND COMMUNITY ENVIRONMENTS

Gram-negative bacterial infections represent a major global medical challenge. Due to a wide array of virulence factors, these bacteria can cause infections across all age groups. Individuals with impaired nonspecific immune defense are most commonly affected. In addition, Gram-negative organisms can exhibit both intrinsic and acquired resistance to various classes of antibiotics. The spread of antimicrobial resistance is driven by the selective pressure resulting from the extensive use of antibiotics in both human medicine and agriculture. Colonization of the human host with multidrug-resistant strains constitutes a risk factor for the subsequent development of hard-to-treat infections, both in the community and in healthcare settings. Antimicrobial resistance is directly responsible for over 1.2 million deaths annually and is associated with the mortality of more than 3.6 million patients worldwide each year.

This doctoral thesis investigates the involvement of Gram-negative pathogens in the development of infections across three typical clinical-epidemiological scenarios, ranging from community-acquired infections, where antimicrobial resistance is relatively low, to colonization with resistant organisms in hospitalized patients, and the prevalence of secondary infections in critically ill COVID-19 patients admitted to intensive care units.

The first study describes the serologic and genetic diversity of diarrheagenic *Escherichia coli* strains isolated from children under the age of two. This retrospective study included diarrheagenic *E. coli* strains isolated between May 2016 and July 2019 from stool samples of children aged 0–2 years, hospitalized with acute diarrheal syndrome at the Mureș County Clinical Hospital. The isolated strains were tested using anti-O sera to identify frequently encountered *E. coli* pathotypes, namely Shiga toxin-producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC), and to determine the circulating serogroups. The presence of the *hlyA* gene, characteristic of enterohemorrhagic strains, was assessed by PCR in *E. coli* strains belonging to serogroup O157. Genetic similarity of the isolates was evaluated using ERIC-PCR. Of the 130 strains included in the study, more than half (50.8%) were classified as STEC. The dominant serogroup was O157, with only one strain identified as enterohemorrhagic. The strains exhibited marked genetic heterogeneity, suggesting the absence of epidemiological clusters or outbreaks.

The second study assessed colonization with multidrug-resistant organisms in patients admitted to hospital wards with high-risk for healthcare-associated infections. Colonization screening was conducted at Mureș County Clinical Hospital between January 3 and June 21, 2017, using chromogenic media to identify multidrug resistant bacteria. For Gram-negative bacteria, carbapenem resistance and extended-spectrum beta-lactamase (ESBL) production were specifically monitored. A total of 440 multidrug-resistant Gram-negative isolates were identified. The majority of ESBL-producing strains were *E. coli* and *Klebsiella pneumoniae*, while carbapenem-resistant strains were predominantly *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *K. pneumoniae*. Among carbapenem-resistant isolates, 51% produced metallo-beta-lactamases, and 39% produced oxacillinases, with OXA-48 accounting for approximately 30% of all oxacillinases. No KPC-producing bacteria were detected during the screening period. ESBL phenotypes were less common in isolates from ICU

patients compared to surgical ward patients. Conversely, the proportion of carbapenem-resistant *Enterobacterales* was substantially higher in ICU settings. Persistant colonization was observed in some cases, with multidrug-resistant isolates detected in serial screening samples collected more than 50 days apart.

The third study examined the occurrence of secondary bacterial infections in patients with severe COVID-19 admitted to a tertiary intensive care unit. This clinical-epidemiological scenario presents numerous risk factors for infections with multidrug-resistant organisms, including the ICU environment, frequent use of invasive procedures, the patients' poor clinical condition, and immunosuppressive therapy. Between 01.08.2020 and 31.012021, a total of 243 patients were admitted to the COVID Support Unit in Târgu Mureș. Of these, 59 patients (24.3%) developed secondary bacterial infections. The most frequently isolated pathogens were *A. baumannii* and *K. pneumoniae* (31.1% and 18.9%, respectively), with most strains being multidrug-resistant. The prevalence of chronic obstructive pulmonary disease (COPD) was higher among patients with bacterial co-infections. Secondary bacterial infections were associated with prolonged ICU stay and increased mortality. The overall mortality rate at the COVID Support Unit during the study period was 84.4%. The need for mechanical ventilation and the occurrence of secondary infections, along with demographic and laboratory parameters (age, sex, C-reactive protein, lactate dehydrogenase, procalcitonin, and ferritin), were significantly associated with mortality. A logistic regression model based on these variables showed good predictive accuracy for mortality, with a sensitivity of 79%, specificity of 87%, and an area under the receiver operating characteristic curve (AUROC) of 0.896 (95% CI: 0.889–0.902).

This dissertation provides a comprehensive overview of the role of Gram-negative bacteria as infectious agents and colonizers across various clinical-epidemiological contexts—from community-acquired bacterial enterocolitis to multidrug-resistant colonization in hospitalized patients and secondary bacterial infections in critically ill ICU patients.

The findings underscore the critical importance of screening for multidrug-resistant organisms and implementing robust infection prevention and control practices, both in community settings and within healthcare facilities.