PhD thesis abstract

Upper respiratory tract infections, a clinical and laboratory challenge

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The thesis analyzes in detail upper respiratory tract infections, a major clinical and laboratory problem with significant impact on public health. Respiratory infections are extremely common in all age groups, and their etiology is varied, including viral, bacterial, fungal or parasitic agents. The SARS - CoV - 2 pandemic has drastically changed the diagnostic approach , by the widespread introduction of rapid , sensitive and specific PCR tests , which have become the standard for the identification of respiratory viral agents.

The respiratory microbiome is a central element in maintaining airway health. The respiratory flora is formed from birth and is influenced by the maternal microbiome, type of delivery, diet, exposure to communities and environmental factors. The upper respiratory tract hosts a diverse microbiota, while the lower tract has a low microbial density, but is not sterile. There are methods of defense of the body, all with well-defined roles. Respiratory mucus plays an essential role in the capture and elimination of inhaled particles, and its changes, such as hypersecretion or increased viscosity, affect mucociliary clearance and favor the colonization of pathogens.

Bacterial competition is a fundamental mechanism by which the respiratory microbiota limits pathogen colonization. Commensal bacteria compete for adhesion sites and nutrients, produce antimicrobial substances and influence biofilm formation. Bacteriocins have great potential as promising alternatives to antibiotics, having activity against multidrug-resistant pathogens such as MRSA, VRE or *C. difficile* . Some bacteriocins can modify the phenotype of target bacteria, influencing motility, exopolysaccharide production and biofilm structure. Bacterial secretion systems, in particular T3SS, T4SS and T6SS, are involved in both pathogenicity and intermicrobial competition, facilitating the

transfer of toxins and resistance genes. A relevant example is Stenotrophomonas maltophilia, which uses T4SS to destroy bacteria such as *E. coli* , *K. pneumoniae* or *P. aeruginosa* .

Respiratory dysbiosis occurs as a result of antibiotic treatment, chronic inflammation or respiratory diseases such as COPD, asthma, cystic fibrosis or primary ciliary dyskinesia. This leads to a decrease in microbial diversity, an increase in bacterial load and colonization with opportunistic pathogens, favoring severe infections. A central element of the thesis is the formation of biofilm, an essential process in the pathogenicity of respiratory bacteria, especially in mechanically ventilated patients. Biofilm confers increased resistance to antibiotics and protection against the immune system, being a major factor in persistent infections. The thesis analyzes the biofilms produced by *K. pneumoniae* , *A. baumannii* , *P. aeruginosa* and *S. maltophilia* , highlighting the differences between clinical isolates and isolates from screening samples.

Diagnosis of respiratory infections includes direct examination, bacterial culture, correlation with biomarkers such as procalcitonin and modern methods represented in particular by molecular tests. Healthcare-associated infections, especially respiratory infections, are common in intensive care units, where patients are exposed to multidrug-resistant bacteria. Most pathogens have multiple resistance mechanisms, which are difficult to treat and limited.

The thesis experimentally presents biofilm formation and resistance to carbapenemase -producing Gram - negative bacilli. Comparative analysis between screening and pathological isolates revealed a greater capacity for biofilm formation and a more complex resistance profile in clinical isolates.

The thesis also includes the development of a Triplex PCR endpoint test for the detection of SARS - CoV - 2, designed to be rapid, accurate and cost-effective, adapted to laboratories with limited resources. The process includes sample selection , primer design, optimization of simplex and triplex reactions and statistical validation . The test has proven effective in the simultaneous detection of multiple viral genes.

The last part of the thesis includes an epidemiological analysis of carbapenem-resistant infections in intensive care units, evaluating the prevalence, genetic relatedness of strains and clinical correlates, such as comorbidities, treatments and mortality. The results show the circulation of multiresistant clones and their major impact on the prognosis of patients.