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DEVELOPMENT OF METHODOLOGY FOR THE MOLECULAR DETECTION AND IDENTIFICATION OF BACTERIA AND THE APPLICATION ON PATIENTS' CLINICAL SAMPLES

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ABSTRACT

The present thesis describes the development of three multiplex Polymerase Chain Reaction (PCR) assays and evaluates their accuracy as molecular techniques in comparison to classical techniques, as well as, their application on clinical samples.

The first part presents the development of a multiplex PCR assay for the simultaneous detection and identification of *H.influenzae, P.aeruginosa, S.aureus, Streptococcus* spp. The developed multiplex PCR assay is of high specificity and sensitivity and can be applied directly on patients' clinical samples, such as cerebrospinal fluid, whole blood and pleural fluid. In addition, it proved to be a useful diagnostic tool for the early confirmation of severe infectious diseases such as meningitis, septicaemia and pneumonia. In contrast, it was found that for the evaluation of respiratory tract infections, the clinical samples obtained (bronchoalveolar lavage, sputa, ear fluid) need thorough sampling by the clinician because the presence of the microorganisms from the normal flora will confuse the diagnosis by the molecular techniques.

The second part presents the development of a multiplex PCR for the simultaneous identification of *S.pyogenes* and *S.agalactiae.* This PCR was applied to all clinical samples positive for *Streptococcus* spp. The assay showed 100% sensitivity and specificity in comparison to culture methods and revealed *S. pyogenes* was the second microorganism responsible for causing respiratory tract infections, following *S.pneumoniae*.

Respiratory tract infections are usually treated, apart from penicillin, with macrolides-lincosamides-streptogramines B, known as MLS_B antibiotics. The third multiplex PCR assay which is presented was developed for the detection of genes responsible for MLS_B antibiotic resistance. This assay was combined with the multiplex PCR for the identification of *S. pyogenes* and *S. agalactiae* in one multiplex PCR assay. The new multiplex PCR assay proved to be highly sensitive and specific for the identification of *S. pyogenes* and *S. agalactiae*, as well as, for their resistance in MLS_B antibiotics.

Overall, the PCR assays described are simple and easily implemented methods that can be applied on a wide range of clinical samples. Their high specificity and sensitivity proved them as useful diagnostic tools, especially wherever emergent identification is needed for treatment.