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Utilization of a new multiplex-PCR for rapid determination of *Staphylococcus aureus* virulence and resistance to methicillin

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Methicillin resistant *Staphylococcus aureus* (MRSA) has been historically associated with hospital infections and it is among the most frequent infections caused by β-lactam resistant Gram-positive microorganisms. However, it has been found community acquired MRSA infections as a recently emerging disease. The ideal system for the identification of MRSA outbreaks in Clinical Pathology Laboratories (CPL) should be standardized, reproducible, low cost, sensitive and rapidly available. It has also been known the importance of the *mec*A gene in the determination of the methicillin resistance. The main objective of the present work is to develop an alternative method for antimicrobial susceptibility determination. A new multiplex-PCR (mPCR) method was used with specific primers for *S. aureus* 16 rRNA gene and for the gene *mec*A. It was included in the mPCR a set of primers for the virulence Panton-Valentine Leukocidin (PVL) genes, *lukS/F-PV*, since they have been assuming an important role in the virulence of community acquired infections caused by staphylococci. The method proposed in the present work may be used routinely in the CPL due to its simplicity, information, reproducibility and fastness.

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Expression of frutalin in *Pichia pastoris* and evaluation of its potential as a cancer marker

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One of the most interesting biological properties of frutalin (α-D-galactose-binding jacalin-related lectin from *Artocarpus incisa* seeds) is its potential use in cancer diagnostic due to its ability to interact with galactose complexes of cancer cells surfaces. Hence, the availability of frutalin in large scale will be necessary to facilitate its further application. However, its isolation and purification from plants is time-consuming and results in low yields, as well as in a heterogeneous mixture of different lectin isoforms. To overcome this limitation, frutalin optimized synthetic gene was cloned and expressed in the methylotrofic yeast *Pichia pastoris*. Frutalin was expressed in *P. pastoris* as a single chain protein since the 4-amino-acid linker peptide, that connects α and β chains, was not cleaved. Moreover, the signal sequence used, the *Saccharomyces* α-factor preprosequence, was not completely removed and part of recombinant lectin was highly N-glycosylated. Nevertheless, recombinant lectin ability to bind galactose was maintained. Preliminary immunohistochemical studies for evaluate its potential as a cancer marker revealed that recombinant frutalin is able to recognize prostate cancer cells in the same conditions as native frutalin. It preferentially binds prostate neoplasic cells, rather than hyperplasic and normal cells, showing its potential as tumour marker, underlying its putative role in diagnosis of prostate cancer.