Bioluminescense method for high-throughput screening of compounds against *Mycobacterium tuberculosis*

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There has been an increase of *Mycobacterium tuberculosis* strains that are resistant to the current anti-TB agents, mainly through acquired resistance by therapeutic failure, This fact has underscored the need of a quick development of antimycobacterial drugs that are more effective than those currently in use. Moreover, new methodologies to determine the bactericidal activity of these compounds have been proposed. This study describes the use of bioluminescent strains of *Mycobacterium tuberculosis* H37Rv – ATCC 27974 and *Mycobacterium tuberculosis* Erdman ATCC 35801 both containing the plasmid pSMT1 constructed with *luxA* and *luxB* genes from *Vibrio harveyi* in a screening to evaluate the antimycobacterial activities of anti-TB agents. The standardization of the technique was performed using isoniazid and rifampicin, as a drug standard and the results of Minimal Inhibitory Concentration (MIC) were 0.03 μg/mL and 0.03 μg/mL, respectively. These values were totally compatible with those obtained with Microplate Alamar Blue Assay (MABA). The standardization of the bioluminescence measurement of intracellular antimycobacterial activity was performed using the J774 murine macrophage-like cell line infected with *Mycobacterium tuberculosis* Erdman containing the plasmid pSMT1 and rifampicin as a drug standard and the result of MIC was 0.16 μg/mL similar with those obtained with the technique of colony forming unit (CFU). A total of 32 compounds were evaluated, 19 crude plants extracts and 13 synthetic compounds and the results of Minimal Inhibitory Concentration were compared with those obtained with the MABA. 02 crude plants extracts and 02 synthetic compounds were evaluated and the results of MIC and percent of inhibition were compatible with those obtained with the CFU technique. The overall agreements between the MICs obtained by MABA and the results obtained with the luciferase reporter strain of *Mycobacterium tuberculosis* and with bioluminescence measurement of intracellular antimycobacterial activity and the CFU technique encourage the use of this recombinant mycobacteria in high-throughput screening of compounds against *Mycobacterium tuberculosis*.

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Intestinal carriage of yeasts by children in hospitalar setting

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At the last decades the nosocomial infections caused by yeasts raised significantly especially by *Candida* yeasts. The infections source can be endogen or exogenous, since spores of unicellular and multicellular are kept viable for months and several yeasts species are found in skin and mucosa of healthy people. In a saprophytic state yeasts are found in the human gastrointestinal tract but the relationship between the presence of these microorganisms and their pathology is associated with several facts such as: number, variety of sites colonized, effective use of antibiotics, associated infections caused by another microorganisms and mainly disturbance due to lack of immunity and metabolic. Yeasts in the gastrointestinal tract can be transmitted fecal-oral direct or indirectly from an individual to another. The transmission of a strain in a

saprophytic state to a host can result in colony followed by infection. The infection can be serious depending on the host conditions and the etiologic agent that includes virulent factor and resistance to antifungal drugs. These attributes are important to *Candida albicans* in which enzymes with phospholipase activity are responsible for virulent factors. Resistance phenotypes, otherwise it should occur more frequently in non-*albicans* species. Concerning the possibility of an endogen disease and the spread of virulent and resistant strains, from the gastrointestinal colony, studies that contribute to determine these agents that constitute the microbiota of patients, are important to know the natural story of nosocomial infections caused by yeasts. This work aims at evaluating the intestinal tract as a source of hospital infections by yeasts describing the remaining species in the first hours and a possible change depending on the time that may happen to virulent phenotypic and resistance to ant fungi. Two hundred eighty one yeast samples from sixty-six children attended in pediatric and semi-intensive units in 2 public hospitals located in São Paulo and Guarulhos cities in Brazil were analyzed. The fecal samples were collected at the first hours after and during their arrival at the hospital. To identify the yeasts according to their gender and species traditional methods were used, analyzing morphological and physiological aspects. The ability to produce enzymes phospholipase and proteinase was verified the same way it was proposed by Price et al.1982 and Ruchel et al.1982. The sensibility to antifungals: amphotericin B (AMB), fluconazole (FZ), ketoconazole (CZ) e nistatin (NIS), was analyzed by the diffusion technical by disks (CECON São Paulo, Brazil). Resistant samples or with intermediate sensibility were confirmed by micro-dilution method according to NCCLS (1997) modified by EUCAST (2002). The isolated species were: *Candida tropicalis* (30%), *C.parapsilosis* (27%), *C.krusei* (4%), *Trichosporon cutaneum* e *T.inkin* (3%), *Rhodotorula mucilaginosa* e *R.glutinis* (2%), *C.guilliermondii* (2%), *C.glabrata* (1%) and *C.kefyr* (1%). Enzymatic activity was verified in most of the 84 *C.albicans* samples being 96% of phosfolipase and 95% of proteinase production. Among the non-*albicans* species of *Candida* it was observed 97% of phospholipase and 67% of proteinase activity. Less sensitive samples to azoic drugs including resistant or SDD sensibility, which depends on the achieved dose, were found in 4.3% of the 281 samples of yeast. The hugest percentage was observed in *C.krusei* (90%). We can conclude that different yeast species occur in stools of pediatric population hospitalized, including virulent strains and antifungal resistant phenotypes. The persistent of these phenotypes in the intestinal tract during hospitalization period may represents a risk factor contributing to endogen infection, or play a role in dissemination of potential pathogens inside a nosocomial evironment.

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Contribution to the immunodiagnosis of human leptospirosis: emphasis to monoclonal antibodies

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The best serological test for leptospirosis laboratory diagnosis remains the microscopic agglutination test (MAT). Because of the complexity of MAT, we have been developed some rapid screening tests for leptospiral antibodies detection in the acute phase of infection. In the decade of 80, a passive hemagglutination test employing polysaccharide fractions of leptospires was considered appropriate for early diagnosis, but its antigen preparation included "common antigens" recognized by antibodies from 4% of healthy individuals. A new ELISA (enzyme-linked immunosorbent assay) employing proteinase K resistant immunodominant antigens was developed and its potential diagnosis evaluated. This technique, the PK-ELISA, presented 89.9% sensitivity and 97.4% specificity, and satisfied the requeriments needed for serological screening tests of human leptospirosis. However, some of the reagents used in its antigen preparation are imported and very unstable. So, it was proposed, in a "Cooperative Research Accordance" between Instituto Adolfo Lutz and Laboratório Fleury, to try new approaches with monoclonal antibodies. Two hibridomas secreting specific