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.

*Available in the library of Conjunto das Químicas da USP
Site: www.teses.usp.br and in the library
of Instituto Adolfo Lutz
e-mail: talarico_rico@ig.com.br

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

Ribeiro, M. A. **Contribution to the immunodiagnosis of human leptospirosis: emphasis to monoclonal antibodies.** São Paulo, 2003. [Tese de Doutorado – Faculdade de Ciências Farmacêuticas da Universidade de São Paulo]. Orientadora: Profa. Dra. Hermínia Yohko Kanamura.

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

monoclonal antibodies (MAb) were selected: one, against an epitope detected in 16 of 23 members of the genus *Leptospira* (clone A12P4) and the other, specific to the icterohaemorragiae serogroup (clone H7P1). The MAb A12P4, a G2 (IgG2B) immunoglobulin, reacted with an epitope present in the 16-18 kDa components of icterohaemorragiae serogroup and with the 75-84 kDa components of serovars copenhageni and canicola, after whole-cell lysates of the leptospires were separated by sodium dodecyl sulfate- polyacrylamide gel electrophoresis. The MAb H7P1, which is an IgG, reacted with an epitope common to several fractions of molecular weight above 21 kDa of strain RGA and with the 21-22 kDa and the 75-82 kDa components of strain M-20. Both monoclonal antibodies were employed in enzyme immunoassays for detecting specific antibodies in serum samples serially colleted from 52 patients with leptospirosis, and from the control group, which consisted of sera from 57 patients with other diseases included in the differential diagnosis, and from 68 healthy individuals. These tests, however, were not satisfactory. A new ELISA was developed in the present study employing an antigen suspension "AgMc", purified by affinity chromatography with CNBr-activated Sepharose 4B coupled to the monoclonal antibodies described above. The results obtained with this test were compared to the MAT and to the classical IgM ELISA (ELISA c). The new method, "AgMc ELISA", presented serological indices, relatively to reference test MAT, of 80.70 % and 83.33 % of sensitivity and specificity, respectively; positive and negative predictive values of 69.70 % and 90.10 %, respectively, and general agreement index of 82.49 %. So, this test was not considered a promising approach to rapid diagnosis of human leptospirosis. Moreover, the proportion of patients diagnosed as having leptospirosis by the "AgMc ELISA" and the MAT differ significantly. The possible explanations for the results obtained are discussed.

*Tese disponível na Biblioteca
Digital da USP site www.teses.usp.br
e-mail: ribeiro_ma@hotmail.com

Experimentação Animal: princípios éticos e legislação

Sarmento, E. O. **Experimentação Animal: princípios éticos e legislação.** São Paulo; 2002. [Dissertação de Mestrado - Faculdade de Saúde Pública - Universidade de São Paulo]. Orientador: Prof. Dr. Paulo Antônio de Carvalho Fortes.

Em todo mundo, milhares de animais são utilizados em pesquisas, testes biomédicos e práticas de ensino, contribuindo para melhoria da qualidade de vida de seres humanos. No século XX, intensificou-se o debate em torno da moralidade do uso de animais em experimentos, o que se refletiu numa maior preocupação da sociedade com o bem-estar animal. Como resultado, ocorreram mudanças significativas nas políticas, leis e regulamentações relacionadas à proteção de animais, que passam a estabelecer recomendações básicas e critérios para utilização, com o objetivo de limitar a dor e o sofrimento impostos aos animais destinados à experimentação. Em geral, as leis e regulamentações incorporaram os princípios éticos propostos por Russell e Burch (3R's), que indicam a redução do uso (*reduction*), a adoção de métodos alternativos (*replacement*) e o refinamento das técnicas envolvidas na experimentação animal (*refinement*). À luz dos princípios dos Três Rs são analisadas leis e regulamentações de países da Europa e das Américas. O Reino Unido possui a lei pioneira e tradição histórica na proteção de seus animais, centralizando no governo as ações de controle da experimentação animal, porém ultimamente tem investido na organização de comitês de ética institucionais. Nos Estados Unidos da América o sistema de controle é realizado através de políticas públicas e do funcionamento de comitês de ética para cuidado e uso de animais. O Canadá, pioneiro na implantação voluntária de comitês de ética voltados ao bem estar animal, conta com a supervisão de um conselho nacional para elaboração de políticas e regulamentações pertinentes. No Brasil, as regulamentações vigentes não garantem eficácia à promoção do bem estar ou não tratam especificamente da pesquisa com animais. Consideramos que a efetivação de medidas legais, a conscientização dos pesquisadores (3Rs), a implementação de comitês de ética são essenciais para garantia do bem-estar e a excelência das atividades técnico-científicas desenvolvidas em animais.

Dissertação disponível na biblioteca da Faculdade de Saúde Pública -USP
e-mail: evelynoliver@bol.com.br
evelynoliver@ial.sp.gov.br