expected. Repeatedly, a small artificial protein originating from the bank construction was identified, which interacted specifically with Pmt6p. Surprisingly, the screening also revealed an interaction between Pmt2p and Alg9p, which was confirmed by co-immunoprecipitation. Alg9p is involved in N-glycosylation and its interaction with Pmt2p is a first hint for a functional linkage of protein O- and N-glycosylation.

C153
ACTIVITY OF ANIDULAFUNGIN AGAINST DIFFERENT CLINICAL ISOLATES OF CANDIDA SPP. IN SPAIN
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Introduction: Anidulafungin is an echinocandin antifungal agent with potent activity against Candida spp. by inhibition of 1,3-β-D-glucan synthase in the fungal cell wall. Like other echinocandins display a broad spectrum of action, even against isolates resistant to amphotericin B and fluconazole. Objective: To evaluate the antifungal activity of anidulafungin against Spanish clinical isolates of Candida spp. Methods: We assessed the in vitro activity of anidulafungin (Pfizer) against 1126 clinical isolates of Candida albicans (n= 661), Candida parapsilosis (n= 175), Candida glabrata (n=125), Candida krusei (n= 45) and Candida tropicalis (n= 112). Amphotericin B (Sigma), caspofungin (Merck), fluconazole (Pfizer) and voriconazole (Pfizer) were tested in parallel against all isolates. We performed antifungal susceptibility testing according to the Clinical and Laboratory Standards Institute M27-A2 method and used a 24-h (and a 48-h) prominent inhibition endpoint for determination of the MIC. MIC50 and MIC90 results were the concentrations of antifungal agent necessary to inhibit 50% and 90% of the isolates, respectively. Candida parapsilosis ATCC 22019 and Candida krusei ATCC 6258 were included as QC isolates. Results: Overall anidulafungin was very active against most species of Candida. MIC90 was 0.06 micrograms per milliliter at 24 h and 0.25 micrograms per milliliter at 48 h for Candida albicans, Candida tropicalis, Candida glabrata and Candida krusei blood isolates. Anidulafungin activity was comparable to voriconazole and caspofungin activities and was higher than fluconazole and amphotericin B ones. However, a lower activity was observed against Candida parapsilosis isolates where voriconazole activity was very good. Results by species, expressed as anidulafungin MIC50/MIC90 (micrograms per milliliter) at 48 h were as follows: Candida albicans, 0.046/0.293; Candida tropicalis, 0.023/0.125; Candida glabrata, 0.052/0.216; Candida krusei, 0.034/0.182; and Candida parapsilosis, 1.296/1.889. The general susceptibility pattern revealed that most of the isolates (87.94%) were sensible to fluconazole but this rate decreased to 68.84% when the isolate considered was not a C. albicans. Voriconazole and anidulafungin had an excellent susceptibility pattern with less than 0.5% of the isolates resistant to those drugs. Anidulafungin showed excellent activity with fluconazole-resistant isolates. Conclusion: Intrinsic activity of anidulafungin was bigger than the other echinocandins with similar in vitro antifungal susceptibility profile. Both characteristics make anidulafungin an excellent antifungal drug against most of the species tested in this study.

A154
PROTEOMIC STUDY OF THE RESPONSE OF MACROPHAGES TO CANDIDA ALBICANS. EFFECT ON TNF-A AND GALECTIN-3
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Host-pathogen studies open interesting opportunities for the search of new virulence determinants and new targets for antimicrobial therapies. In our group, C. albicans response to macrophages has already been studied using genomics and proteomics (Fernández-Arenas et al., 2007, MCP, 6: 460-78). To study the host response, we have chosen the murine macrophage cell line RAW 264.7, in the light of the importance of macrophages for optimal host protection against C. albicans systemic infections. We have performed a proteomic study about the induced expression/repression of proteins from macrophages when they are in contact with C. albicans, based on 2-DE, comparison between different gels and protein identification. RAW 264.7 cells were allowed to interact with C. albicans SC 5314 cells for 45 min, and an important differential protein expression was observed in these macrophages compared to control ones. Many processes seemed to be affected: cytoskeletal organization, oxidative responses (superoxide and nitric oxide production) and protein biosynthesis and refolding (Martínez-Solano et al., 2006, Proteomics S1, S133-144). Differences in the macrophages response to either live, heat inactivated (HI) cells or components from C. albicans and from other pathogens have been described. Therefore, the response of RAW 264.7 cells to HI C. albicans SC5314 cells during 45 min has been studied and compared with the previous results against live yeasts. Some of the proteins with differential expression were common to both stimuli, but there were differences. Many of the different proteins have been described as related to the inflammatory response, more precisely, to TNF-α secretion. The proteomic data on this subject pointed to an overall anti-inflammatory response against HI cells, while against live cells most of the differences in protein expression point to an inflammatory response. This hypothesis was corroborated by measurement of the TNF-A levels, showing an increase in the secretion of this cytokine in the presence of live cells at 45 min and 3h of interaction, while this was not observed with HI cells.

B155
IDENTIFICATION OF CANDIDA SPECIES ISOLATED FROM PATIENTS IN ‘HOSPITAL DE SÃO MARCOS’
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Candidiasis is increasing as one of the major hospital infections. Most of these infections have been attributed to Candida albicans. However,
recently non-Candida albicans Candida (NCAC) species have been increasingly identified as candidiasis agents. Thus, it is of utmost importance to identify these new species in order to control the dissemination of infection. Moreover, these NCAC species are acquiring resistance to the antifungal agents more commonly used. The main aim of this study, carried out in the Clinical Pathology Laboratory of Hospital de São Marcos (Braga, Portugal), was to evaluate the prevalence and distribution of Candida spp. among clinical specimens. Moreover, this work also aimed at the comparison of three biochemical methods (Auxacolor, ID32C and Vitek) with a molecular method (PCR), for Candida species identification. Samples were collected from different origins, namely, sputum/bronchial wash/bronchoalveolar lavage, vaginal swab, hemoculture, catheter tip and urine, among others. This clinical isolates (229) were identified by Auxacolor and ID32C manual systems and by Vitek, an automated system. The results were compared with a molecular identification performed by Polymerase Chain Reaction (PCR) and gel agarose electrophoresis. From the results obtained it was possible to observe that C. albicans and NCAC species were detected in an equivalent percentage. Among NCAC species, C. parapsilosis was found in a higher percentage (13.9%), followed by C. tropicalis (10.5%), C. glabrata (9.1%), C. krusei (9.9%), C. dubliniensis (0.4%) and other unidentified Candida species (14.8%). The biochemical methods used in the identification differed significantly from molecular identification, especially the ID32C system. This manual method was the less accurate and, therefore, should be replaced by Auxacolor, which offered better results. Moreover, the molecular method is a promising technique for the rapid and accurate identification of Candida species in clinical routine. This study highlights the importance of Candida epidemiological studies, since, maybe due to the new methodology for yeast identification or to the emergence of new species, the prevalence of Candida albicans is changing.

**C156**

**DETERMINATION OF CANDIDA MINIMAL INHIBITORY CONCENTRATION BY E-TEST**


The incidence of Candida infections has been increasing, and although C. albicans is still the most prevalent species, an epidemiological shift of these pathogens to non C. albicans Candida species has been observed. Since these Candida species present variable levels of susceptibility to antifungal agents, there is an increasing need for rapid and precise methods for susceptibility testing, such as the E-test method. To assure the correct performance of these tests, and the accuracy of the results, it is extremely important to perform quality control assays using reference strains, which have already he minimum inhibitory concentration (MIC) values established. Thus, the aim of this work, performed in the Service of Clinical Pathology of Hospital de São Marcos (Braga, Portugal), was to compare the values of MIC obtained with the E-test for reference strains with the literature ones. Since the E-test protocol does not involve a precise control of the inoculum volume, which could affect susceptibility results, it was also a goal of this study to assess the influence of the inoculum volume in the E-test readings. The susceptibility of ATCC strains C. albicans 90028, C. parapsilosis 22019 and C. krusei 6258 to the antifungal agents (Amphotericin B, Fluconasol, Caspofungin, Fluconazole, Itraconazole, Voriconazole, Ketoconazole and Posaconazole) was assessed by E-test. MIC determinations were obtained from the average of 20 assays performed in the same day. Moreover, E-test was performed with four different volumes (50, 150, 300 and 1500 μL) of inoculum on 150 mm agar plates. MICs obtained for the ATCC strains were generally lower than those established by NCCLS for all antifungal agents, except for Caspofungin, whose MICs were similar to the established. Thus, the results highlight the idea that MIC values should be revised. Considering the influence of the inoculum volume in E-test method, significant differences were mainly detected between the most divergent volumes (50 and 1500 μL).

Furthermore, E-test results using the normal procedure (swab) were more similar to the MICs obtained with the lower volumes. Therefore, this work indicates that MIC values obtained by the E-test method for the reference strains assayed is lower than the established by NCCLS and that the inoculum volume does not influence E-test MIC readings, as long as it is not too high.

**A157**

**BIOFILM FORMATION AND HYPOXIA IN CANDIDA PARAPSILOSIS**

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The virulence of the pathogenic yeast, Candida parapsilosis, is closely associated with its ability to form biofilms on indwelling medical devices. Biofilms are low oxygen environments, and we showed that the cell growth of C. parapsilosis is greatly reduced in 1% O2. We investigated the correlation between transcriptional changes occurring during growth in biofilms compared to growth in hypoxic environments. Biofilms were developed in fermentors for 24 h on Thermaxon slides in continually circulating SD media, and compared to planktonic cultures. Transcriptional profiling was carried out using partial genomic microarrays, representing approximately 3,900 open reading frames. In separate experiments, cells were grown in high glucose media in either 20% or 1% O2, and the transcriptional profile was determined using a new version of arrays representing 5,900 open reading frames (based on our recent annotation of the C. parapsilosis genome sequence). Our analysis shows that there is a significant correlation between the two data sets. Expression of the ergosterol pathway genes ERG1, ERG5 and ERG11 is increased in both biofilms and hypoxia. Changes in expression of ERG1 and ERG11 were confirmed by real-time PCR in both conditions. The expression of some cell wall genes (including RBT1 and CSA1), and genes involved in the glycolytic pathway (including PFK2 and PGK1) is also increased. We identified genes for which expression is specific to either biofilm or hypoxic conditions, some are particularly strongly regulated. Expression of cpar903, an ortholog of orf119.822, is increased >200-fold in biofilms, whereas expression of cpar2047 (a gene with no orthologs in other Candida species) is induced >100-fold in hypoxia. We have generated knockouts of RBT1 and cpar903 using a SAT1-flipper cassette, and the effect on biofilm formation is currently being studied. T. Rossignol and C. Ding contributed equally to this work.

**B158**

**INDUCTION OF RESISTANCE BY AZOLES IN CANDIDA ALBICANS AND C. PARAPSILOSIS: EXPERIMENTAL ASSAYS**

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**Introduction.** Along the last decade Candida species have emerged as major opportunistic pathogens, mainly due to the increase of immunocompromised patients. In a two year survey of fungemia conducted in Portugal, C. albicans and C. parapsilosis were respectively the first and second most frequently isolated fungal agents. Fluconazole represented a