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 S. 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-Candida 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 thave 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, Fluconosin, Caspofungin, Fluconazole, Itaconazole, 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**

**T. Rossignol**, **C. Ding**, **C. d’Enfert**, **G. Butler**; ‘Institut Pasteur, Paris, FRANCE, ‘UCD School of Biomolecular and Biomedical research, Conway Institute, Dublin, IRELAND.

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 50 h on Thermonax 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 cpd903, an ortholog of orf19.822, is increased >200-fold in biofilms, whereas expression of cpd2047 (a gene with no orthologs in other Candida species) is induced >100-fold in hypoxia. We have generated knockouts of RBT1 and cpd903 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**

**S. Costa-de-Oliveira**, **A. P. Silva**, **A. S. Dias**, **A. G. Rodrigues**, **C. Pina-Vaz**; ‘Department of Microbiology, Faculty of Medicine, University of Porto, Porto, PORTUGAL, ‘Department of Microbiology, Faculty of Medicine, University of Porto; ‘Department of Plastic and Reconstructive Surgery Hospital S. João, Porto, PORTUGAL, ‘Department of Microbiology, Faculty of Medicine, University of Porto; Department of Microbiology Hospital S. João, Porto, PORTUGAL.

**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
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 (6.9%), 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

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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 Candida 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 the 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, Fluconysin, 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 different inoculum 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

T. Rossignol1, C. Ding1, C. d’Enfert1, G. Butler2; 1Institut Pasteur, Paris, FRANCE, 2UCD School of Biomolecular and Biomedical research, Conway Institute, Dublin, IRELAND.

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 50 h on Thermoxan 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 PKF2 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 orf19.822, 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

S. Costa-de-Oliveira1, A. P. Silva1, A. S. Dias1, A. G. Rodrigues2, C. Pina-Vaz1; 1Department of Microbiology, Faculty of Medicine, University of Porto, Porto, PORTUGAL, 2Department of Microbiology, Faculty of Medicine, University of Porto; Burn Unit, Department of Plastic and Reconstructive Surgery Hospital S. João, Porto, PORTUGAL.

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