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INFECÇÃO SANGUÍNEA POR CANDIDA EM PACIENTES COM NEOPLASIA HEMATOLÓGICA: TAXONOMIA POLIFÁSICA E SUSCETIBILIDADE ANTIFÚNGICA

BLOODSTREAM INFECTION BY CANDIDA IN PATIENTS WITH HEMATOLOGIC NEOPLASIA: POLYPHASIC TAXONOMY AND ANTIFUNGAL SUSCEPTIBILITY

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RESUMO

As infecções de corrente sanguínea por espécies de Candida têm sido identificadas como importantes causas de óbitos em pacientes com neutropenia submetidos à quimioterapia para tratamento de neoplasias hematológicas. Este estudo teve como objetivo verificar a ocorrência destas infecções em pacientes internados no serviço de hematologia e oncologia de um hospital público especializado no tratamento de câncer no Nordeste do Brasil. Um total de 105 amostras clínicas de 62

pacientes com neoplasias hematológicas foram analisadas no Laboratório de Micologia Médica da Universidade Federal de Pernambuco. Apenas 7 indivíduos estavam no ambiente de UTI. O diagnóstico micológico foi realizado por meio de automação (BACTEC 9120 / PHOENIX ™), identificação proteômica (MALDI-TOF MS) e análise molecular (PCR). O teste de susceptibilidade antifúngica seguiu as recomendações do Clinical & Laboratory Standards Institute. Dentre as amostras estudadas, nove cepas (8,57%) eram do gênero Candida, sendo seis C. tropicalis e três C. albicans. Os isolados foram completamente sensíveis aos antifúngicos testados. Óbitos ocorreram em 66,6% dos casos. Pacientes com neoplasias hematológicas internados em terapia intensiva e em estado de choque séptico apresentam maior risco de ocorrência de infecções de corrente sanguínea e óbito por espécies de Candida.

Palavras-chave: Candida. Sepse. Neoplasia Hematológica. Susceptibilidade Antifúngica.

ABSTRACT

Bloodstream infection (BSI) by species of Candida has been identified as an important cause of death in patients with neutropenia who undergo chemotherapy for the treatment of hematologic malignancies This study aimed to verify the occurrence of bloodstream infections by Candida species in patients admitted to the haematology-oncology service of a public hospital specialized in the treatment of cancer in Northeast Brazil. A total of 105 clinical samples from 62 patients with haematological malignancies were analyzed at the Laboratory of Medical Mycology at the Federal University of Pernambuco. Only 7 of 105 individuals were in the ICU environment. The mycological diagnosis was performed through automation (BACTEC 9120 / PHOENIX™), proteomic identification (MALDI-TOF MS) and molecular analysis (PCR). The antifungal susceptibility test followed the bloodstream infection recommendations of Clinical & Laboratory Standards Institute. Among the samples studied, nine strains (8,57%) were of the genus Candida, being six C. tropicalis and three C. albicans. The isolates were completely susceptible to the antifungal agents tested. Deaths occurred in 66,6% of the cases. Patients with hematologic malignancies hospitalized in intensive care and the state of septic shock present a higher risk of occurrence of BSI by Candida and death by this opportunistic pathogen.

Keywords: Candida. Sepsis. Hematologic Neoplasm. Drug Resistance, Fungal.

INTRODUCTION

Bloodstream infection (BSI) by species of the yeast genus Candida has been identified as an important cause of death in patients with neutropenia who undergo chemotherapy for the treatment of hematologic malignancies (Walsh et al., 2004). The genus Candida is responsible for nearly 80% of fungal infections in critical patients and the fourth most common cause of bloodstream infections (Coombo et al., 2006; Pappas et al., 2009; Chen et al., 2012; Ruhnke et al., 2012). The incidence of invasive candidiasis is reported around 8% in hospitalized patients and 40-60% mortality on them (Colombo et al., 2003; Ahmadi et al., 2014; Barreti et al., 2013; Quindós, 2014; Barretti et al., 2014). The suppression of immunity due to hematologic malignancies, and/or due to biologic immunosuppression via the treatment is responsible for the destruction of the normal immune barriers, as a result of which Candida may enter the bloodstream. Gut translocation is another important mechanism of bloodstream invasion. The use of broad-spectrum antibiotics, central venous catheter insertion and admission in the Intensive Care Unit has been appointed as risk factors for these infections (Ahmadi et al., 2014).

C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. krusei, C. guilliermondii e C. lusitaniae have been the most studied species and also of greater clinical interest in patients under treatment of hematologic malignancies. (Colombo et al., 2013). In cases of invasive candidiasis, C. albicans remains the most frequent species (Ahmadii et al., 2014; Colombo et al., 2013; Mímica et al., 2009; Taj-Aldeen et al., 2014; Guo et al., 2020), however, Candida non-albicans species have emerged, promoting infections due to increased prophylactic use of antifungal drugs in critically ill patients (Chen et al., 2012; Barretti et al., 2013; Pfaller et al., 2002), which may be contributing to the emergence of resistant species that hinder the treatment of BSI (Alves et al., 2010).

The analysis of the geographic differences of Candida species and the in vitro susceptibility profile to antifungal agents are important to evaluate changes in the incidence of these species and the behaviour towards new drugs (Mímica et al., 2009; Yan et al., 2019). Thus, epidemiologically and therapeutically, it is essential to identify species-level yeasts to monitor rates of infection related to health care and to allow the early diagnosis of outbreaks of Candida (Quindós et al., 2014). Studies have shown the use of rapid and low-cost techniques, such as mass spectrometry (MALDI-TOF MS) (Buchan et al., 2013; Spanu et al., 2012) and precise techniques such as polymerase chain reaction (PCR) (Teixeira et al., 2014). Such procedure is fundamental to define the therapeutic regimen, to reduce hospitalization time and hospital costs (Quindós, 2014). Thus, this research aimed to diagnose BSI by Candida species by polyphase taxonomy, through automatic diagnosis methods and molecular analysis (PCR); besides, in vitro verification of the antifungal susceptibility.

METODOLOGY

Study Design

This study assessed patients diagnosed with hematologic malignancies of both genders, aged 18 to 70, admitted to the haematology-oncology service of a public health reference hospital in the treatment of cancer in Northeast Brazil.

Identification of yeasts by mass spectrometry (MALDI-TOF MS)

The identification of yeasts by MALDI-TOF MS was performed according to the protocol of the Bruker Daltonics (Bremen, Germany). The isolates were deposited in duplicate

and the matrix was crystallized by drying at room temperature for 5 minutes [30]. In the identification stage, the equipment used was the MALDI TOF Autoflex III Mass Spectrometer (Bruker Daltonics Inc., USA / Germany) equipped with one laser of Nd: YAG (Yttrium grenade and doped aluminium with neodymium; Nd: Y3Al5O12). The mass range of 2.000 a 20,000 Da was recorded using a linear model with a delay of 104 ns and an acceleration voltage of 20 kV. The resulting peak lists were exported to the MALDI Biotyper ™ 3.0 software (Bruker Daltonics, Bremen, Germany), where the final identifications were reached. For interpretation of the test, the ranges of protein values were analyzed, where values above 2.000 (> 2.0) show identification of the species level and intervals between 1.700 e 1.999, recognition at the gender level. Values below 1.700 (<1.7) are not possible to identify (Fredrickis et al., 2005; Lima-Neto et al, 2014).

Molecular Analysis by Polymerase Chain Reaction (PCR)

For DNA Extraction, the isolates were harvested on plates containing Sabouraud Dextrose agar (SDA) added with chloramphenicol and kept at 37°C for five days. Each cell mass obtained was transferred to threaded extraction tubes containing glass beads. DNA was extracted according to the methodology proposed by Fredricks et al., 2005. The extracted DNA was quantified in NanodropTM 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham-USA) and the concentration adjusted to 10ng/µL.

A couple of species-specific primers for C. albicans (sense: CAL5-TGTTGCTCTCTCGGGGGCGGCCG and anti-sense: NL4CAL-AAGATCATTATGCCAACATCCTAGGTAAA) and another for C. tropicalis (sense: CTR22-TGGGCGGTAGGAG AATTGCGTTA and anti-sense: NL4CTR1-TAAGATCATTATGCCAACATCCTAGGTATA) in the concentration of 0,5 µM each, were used. The PCR was performed in Techne™ TC-512 (Techne, United Kingdom) following the conditions of initial denaturation at 94°C for 5 min, with 30 cycles of 94°C for 30 sec, 50°C for 60 sec, 72°C for 90 sec, and a final extension at 72°C for 10 min. Amplification was performed using as controls the American Type Culture Collection (ATCC) 90028 of C. albicans and ATCC 750 of C.tropicalis. The amplification products were separated by agarose gel electrophoresis at 1% With 1X TAE buffer (Tris-Acetate-EDTA) for 40 min at 3V / cm. The gel was stained with GelRed TM and photographed under ultraviolet light.

Antifungal susceptibility

The isolates were tested by the broth microdilution method (M27-A3) recommended by the Clinical and Laboratory Standards Institute (CLSI, 2008 S4, 2012). Commercial antifungal agents used in the form of salts were Amphotericin B (Bristol-Myers Squibb, Princeton, USA), Echinocandins: Caspofungin (Cancidas Merck Sharp & Dhome Pharmaceutical LTDA.), Anidulafungin (Pfizer, New York, USA) and Micafungin (Astellas Farma Brasil Importação e Distribuição de Medicamentos LTDA.) solubilized in dimethylsulfoxide (DMSO) and Fluconazole (Pfizer, New York, USA), diluted in distilled water. Different concentrations of such antifungals were prepared and used in intervals of 0,03 to 16 µg.mL-1 for amphotericin B and echinocandins; 0,125 to 64 µg.mL-1 for fluconazole. The yeast species were maintained in Sabouraud Dextrose agar medium (Difco, USA) and incubated at 35 ° C for up to 24 hours. The suspensions of the samples of the isolates, prepared in saline, and their density adjusted according to the scale 0.5 of McFarland in 90% of the transmittance using a spectrophotometer at 530 nm. The volume of the inoculum was adjusted to 5.0 mL of sterile saline solution and then diluted in medium RPMI 1640 for a concentration of 2-5x103 cels/mL.

In the susceptibility tests, 96-well microtiter plates (TPP; Trasadingen, Switzerland) were used. The inoculum was added to the wells containing the drugs to be tested, and, as controls, the ATCC 90028 of C. albicans and ATCC 750 of C. tropicalis. Plates were incubated at 35 ° C for 48 hours for Minimum Inhibitory Concentration (MIC) determination. The MICs for amphotericin B were determined for 100% (CIM = 0,25-1,0 µg/mL) and of the fluconazole (CIM <= 8 µg/mL) and echinocandins (anidulafungin: CIM < =1,0 µg/mL; micafungin: CIM <= 1,0 µ g/mL; caspofungin: CIM < =1,0 µg/mL) to □50% of inhibition in relation to the control wells, by visual reading.

Ethical Statements

The study was submitted and approved by the ethics committee from the Health Sciences Center of the Federal University of Pernambuco under protocol number: 3650714.0.0000.5208. The authors declare that the procedures followed the regulations established by the local ethics committee of the Federal University of Pernambuco and the Helsinki declaration of the world medical association.

Statistical analysis

The descriptive statistical analysis was performed in the GraphPad Prism 6 program using Fisher's exact tests and Odds ratio calculation (95% confidence interval for p

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RESULTS

We analysed 105 samples from 62 patients with hematologic malignancies (36 men and 26 women between 18 and 70 years old). The isolation of yeasts of the genus Candida occurred in nine individuals (8.57%), with Candida tropicalis (n= 6) and Candida albicans (n=3). These results were obtained through automation and mass spectrometry analysis, and they were confirmed by molecular analysis. Various risk factors for BSI by Candida were analyzed, and ICU admission showed a statistically significant association with the occurrence of this infection (p = 0,0008) (Table I). Candidemia caused death in four individuals (p = 0,03) and septic shock was a risk factor for death in these patients (p = 0.0476) (Table II). At the antifungal susceptibility test, Candida isolates were susceptible to all antifungals used: amphotericin B (CIM < 0,5 ug/ml), caspofungin (CIM < 0,25 ug/ml), anidulafungin (CIM < 0,06 ug/ml), micafungin (CIM < 0,06 ug/ml) and fluconazole (CIM < 1 ug/ml).

DISCUSSION

BSI caused by yeasts of the genus Candida is the most important opportunistic mycosis in the world with increasing morbidity and mortality (Chen et al., 2012; Ahmadi et al., 2014; Barretti et al., 2013; Admikary & Joshi, 2011; All Thagafi et al., 2014; Quindós, 2018). Its etiological pattern varies according to pertinent geographical variations as documented in several countries (Colombo et al., 2006; Admikary & Joshi, 2011). The incidence of BSI by species of the Candida genus found in this study can be considered elevated when compared to similar studies in different geographic regions (Chen et al., 2012; Chander et al., 2013).

Al Tthaqafi et al. (2014) evaluated patients with hematologic malignancy and BSI by Candida and found C. albicans as the most frequent species (34,1%) followed by C.tropicalis (15,5%), C. parapsilosis (11,9%) and C. glabrata (9,1%). There was a mortality rate of 57.8% for Candida non-albicans species (Al Thagafi et al., 2014) in Taiwan, and Tang et al. (2014) studied cancer patients and, among the species of Candida found in hematologic malignancies, C. albicans was the most common (63,0%) followed by C. tropicalis (22,2%),

C. parapsilosis (7,4%) and C. glabrata (7,4%). The high mortality rate (50.8%) was associated with the absence of antifungal treatment (Tang et al., 2014). Although several studies have shown C. tropicalis as the first or second species of Candida non-albicans more incident, the present study obtained results similar to those performed in North and South of India and Taiwan that identified C. tropicalis as the most frequent species in patients with hematologic malignancies (Chen et al., 2012; Al Thagafi et al., 2014; Chander et al., 2013).

Regarding the risk factors, there was a significant association between ICU admission and BSI by Candida species, justifying, thus, the high incidence of death. Unfortunately, high mortality due to Candida in BSI is a fact that is also reported in other studies (Adhikary & Joshi, 2011; Al Thagafi et al., 2014; Tang et al., 2014; Li et al., 2015). The association between septic shock and death found in our study was also evidenced by Chen et al. (2012) who reported that patients in septic shock had a worse prognosis and a higher risk of Candida infections. For antifungal susceptibility, our results were compatible with several studies showing isolates of C. albicans and C. tropicalis susceptible to amphotericin B, fluconazole (Pfaller et al., 2012; Adhikary & Joshi, 2011; Beilly et al., 2016; Kim et al., 2014; Mirhendi et al., 2020) and echinocandins (Jeong et al., 2016; Mousset et al., 2014). Thus, the importance of monitoring Candida BSI in patients admitted to the ICU is emphasized in order to initiate appropriate treatments and avoid unfavorable outcomes.

CONCLUSIONS

In summary, the occurrence of BSI by Candida and death by this opportunistic pathogen increases in patients with hematologic malignancies hospitalized in ICU and state of septic shock. It is concluded that the data of the study are relevant to the clinical management of patients with Candidaemia and show the need for urgency and precision in the diagnosis, with identification at the level of the species to obtain therapeutic success. Studies with larger sampling are needed to further elucidate the incidence and epidemiology of Candida.

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