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MICROBIOLOGY

Distribution of genetically characterized yeasts and its antifungals susceptibility in the hospital environment

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Abstract: *Candida* spp. is one of the main pathogens associated with nosocomial infection in Brazil and worldwide. The aim of this study was to evaluate the distribution of *Candida* yeasts in the ICU and their susceptibility to the antifungal agents terbinafine and fluconazole. The samples were collected by swabbing nine surfaces in the ICU of a hospital located in Pelotas, RS. These isolates were genetically characterized by sequencing the internal transcript spacer (ITS) using the primers ITS1 and ITS4. The test against antifungals was performed by Microdilution in Broth (CLSI-M27-A4). 64 yeasts identified as *Candida parapsilosis* (45.31%; n = 29), *Meyerozyma* (*Pichia*) *guilliermondii* (28.12%; n = 18), *Claviceps lusitaneae* (25%; n = 16) and *Candida tropicalis* (1, 56%; n = 1) mostly at the counter used for handling medicines and food distribution (68.75%; n = 44). Susceptibility to antifungals varied between species. These results describe potentially pathogenic *Candida* species as contaminants in the ICU environment. The study environment is a potential source of exogenous infection for hospitalized patients.

Key words: *Candida* spp., environmental contamination, exogenous infection, nosocomial infection.

INTRODUCTION

The hospital environment is configured as an exogenous source of infection when selecting resistant infectious agents together with vulnerable individuals (Nogueira et al. 2009). Microorganisms present in this environment are capable of influencing the microbiota of healthy individuals, as well as promoting their replacement by more virulent species through cross-transmission (Bonassoli & Svidzinski 2002).

Candida spp. is considered one of the main fungi involved in HIs (Diongue et al. 2015, ANVISA 2016). The cross-transmission of *Candida* spp. was verified, and the same multilocus genotype was shared by isolates recovered from the hand of a health professional, from the hospital environment and from patients at the same institution, suggesting these sources as possible routes of transmission and that infections by *C. parapsilosis* may be mainly related exogenous transmission to the patient (Sabino et al. 2015).

This study aimed to verify the presence of *Candida* spp. on surfaces in a hospital environment, to identify possible sources of exogenous contamination, as well as their susceptibility to the antifungals used in the clinical routine.

MATERIALS AND METHODS

Samples

Samples were collected in a General Intensive Care Unit (ICU) of a public hospital in the city of Pelotas, RS, Brazil. The research was carried out five days a week during the morning, one hour after disinfecting the environment.

Nine different points were collected in the ICU, totaling 45 samples. The locations selected for collection were:

- tables on each bed (BE) (n = 6),
- workbench for disposal of materials (B1) (n = 1),
- counter for receiving and distributing medicines and food (B2) (n = 1) and,
- contaminated material disposal bench (B3) (n = 1).

The collected material was obtained through the friction of sterile swabs in the selected places. The swab was kept in a sterile tube containing peptone water and immediately forwarded to the Microbiology and Bioprospecting Laboratory of the Institute of Biology (UFPel), where dextrose agar plus chloramphenicol were sown on 37°C for 48h. After this period, macro and microscopic visualization of the isolate and selection of colonies with yeast-like characteristics was performed.

Identification

The genomic DNA of yeast isolates was extracted at the Technological Development Center of the Faculty of Biotechnology/UFPel, through the *in house* technique adapted using Breaking buffer, Phenol:Chloroform:Isoamyl alcohol and glass pearls.

The spacer internal transcribed (ITS) from rDNAwas amplified using primers ITS 1 (5'-TCC GTA GGT GAA CCT GCG G-3') e ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (Irinyi et al. 2015). Reactions were performed in a thermocycler (Ampliterm-ThermalCyclers) under the following conditions: initial denaturation of 94°C for 5 minutes, 30 cycles of 94°C for 1 minute; annealing at 55°C for 1 minute and extension at 72°C for 1 minute; final extension at 72°C for 10 minutes.

Amplification products were purified by Kit illustra GFX PCR DNA and Gel Band Purification (GE Healthcare). O DNA was quantified by the fluorimetric method in a spectrophotometer Qubit (Thermo Fisher Scientific, Wilmington, DE, USA), DNA fragments were analyzed in sequencer ABI 3730 DNA Analyser (Applied Biosystems, Foster City, CA, USA) with kit BigDye Terminator v3.1 Cycle Sequencing (Applied Biosystems). The fragments were sequenced in both directions to increase the quality of the data. The sequences were assembled using the software Vector NTI (Vector NTI, InforMax, Inc, USA) and analyzed through the platform BLASTN (http:\\www.ncbi.nlm.nih.gov/blast).

Descriptive statistical frequency analyses with SPSS statistical software 20.0 were carried out to determine the distribution of *Candida* species and descriptive analyses of susceptibility front of antifungals was determined.

Susceptibility

The antifungal action of fluconazole (FLU) and terbinafine (TER) were analyzed with the Broth Microdilution test to determine the Minimum Inhibitory Concentration (MIC) according to document M27-S4 (CLSI 2012), in concentrations between 64 µg / mL and 8 µg / mL. Ten different concentrations were used, diluted 1: 2 in liquid RPMI. Fungal inoculums, obtained from overnight culture on Sabouraud dextrose agar (SDA), were resuspended in tubes containing 5 mL of sterile saline to obtain turbidity comparable to that of the 0.5 McFarland Standards, approximately 10^6 CFU / mL. The plates were incubated at 36 ° C for 48 hours.

MIC was determined by visual comparison of the growth or not of the microorganism in relation to the control-positive well. The Minimum Fungicidal Concentration (CFM) was determined by transferring 5µl of each negative well on Sabouraud dextrose Agar and the plates were incubated at 36°C for up to 48 hours. CFM was defined as the lowest concentration at which no growth was observed.

RESULTS AND DISCUSSION

64 fungal isolates were observed on the nine surfaces of the ICU, which were obtained as described in Figure 1. Bench 2 showed a relevant result ($p<0.05$) because it is the place of distribution of food that will be administered orally to hospitalized patients. This can promote the distribution of microorganisms to patients, an exogenous yeast transmission. The presence of yeast on tables near the beds also facilitates the transmission of pathogens through medical devices that will be used in patients.

The possibility of the hospital environment causing exogenous infection is considered low, however, it can act on secondary cross-contamination, by the hands of health professionals or by means of medical and surgical instruments, which in contact with contaminated surfaces can transfer microorganisms to hospitalized people (ANVISA 2010).

Environmental microorganisms can have different origins and remain in place due to inadequate hygiene practices, presence of organic matter, humidity, use of air conditioning, nebulizers, presence of plants and food, among other factors. Because of this, there is a need to maintain health environments more broadly and continuously (Cordeiro et al. 2010, ANVISA 2010). The studied sites may have been contaminated by these factors.

In this study, four yeast species were identified, all of the *Candida* genus (Figure 2), in their sexual and asexual form. *Candida parapsilosis* was the most prevalent species, totaling 45.3% of the isolates, followed by *Meyerozyma (Pichia) guilliermondii* (28.1%), *Claviceps lusitaneae* (25%) and *Candida tropicalis* (1.56%). *C. albicans* was significantly higher than the other species.

Candida species are identified as one of the main microorganisms involved in hospital infections in Brazil and worldwide. It is considered one of the most relevant and frequent fungal agents associated with bloodstream infections (ANVISA 2014, 2016, Ruiz & Richini 2016, Ahangarkani et al. 2020). According to ANVISA (2016), in 2015, *Candida* spp. it appears as the sixth most reported microorganism among the etiologic agents of bloodstream infections in patients hospitalized in an adult ICU, or the third in neonates and in a pediatric ICU. It is the only fungus of a species isolated in these situations. Its pathogenicity factors, combined with its ability to survive for hours in the environment, have provided high rates in several studies (ANVISA 2010, Goemaere et al. 2018).

Candida spp. infections are superficial or invasive manifestations and affect individuals exposed to different risk factors (Colombo & Guimarães 2003). In cases of invasive candidiasis, these factors may be associated with the host and health care (Yapar 2014). Aspects such as immunosuppression, skin and mucosal rupture, defects in the number and function of neutrophils or cell-mediated immunity, metabolic dysfunction, age extremes (Pfaller & Diekema 2007), in addition to antibiotic therapy, corticotherapy, chemotherapy, surgery, catheterization, use of tubes and catheters (Costa et al. 2008) are factors that contribute to the increase in infections by opportunistic fungi.

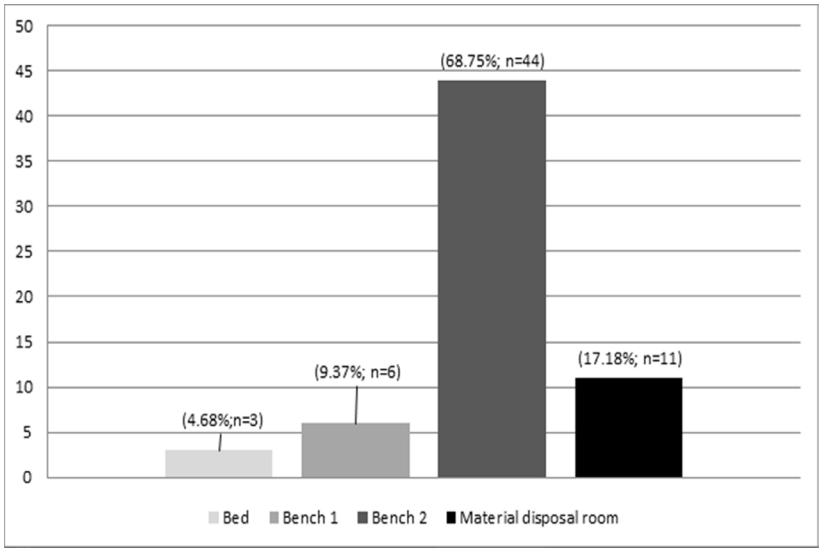

Figure 1. Distribution of the number of yeasts isolates according to the location of the surfaces located in the ICU of the hospital located in the city of Pelotas RS.

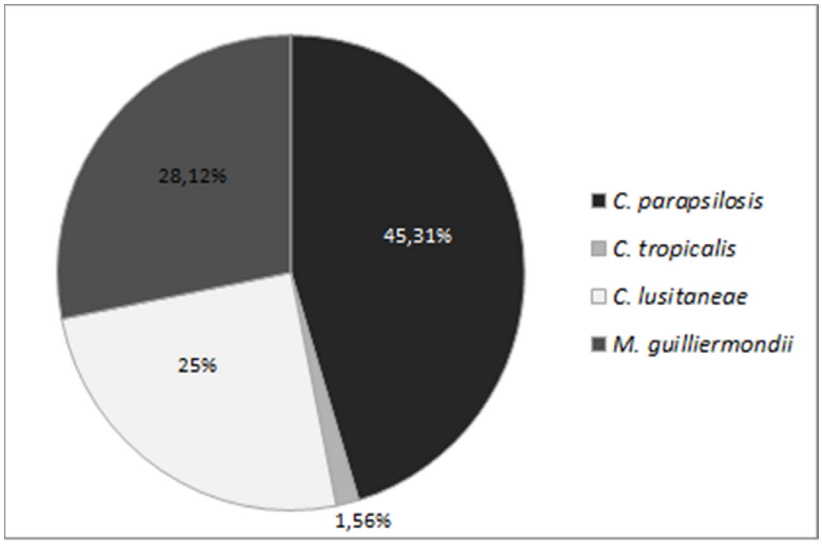

Figure 2. *Candida* spp. on hospital ICU environment surfaces of the hospital located in the in the city of Pelotas RS.

Currently, *C. albicans* is still considered a more prevalent species in cases of candidemia, however, there is a tendency to increase infections by other species of the genus, with emphasis on *C. parapsilosis* (Pfaller & Diekema 2007, Goemaere et al. 2018, Ahangarkani et al. 2020). In a study by Storti et al. (2012) this species was the third most frequent, as well as in the retrospective study on candidemia in Brazilian hospitals (Colombo et al. 2014). Fu et al. (2017), observed *C. parapsilosis* as the fourth most isolated non-*albicans* species, indicating the importance of the genus in nosocomial infections.

In the present study, species already described in environmental and clinical samples were identified (Storti et al. 2012, Colombo et al. 2014, Fu et al. 2017, Goemaere et al. 2018). These authors used biochemical methods to identify the isolates, except for Goemaere et al. (2018) who used ITS region sequencing.

According to the antifungals used, it was possible to observe differences regarding the susceptibilities of the substances (Table I).

Table I. Variation range of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (CFM) of fluconazole and terbinafine against yeasts isolated in a hospital environment in the city of Pelotas RS

Yeasts	Samples (n)	Fluconazole MIC / CFM (µg/mL)	Terbinafine MIC / CFM (µg/mL)
C. parapsilosis	29	0,48 - > 64 / 0,18 - 15,6	0,03 - 3 / 0,075 – 3
C. tropicalis	1	11, 7 / > 64	4 / > 8
M. guilliermondii	18	7,81 - > 64 / 3,9 - > 64	0,37 - > 8 / 0,31 - > 8
C. lusitaneae	16	0,36 - 7,81 / 0,36 - 7,81	0,09 - 6 / 0,75 - > 8

According to CLSI (2002), *Candida* species inhibited in concentrations below 8 µg / mL are considered sensitive to fluconazole, resistant when inhibited in concentrations above 64 µg / mL and with dose-dependent sensitivity in concentrations of 16 -32 µg / mL. In our study, *C. lusitaneae* was more susceptible to fluconazole, with MIC and CFM between 0.36 - 7.81 µg / mL. The other species also presented resistant isolates. With terbinafine, isolates of *C. parapsilosis* were sensitive to the concentrations used, with an interval of 0.03 - 3 µg / mL. In relation to the other species, a higher concentration was necessary for inhibition to occur. *C. tropicalis* was the species least susceptible to the products used.

Although the literature indicates resistance to fluconazole by several species of fungi, its administration in the treatment of infections by *Candida* spp. it is very common in Brazilian hospitals and worldwide (Pfaller & Diekema 2007, Colombo et al. 2014).

Resistance to fluconazole was verified globally, as reported by Pfaller & Diekema (2007) who observed increased resistance of species such as *C. guilliermondii, C. glabrata, C. rugosa* and *C. famata* in recent years, and by Fesharaki et al. (2013), although MICs were low, the terapy with fluconazole failed in a patient with endocarditis caused by *Candida* species. In the study by Goemaere et al. (2018), the authors attributed prior exposure to fluconazole as one of the factors associated with candidemia caused by less susceptible species. In front of the azole groups, *Candida* spp. may have different resistance mechanisms, such as biofilm formation, presence of efflux pumps, modification of the target enzyme and inactivation of the drug by modification, and there may be more than one resistance factor (Kanafani & Perfect 2008, Quintero 2010)

Regarding terbinafine, it is used in antifungal therapy and applied in the treatment of various fungal pathologies (Mahmoudabadi et al. 2015, Babu et al. 2017). Resistant strains have been described by Gamarra et al. (2014), Mahmoudabadi et al. (2015) and Hu et al. (2017), who evaluated its effectiveness against vulvovaginal candidiasis, invasive candidiasis and balanoposthitis, respectively. For White et al. (1998) the resistance of *C. albicans* to terbinafine may be related to the low intracellular accumulation of the drug in the fungal cell, which would result in isolates with less susceptibility to the drug.

The occurrence of different species of *Candida* in the hospital environment with resistance to antifungals, can be a complicating factor in the treatment and recovery of patients. Therefore, considering the presence of these microorganisms to analyze the possibility of proposing stricter measures in the hospitals in the city through the authorities it be important.

CONCLUSION

It is concluded, therefore, that the presence of yeast fungi is real on the surfaces of the ICU environment, and it is important to analyze the possibility of proposing more stringent measures in hospitals through the authorities.

The presence of these microorganisms makes it an exogenous source of hospital infection and exposes immunocompromised individuals to isolates resistant to antifungals in common use in the health system.

Candida parapsilosis was the most prevalent species. Of the evaluated sites, on the bank used for drug handling and food distribution showed a relevant result.

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