



Antifungal susceptibility profile of different yeasts isolates from wild animals, cow's milk with subclinical mastitis and hospital environment

J. F. Mendes^{a*}, C. L. Gonçalves^b, G. F. Ferreira^b, I. A. Esteves^b, C. H. Freitas^b,
J. P. V. Villarreal^b, J. R. B. Mello^c, M. C. A. Meireles^a and P. S. Nascente^b

^aCentro de Pesquisa e Diagnóstico em Micologia Veterinária – MICVET, Departamento de Veterinária Preventiva, Faculdade de Veterinária, Universidade Federal de Pelotas – UFPel, Campus Universitário, s/n, CEP 96160-000, Capão do Leão, RS, Brazil

^bLaboratório de Micologia e Bioprospecção, Departamento de Microbiologia e Parasitologia, Instituto de Biologia – IB, Universidade Federal de Pelotas – UFPel, Campus Universitário, s/n, CEP 96160-000, Capão do Leão, RS, Brazil

^cDepartamento de Farmacologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul – UFRGS, Sarmiento Leite, 500/200, Cidade Baixa, CEP 90050-170, Porto Alegre, RS, Brazil

*e-mail: josiara.mds@hotmail.com

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Abstract

Yeast infections have acquired great importance due to increasing frequency in immunocompromised patients or patients undergoing invasive diagnostic and therapeutic techniques, and also because of its high morbidity and mortality. At the same time, it has been seen an increase in the emergence of new pathogenic species difficult to diagnose and treat. The aim of this study was to determine the *in vitro* susceptibility of 89 yeasts from different sources against the antifungals amphotericin B, voriconazole, fluconazole and flucytosine, using the VITEK® 2 Compact system. The antifungal susceptibility was performed automatically by the Vitek® 2 Compact system. The origin of the yeasts was: Group 1 - microbiota of wild animals (W) (26/89), 2 - cow's milk with subclinical mastitis (M) (27/89) and 3 - hospital environment (H) (36/89). Of the 89 yeasts submitted to the Vitek® 2 test, 25 (20.9%) were resistant to fluconazole, 11 (12.36%) to amphotericin B, 3 (3.37%) to voriconazole, and no sample was resistant to flucytosine. Regarding the minimum inhibitory concentration (MIC), fluconazole showed an MIC between 1 and 64 mg/mL for the three groups, voriconazole had an MIC between 0.12 and 8 mg/mL, amphotericin B had an MIC between 0.25 and 4 mg/mL for group H and group W respectively, between 0.25 and 16 mg/mL for group M and flucytosine had an MIC equal to 1 µg/mL for all groups. The yeasts isolated from the H group showed the highest resistance to fluconazole 12/89 (13.49%), followed by group W (7.87%) and group M (5.62%). The more resistant group to voriconazole was followed by the M and H groups, the W group showed no resistance to this antifungal. Group H was the least resistant (2.25%) to amphotericin.

Keywords: antifungal, yeast, Vitek® 2 Compact.

Perfil de suscetibilidade antifúngica de diferentes leveduras isoladas da microbiota de animais silvestres, leite com mastite subclínica e ambiente hospitalar

Resumo

As infecções por leveduras têm adquirido grande importância, devido ao aumento da sua frequência em pacientes imunocomprometidos ou pacientes submetidos a técnicas diagnósticas e terapêuticas agressivas, e devido sua alta morbidade e mortalidade. Paralelamente tem-se observado um incremento na aparição de novas espécies patogênicas difíceis de diagnosticar e tratar. O objetivo desse estudo foi avaliar a suscetibilidade *in vitro* de 89 leveduras de diferentes origens frente aos antifúngicos Anfotericina B, Voriconazol, Fluconazol e Fluocitocina pelo Sistema Vitek® 2. O antifungograma foi realizado automaticamente pelo Vitek® 2 Compact. A origem das leveduras foi: Grupo 1-Microbiota de Animais Silvestres (S) (26/89), 2- Leite com mastite bovina subclínica (L) (27/89) e 3- Ambiente Hospitalar (H) (36/89). Das 89 leveduras submetidas à carta Vitek®, 25 (20.09%) foram resistentes ao fluconazol, oito (8.99%) à anfotericina B, três (3.37%) ao voriconazol, e nenhuma amostra mostrou-se resistente a fluocitosina. O grupo três (H) foi mais resistente ao fluconazol que os demais, já o dois (L) foi mais resistente ao voriconazol e a anfotericina B que

os outros dois. O fluconazol pode ter apresentado maior número de resistências devido ser um fármaco comumente usado principalmente em humanos. As leveduras isoladas de humanos apresentaram maior número de resistências aos fármacos testados do que as leveduras isoladas de animais silvestres. O que pode ocorrer devido a uma maior exposição dos humanos aos fármacos em relação aos animais que vivem isolados em ambientes selvagens e na maioria dos casos nunca teve contato com fármacos de qualquer origem.

Palavras-chave: antifúngicos, leveduras, Vitek® 2 Compact.

1. Introduction

Fungi are microorganisms found in soil, water, vegetables, air, animals and detritus. Many of them have a pathogenic potential to human and animals causing diseases known as mycoses (Trabulsi and Toledo, 1991). The infections caused by fungi had acquired importance in the last decades, not only due to an increase in its frequency in immunocompromised or submitted patients undergoing through aggressive diagnostic techniques and therapeutic treatments, but also due to its high morbidity and mortality. Meanwhile an increase in the emergence of new pathogenic species difficult to diagnose and treat has been observed (Coleman et al., 2008); this increase is not only due to the frequent use of antifungal drugs to treat these kinds of infections but also due to therapeutic drug overdose. This resistance, especially in azole derivatives has been previously described, demonstrating the need of *in vitro* susceptibility studies to evaluate the efficacy of antifungals drugs, before starting a treatment (Sandven, 2000).

Among different fungi species, the gender *Candida* spp., is responsible for around 80% of all nosocomial fungal infections (Spellberg et al., 2006). *Candida albicans* is the most predominant specie present in about 50% of all the isolates, followed by *C. glabrata*, *C. parapsilosis* and *C. tropicalis* present in around 10 to 25% respectively. In Latin America, *C. parapsilosis* figures as the second cause of the mycoses (Spellberg et al., 2006; Höfling et al., 2010).

The antifungal resistance has represented a major challenge for the clinic, by the difficulty to treat candidiasis. The increase of the antifungal resistance may be due to the use of selective therapies with inadequate doses or to the drug's frequent use in the fungal infection prophylaxis, both in human and animals, which may affect the selective clinical resistance (Colombo and Guimarães, 2007; Galle and Gianinni, 2009).

The Vitek® 2 Compact system is an automatized commercial method for antifungal susceptibility analyses, which determinates the spectrophotometric yeast growth allowing the identification of different species, comparing the biochemical profile with a wide data base (Al-Sweih et al., 2009; Mokaddas et al., 2007). This system also incorporates the AST- YS01 plate, which is used to test the yeast against the following antifungal drugs: Amphotericin B, Voriconazole, Fluconazole and Flucytosine.

Once the plate is placed inside the Vitek® 2 Compact system device, using an adequate microorganism suspension, the manipulation it's not necessary. Once the dilutions

are made, the plate is inoculated using a vacuum filling process, then is sealed and placed automatically in the reader/incubator (bioMérieux, 2008). The system integrates a software that validates and interprets the susceptibility test results and determinates them automatically. The growth is based on the measure of the decreased light by an optic scanner. This data is used to determinate the minimum inhibitory concentration (MIC) values of the antifungal drugs tested (Pfaller et al., 2011).

The aim of this study was to evaluate the *in vitro* susceptibility of 89 yeasts from different origins against the following antifungal drugs: Amphotericin B, Voriconazole, Fluconazole and Flucytosine using the Vitek® 2 Compact system.

2. Material and Methods

Eighty-nine different isolated yeasts were evaluated. Yeasts were obtained from the mycology collection of Mycology Laboratory in the Department of Microbiology and Parasitology located in the Biology Institute from the Federal University of Pelotas.

The yeasts isolates were identify and grouped in eight fungal genera, each one with their respective species: *Candida albicans* (11), *C. guilliermondii* (14), *C. famata* (10), *C. parapsilosis* (10), *C. ciferri* (4), *C. catenulata* (2), *C. sphaerica* (2), *C. intermedia* (2), *C. pulcherrima* (2), *C. rugosa* (1), *C. peliculosa* (1), *C. lipolytica* (1), *C. globosa* (1), *Cryptococcus laurentii* (9), *Trichosporon asahii* (2), *Trichosporon* sp. (1), *Sacharomyces* sp. (1), *S. cerevisiae* (3), *Geotrichum klebahnii* (2), *Zigossacharomyces* sp. (1), *Rhodotorula* sp. (8) and *Kodamala ohmeri* (1).

The yeasts used in this study were obtained and isolated from different places and distributed in three different groups: Group 1- Isolates from the wild animals microbiota (W) (26) 29.21%; Group 2- Isolates from cow's milk with subclinical mastitis (M) (27) 30.34% and Group 3- Isolates from human Hospital environment (H) (36) 41.57% (Intensive Care Unit).

The antifungal susceptibility was performed automatically using the Vitek® 2 Compact system. This device was handled according to the manufacturer's instructions. The yeasts, previously stored, were replicated in Sabouraud Dextrose Agar with Chloramphenicol by the depletion technique and incubated at 36°C/48h. After this period the suspensions were made from isolated colonies in disposable tubes containing 3mL of 0.45% Saline Solution adjusted to a 0.5 McFarland standard. To perform the test, the plates

containing the four antifungal drugs were added and inserted in the equipment.

For the antifungal susceptibility test, the Vitek® 2 compact system follows the Clinical and Laboratory Standards Institute (CLSI), European Society of Clinical Microbiology and Infectious Diseases (EUCAST) and Food and Drug Administration (FDA) international standards. In this test, the Vitek® 2 device was configured according to the CLSI documents.

The card of Vitek® 2 – AST – YS01 (Lote 281223610), suitable for the realization of antifungal sensitivity tests are disposable and has four drugs at different concentrations (different dilutions): flucytosine (sensitivity ≤ 1 $\mu\text{g/mL}$), voriconazole (sensitivity 0.125 $\mu\text{g/mL}$, intermediate sensitivity 2 to 4 $\mu\text{g/mL}$, resistant ≥ 4 $\mu\text{g/mL}$), amphotericin B (sensitivity ≤ 0.25 $\mu\text{g/mL}$, intermediate sensitivity 4 to 8 $\mu\text{g/mL}$, resistant ≥ 16 $\mu\text{g/mL}$) and fluconazole (sensitivity ≤ 2 $\mu\text{g/mL}$, intermediate sensitivity 14 to ≥ 32 $\mu\text{g/mL}$, resistant ≥ 64 $\mu\text{g/mL}$). After the loaded plates were placed in the Vitek 2 instrument, the plates were filled with the appropriately diluted yeast suspensions, incubated (for a maximum of 24 h), and reading automatically.

The breakpoints according to CLSI M27 S4 (for *Candida* spp.) for fluconazole are sensitivity ≤ 2 , susceptible-dose dependent (SDD) 4 to ≥ 32 and resistant 8 to ≥ 64 , for voriconazole are sensitivity ≤ 0.12 , SDD 0.25 to 1 and resistant ≥ 1 (CLSI, 2012).

The Vitek® 2 Compact system was also used to perform the yeasts biochemical characterization (assimilation of carbohydrates) and to confirm the identification of the species. For the identification of the species, 64 biochemical tests were performed by measuring the use of carbon and nitrogen sources and by the enzymatic activity as well. The final results were obtained in nearly (average) 18 hours. For the statistical analyses, the chi-square test was performed through the use of the SPSS program.

3. Results

Among the 89 yeasts submitted to the Vitek® card AST, 85 (95.5%) were susceptible to the flucytosine, 71 (79.7%) to the voriconazole, 71 (79.7%) to the amphotericin B and 52 (58.43%) to the fluconazole. According to resistance, 25 (20.09%) were resistant to the fluconazole, 11 (12.36%) to the amphotericin B, 3 (3.37%) to the voriconazole and no sample revealed resistance to the flucytosine. Regarding to the intermediate sensitivity, eight samples (8.99%) were intermediary sensitive to the fluconazole, four (4.49%) to the voriconazole, four (4.49%) to the amphotericin B and no sample was intermediary sensitive to the flucytosine. The rest of the yeasts did not present resistance results, four (4.49%) to the flucytosine, two (2.25%) to the fluconazole, 11 (12.36%) to the voriconazole and three (3.37%) to the amphotericin B, as described in Table 1.

The higher antifungals action spectrum is shown in the following decreasing order: For the flucytosine the susceptibility rate was of 95.5%, for the voriconazole 80%, for the amphotericin B 80% and for the fluconazole 58.8%. For the flucytosine, the most susceptible species were: *C. guilliermondii*, *C. albicans*, *C. parapsilosis* and *C. famata*. The voriconazole presented the genera *Candida* spp. as the most susceptible inside this group, and *Rhodotorula* sp. being the higher resistance sample. *C. laurentii*, showed high susceptibility rates against the fluconazole. Meanwhile the genera *Candida* spp. presented susceptibility variations rates against the amphotericin B, dividing the species inside this genus in two groups: One group having the susceptible species such as *C. guilliermondii*, *C. parapsilosis* and *C. albicans*, and the other group having the resistant species such as *C. ciferri*, *C. famata* and *C. albicans*.

It has been observed a significant difference between the drugs susceptibility profiles and the fungal specie ($p < 0.05$) according with the statistical analyses performed through the chi-square test. The resistance results and the origin of the submitted samples to the tests can be described in Table 2, showing that the isolated yeasts from human

Table 1. Origin and susceptibility of 89 yeasts tested against four antifungals by Vitek® 2 System.

N	Identification	Origin	Species	Flucytosine	Fluconazole	Voriconazole	Amphotericin B
1	LMI 010	H	<i>C. albicans</i>	S	S	S	S
2	LMI 013	H	<i>C. lipolytica</i>	S	S	S	S
3	LMI 023	H	<i>C. laurentii</i>	S	S	-	S
4	LMI 026	H	<i>C. laurentii</i>	S	S	-	S
5	LMI 028	H	<i>T. assahi</i>	S	I	S	I
6	LMI 127	H	<i>Rhodotorula</i> sp.	S	R	I	S
7	LMI 029	H	<i>C. famata</i>	S	S	S	S
8	LMI 012	H	<i>C. guilliermondii</i>	S	R	S	S
9	LMI 145	H	<i>C. albicans</i>	S	S	S	S
10	LMI 136	H	<i>C. laurentii</i>	S	S	-	S
11	LMI 151	H	<i>C. laurentii</i>	S	S	-	S
12	LMI 157	H	<i>C. laurentii</i>	S	I	-	S
13	LMI 158	H	<i>C. laurentii</i>	S	S	-	S

R= Resistant; S= Sensitive; I= Intermediate sensitivity; W= Wild animal; M= Milk; H= Hospital setting.

Table 1. Continued...

N	Identification	Origin	Species	Flucytosine	Fluconazole	Voriconazole	Amphotericin B
14	LMI 159	H	<i>C. laurentii</i>	S	S	-	S
15	LMI 142	H	<i>C. laurentii</i>	S	R	-	S
16	LMI 153	H	<i>C. famata</i>	S	S	S	S
17	LMI 156	H	<i>C. famata</i>	S	S	S	R
18	LMI 131	H	<i>S. cerevisiae</i>	S	S	S	S
19	LMI 132	H	<i>S. cerevisiae</i>	S	I	S	S
20	LMI 143	H	<i>S. cerevisiae</i>	S	R	S	R
21	LMI 133	H	<i>C. parapsilosis</i>	S	S	S	S
22	LMI 134	H	<i>C. parapsilosis</i>	S	S	S	S
23	LMI 135	H	<i>C. parapsilosis</i>	S	S	S	S
24	LMI 139	H	<i>C. parapsilosis</i>	S	R	S	S
25	LMI 130	H	<i>C. parapsilosis</i>	S	R	S	S
26	LMI 125	H	<i>C. guilliermondii</i>	S	S	S	S
27	LMI 126	H	<i>C. guilliermondii</i>	S	S	S	S
28	LMI 150	H	<i>C. guilliermondii</i>	S	R	S	S
29	LMI 154	H	<i>C. cifferi</i>	S	S	S	S
30	LMI 155	H	<i>C. cifferi</i>	S	R	S	I
31	LMI 114	H	<i>Zygosacharomyces</i> sp.	S	I	S	S
32	LMI 137	H	<i>Rhodotorula</i> sp.	S	I	S	S
33	LMI 144	H	<i>Rhodotorula</i> sp	-	-	-	-
34	LMI 141	H	<i>Rhodotorula</i> sp	-	R	S	S
35	LMI 129	H	<i>Rhodotorula</i> sp	S	R	R	S
36	LMI 047	M	<i>C. rugosa</i>	S	R	I	I
37	LMI 122	M	<i>C. albicans</i>	S	I	R	R
38	LMI 124	M	<i>C. albicans</i>	S	S	S	S
39	LMI 046	M	<i>C. laurentii</i>	S	S	-	S
40	LMI 040	M	<i>Trichosporon</i> sp.	-	-	-	-
41	LMI 045	M	<i>C. famata</i>	S	S	S	I
42	LMI 075	M	<i>C. famata</i>	S	S	S	S
43	LMI 120	M	<i>C. famata</i>	S	R	R	R
44	LMI 038	M	<i>Sacharomyces</i> sp.	S	S	S	S
45	LMI 048	M	<i>C. parapsilosis</i>	S	S	S	S
46	LMI 067	M	<i>C. parapsilosis</i>	S	S	S	S
47	LMI 068	M	<i>C. parapsilosis</i>	S	S	S	S
48	LMI 070	M	<i>C. parapsilosis</i>	S	S	S	S
49	LMI 058	M	<i>C. parapsilosis</i>	S	S	S	S
50	LMI 133	M	<i>C. parapsilosis</i>	S	S	S	S
51	LMI 134	M	<i>C. parapsilosis</i>	S	S	S	S
52	LMI 135	M	<i>C. parapsilosis</i>	S	S	S	S
53	LMI 139	M	<i>C. parapsilosis</i>	S	R	S	S
54	LMI 130	M	<i>C. parapsilosis</i>	S	R	S	S
55	LMI 050	M	<i>C. pulcherrima</i>	S	S	S	S
56	LMI 071	M	<i>C. pulcherrima</i>	S	R	S	S
57	LMI 054	M	<i>C. guilliermondii</i>	S	S	S	S
58	LMI 055	M	<i>C. guilliermondii</i>	S	S	S	S
59	LMI 056	M	<i>C. guilliermondii</i>	S	I	S	S
60	LMI 057	M	<i>C. guilliermondii</i>	S	S	S	S
61	LMI 059	M	<i>C. guilliermondii</i>	S	I	S	S
62	LMI 062	M	<i>C. guilliermondii</i>	S	I	S	S
63	LMI 063	M	<i>C. guilliermondii</i>	S	S	S	S

R= Resistant; S= Sensitive; I= Intermediate sensitivity; W= Wild animal; M= Milk; H= Hospital setting.

Table 1. Continued...

N	Identification	Origin	Species	Flucytosine	Fluconazole	Voriconazole	Amphotericin B
64	LMI 064	M	<i>C. guilliermondii</i>	S	S	S	S
65	LMI 061	M	<i>C. peliculosa</i>	S	I	S	S
66	LMI 037	M	<i>Rhodotorula</i> sp.	S	R	S	R
67	LMI 076	M	<i>K. ohmeri</i>	S	R	I	R
68	LPS 022	W	<i>T. assahi</i>	S	R	S	S
69	LMI 091	W	<i>C. albicans</i>	S	S	S	S
70	LMI 092	W	<i>C. albicans</i>	S	S	S	S
71	LMI 104	W	<i>C. albicans</i>	S	S	S	S
72	LPS 005	W	<i>C. albicans</i>	S	S	S	S
73	LMI 098	W	<i>C. albicans</i>	S	R	S	S
74	LMI 102	W	<i>C. albicans</i>	S	R	S	S
75	LMI 081	W	<i>C. albicans</i>	S	R	S	R
76	LPS 009	W	<i>C. famata</i>	S	S	S	S
77	LMI 088	W	<i>C. famata</i>	S	R	S	R
78	LPS 008	W	<i>C. guilliermondii</i>	S	S	S	S
79	LMI 107	W	<i>G. klebahnii</i>	S	S	S	S
80	LPS 016	W	<i>G. klebahnii</i>	S	S	S	S
81	LPS 004	W	<i>Rhodotorula</i> sp.	S	I	S	S
82	LMI 117	W	<i>Rhodotorula</i> sp.	S	R	S	S
83	LPS 007	W	<i>C. sphaerica</i>	S	S	S	S
84	LMI 094	W	<i>C. sphaerica</i>	-	R	I	-
85	LMI 101	W	<i>C. intermedia</i>	S	S	S	S
86	LPS 014	W	<i>C. intermedia</i>	S	S	S	S
87	LPS 006	W	<i>C. catenulata</i>	S	S	S	S
88	LMI 084	W	<i>C. catenulata</i>	S	R	S	S
89	LPS 024	W	<i>C. globosa</i>	S	S	S	S

R= Resistant; S= Sensitive; I= Intermediate sensitivity; W= Wild animal; M= Milk; H= Hospital setting.

Table 2. Profile susceptibility of yeasts from different Origin against them antifungal fluconazole, Flucytosine, voriconazole and amphotericin B obtained by the Vitek® 2 System.

	FLUCONAZOLE (%)			FLUCYTOSINE(%)			VORICONAZOLE (%)			AMPHOTERICIN B (%)		
	I	R	S	I	R	S	I	R	S	I	R	S
W	0.89	7.87	19.10	-	-	26.97	0.89	-	26.97	-	3.37	20.22
M	5.62	5.62	17.98	-	-	37.08	2.25	2.25	23.60	2.25	4.49	22.47
H	7.68	13.49	21.35	-	-	28.09	1.78	1.78	25.84	1.78	2.25	34.84

R= Resistant; S= Sensitive; I= Intermediate sensitivity.

hospitalary environment presented the higher resistance to the fluconazole 12/89 (13.49%) and the higher susceptibility to the flucytosine (100%). The isolated yeasts from cow's milk with subclinical mastitis presented a higher resistance to the amphotericin B 4/89 (4.49%) and to the voriconazole 2/89 (2.25%), and also a higher susceptibility to the flucytosine (100%). The isolated yeasts from the wild animal's microbiota showed a high resistance to the fluconazole 7/89 (7.87%), followed by the amphotericin B 3/89 (3.37%) and both were 100% susceptible to the flucytosine and the voriconazole, Which demonstrates that human isolated yeasts had a major resistance than the yeasts isolated from wild animals to the antifungals used.

Analysis of the minimum inhibitory concentration (MIC) showed that Fluconazole MIC varies between 1 and 64 µg/mL in the three groups, the voriconazole

MIC rates between 0.12 and 8 µg/mL in the H group, between 0.12 and 2 µg/mL in the S group and between 0.12 and 8 µg/mL in the L group, the amphotericin B showed MIC rates between 0.25 and 4 µg/mL in the H group and in the S group and between 0.25 and 16 µg/mL in the L group. Finally, the flucytosine had MIC rates <1µg/mL in all groups.

4. Discussion

The Vitek® MS –Mass spectrometry (bioMérieux) is used for microbial identification being the first and unique mass spectrometer of America approved and certificated by the FDA for the *in vitro* diagnose use. Therefore, beyond being the only system register and approved by the ANVISA for this purpose, now has the certificate and the seal of the FDA (FDA, 2001).

The Vitek® 2 Compact system is a practical and efficient tool used widely by researchers for microbial identification (Melhen et al., 2014; Pereira, 2010; Silva et al., 2013; Zhang et al., 2014).

The Vitek® 2 Compact device monitors the microbial kinetic growth and calculates the respective MIC by an own algorithm used in the susceptibility evaluation against antifungal drugs. Based on the MIC values during the performed assays, these can be compared in a data base containing an average distribution of these concentrations (Joyanes et al., 2001; Pasteran et al., 2011; Soloaga, 2010). The afore mentioned device acts as an useful, accurate and quickly tool in the profile susceptibility detection of microorganisms, helping in the use of prescription and dosage of antibiotics for fungemias (Terasawa, 2006).

These results are similar to the ones found by Crocco et al. (2004) and by Gomes et al. (2010), where the susceptibility of *Candida* spp. was analyzed, showing that the species studied in this genus were susceptible to the amphotericin B with a MIC of < 0.5 µg/mL. Meanwhile the fluconazole MICs varied from 0.12 to 4 µg/mL, and the antifungals itraconazole and voriconazole presented MICs of < 0.03 µg/mL.

In the present study, the microorganisms showed a high resistance against Fluconazole (26.6%) and an intermediate susceptibility (12.2%), as described by Favalessa et al. (2010) attributing the highest Minimum Inhibitory Concentration (MIC) values to the fluconazole, which demonstrates a high resistance in the isolates of *Candida* spp. to this drug. In the susceptibility pattern (method M27-A3 CLSI, 2008), the species: *C. albicans*, *C. tropicalis* and *C. parapsilosis* were considered susceptible to antifungals such as the amphotericin B and to the fluconazole (Rex et al., 2000). The fluconazole is known for having low incidence of collateral effects (compared to the imidazoles) and high activity against most of the *Candida* spp., with the exception of *C. krusei* and *C. glabrata* (Klasterksy, 2004). The amphotericin B, who belongs to the polyene antifungals, is one of the principal drugs of election available for the treatment of invasive fungal infections. However, collateral effects such as nephrotoxicity, dyspnea, hives, chest and abdominal pain and hypoxemia are associated (Oliveira et al., 2016; Lumbresas et al., 2003; Walsh et al., 2002).

The antifungal resistance in yeasts, like any other microorganism, can be classified as intrinsic resistance, natural resistance and acquired resistance. The intrinsic resistance is characterized when all the specie members' are resistant to certain drug or a class of antifungal agent before drug exposure, this is due to genetic expression of this condition. The natural resistance is characterized when some particular kind of strains exhibit resistance. Meanwhile the acquired resistance is developed in response to the drug exposure (Leão et al., 2004; Santos, 2009). The fluconazole is a drug which is known for having a potential action against yeasts from the *Candida* genera. However the repetitive and prolonged fluconazole use in

immunologically compromised patients can developed resistance against this fungi.

Many resistance mechanisms are well known, such as the intrinsic fluconazole resistance expressed by isolates of *C. krusei* and *C. glabrata* (Borowicz et al., 2012), which can explain the higher resistance to this drug, in the present study.

Martins et al. (2010) obtained similar values of those observed in the present study. In such study, they analyzed the antifungal susceptibility of *Candida* spp. obtained from patients with onychomycosis, having 99% of susceptibility against the amphotericin B.

With susceptibility values similar to the amphotericin B (Klepser and Lewis, 2002), the voriconazole was developed at the final period of the 80s decade and its origin was from the idea of obtaining a drug with a broader spectrum action than the fluconazole. The fluconazole has a high antifungal potential against a wide variety of fungi, such as *Candida* spp., *Aspergillus* spp., *Scedosporium* spp. and *Fusarium* spp. (Donnelly and De Pauw, 2004).

Beyond that, its action is more efficient against emergent pathogens, compared to other azoles drugs (Zhang et al., 2014). The flucytosine is efficient in yeasts infections despite of having a short action spectrum, yet susceptible in 100% of the samples tested. The 96.7% (n= 1.202) of the isolates were correctly identified at the species level and 0.2% (n = 2) of the isolates were identified at the genera level, meanwhile the 2.4% (n = 30) of the isolates were not identified by the 2.0 Vitek® MS system, (Zhang et al., 2014), confirming its reliability in the identification.

The four antifungal drugs studied showed different susceptibilities against the yeasts species, wherein fluconazole presented a higher resistance against the three isolated origins, followed by the amphotericin B and the voriconazole, meanwhile the flucytosine presented efficiency against all yeasts present in this study, none of the yeasts showed resistance to the flucytosine. The fact that the Fluconazole had a higher resistance can be associated with the reason of being a drug commonly used mainly in human.

The human's isolated yeasts presented a higher resistance to the tested drugs than the yeasts isolated from wild animals. Which can occur due to the higher drug exposure in human compared to the animals who live isolated in wild environment and most of the cases never had contact with any kind of drugs.

These results also demonstrate the need of susceptibility characterization of the yeasts against the antifungal drugs before any antimicrobial therapy use, in order to avoid the microorganism's resistance and the drug overdose.

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