Revista Brasileira de Biociências
Brazilian Journal of Biosciences

ISSN 1980-4849 (on-line) / 1679-2343 (print)

ARTICLE

Occurrence and susceptibility of yeasts present in the polluted waters of Dilúvio Stream, Porto Alegre, Brazil

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Recebido: 18 de outubro de 2017 Recebido após revisão: 10 de outubro de 2018 Aceito: de dezembro de 2018
Disponível on-line em http://www.ufrgs.br/seerbio/ojs/index.php/rbb/article/view/4077

ABSTRACT: (Occurrence and susceptibility of yeasts from the polluted waters of the Dilúvio stream, Porto Alegre municipality, southern Brazil). Water is one of the natural resources that are essential to vital functions. Nowadays, water pollution has become one of the major issues for the survival of all life forms. The Dilúvio stream, one of the main watercourses in Porto Alegre municipality, southern Brazil, receives not only rainwater, but also domestic and hospital sewage, which favors microbial growth. We investigated the presence of yeasts in water samples collected from Dilúvio stream and assessed virulence factors and the susceptibility of antifungal agents commonly prescribed for the treatment of mycoses. Samples were collected from three sites at two seasons of the year, seeded and isolated on different culture media supplemented with chloramphenicol. To identify the isolates, we performed biochemical assays, evaluated thermotolerance at 37 ºC, and conducted tests for proteinase and phospholipase activity. We also conducted tests to determine their resistance to antifungal agents, including a susceptibility test and minimum inhibitory concentration (MIC) evaluation using different drugs. Our results showed that Ascomycota prevailed among the isolates, 25% of which were potentially opportunistic yeasts. The susceptibility test revealed that the isolates were resistant to fluconazole and voriconazole, while the MIC test revealed isolate resistance to fluconazole and to itraconazole.
Keywords: Yeasts, environmental samples, antifungal agents, susceptibility test.

RESUMO: (Ocorrência e susceptibilidade de leveduras presentes nas águas poluídas do Arroio Dilúvio, Porto Alegre, Brasil). A água é um dos requisitos naturais essenciais de todas as funções da vida. Hoje, a poluição da água tornou-se um dos principais problemas para todas as formas de vida. O Arroio Dilúvio é um dos principais cursos de água em Porto Alegre- Brasil e recebe águas residuais domésticas, hospitalares e águas pluviais, o que favorece o crescimento de micro-organismos. Este estudo investiga a presença de leveduras em amostras de água coletadas do arroio Dilúvio e avalia os fatores de virulência e a susceptibilidade de agentes antifúngicos comumente prescritos no tratamento de micoses. As amostras foram coletadas em três locais, em duas estações do ano, semeadas e isoladas em diferentes meios de cultura suplementados com cloranfenicol. Para identificar os isolados foram realizados ensaios bioquímicos, termotolerância a 37 ºC, proteinase e atividades de fosfolipase. Os testes para determinar a resistência aos antifúngicos incluíram um teste de susceptibilidade e avaliação da concentração inibitória mínima (CIM) usando diferentes agentes antifúngicos. Os resultados mostraram que Ascomycota prevaleceu entre os isolados e que 25% dos quais eram leveduras potencialmente oportunistas. O teste de susceptibilidade revelou que os isolados eram resistentes ao fluconazol e voriconazol, enquanto que o teste de concentração inibitória mínima observou-se resistência ao fluconazol e itraconazol.
Palavras-chave: leveduras, amostras ambientais, agentes antifúngicos, teste susceptibilidade.

INTRODUCTION

Water is of vital importance to the development of populations and environment. Therefore, the preservation, quality, and availability of this natural resource are indispensable. The quantity and quality of water available for various uses are fundamental to the economic and social development of a city (Bertoldo *et al*. 2004). However, the impact of urbanization is a determining factor in the loss of quality and degradation of available water resources in the major urban centers (Vieira *et al.* 2006, Silveira 2000).

Contamination of water available for consumption poses a risk to public health since the use of such contaminated water is one of the main causes of hospital admissions. The lack of basic sanitation and the spread of pollutants in lakes, rivers, and streams are the main factors contributing to the spread of opportunistic and pathogenic microorganisms that can be a health risk to the population.

Yeasts are unicellular fungi that have a crucial role for humanity, with diverse applications. These fungi can be found from many environments such as soil, marine and freshwater water, free or in association with sediments, in other living organisms, extreme environments, oceans, soil and organic matter (Hagler *et al.* 1987, Gadanho *et al.* 2006). They are common inhabitants of aquatic environments, and their density and species diversity depend on the water type and purity.

Several studies have reported the occurrence and the characterization of yeasts in drinking water, bottled mineral water, tap water, as well as in waters sampled in rivers and lakes that are part of supply systems.

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These investigations show the importance of knowing the microbiological diversity in these environments (Kanzler *et al.* 2008, Medeiros *et al.* 2008, Bradão *et al.* 2010, Yang *et al*. 2011, Pontara *et al*. 2011, Coelho *et al*. 2010, Ishida *et al*. 2013). Diverse previous studies have described the correlation of industrial, domestic, and agricultural contaminants present in river and lake waters to the isolation of opportunistic yeasts that are resistant to antifungal agents (Medeiros *et al.* 2008, Pontara *et al*. 2011, Medeiros *et al.* 2012).

In this scenario, the present study evaluates the presence of yeasts in water samples collected in Dilúvio Stream one of the most important watersheds in the Porto Alegre region, Rio Grande do Sul, Brazil. Virulence factors, thermotolerance at 37 °C, and susceptibility pattern to different antifungal agents were assessed to characterize potentially opportunistic yeasts in this environment.

MATERIALS AND METHODS

Sample Collection and isolation

Samples were collected in September 2012 (winter) and February 2013 (summer). Collection sites were selected considering the presence of hospitals along Dilúvio Stream. Collection points were: (1) Crossing of nearby Ipiranga Avenue with Érico Veríssimo Avenue; (2) Santana street with Ipiranga Avenue; (3) Ipiranga Avenue with Nelson Duarte Brochado Street (Fig. 1).

The watershed of Dilúvio Stream is one of the most important in Porto Alegre region, state of Rio Grande do Sul, Brazil. This importance lies in the economic and social role that this stream has in the city (Basso *et al.* 2011). The stream runs for 17,605 m, through highly urbanized neighborhoods flowing into Guaíba Lake. The stream receives domestic sewage and every year, near 50,000 m³ of silt and solid waste is leached and discharged in Dilúvio Stream (DEP 2016).

Serial dilutions were prepared, and 100 µL aliquots of each dilution and the crude sample were seeded on Petri dishes containing the culture media Sabouraud agar and YMA agar (1% glucose, 0.5% peptone, 0.3% malt extract, 0.3% yeast extract, 2% agar) supplemented with 0.2% chloramphenicol. Plates were kept at room temperature for five days. Yeasts colonies were then selected based on morphological characteristics and streaked on plates containing the same culture media. Purified samples were stored in glycerol 20% and kept at -20 ºC.

Physicochemical analysis

Water samples were kept under refrigeration and sent to a specialized laboratory for the analysis of biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total nitrogen analyses.

Characterization of yeasts

Yeasts were identified based on morphological and physiological assays as described by Yarrow (1998). Urease activity was evaluated using the solid medium in a slanted tube containing urea 10%. After inoculation incubation took place at 25 ºC for three days. Diazonium Blue B test (DBB) was conducted as described by Yarrow (1998). This assay was used to separate de filo Ascomycota from the Basidiomycota.

Thermotolerance at 37 ºC

Isolates were inoculated on GPY medium (8% glucose, 1% peptone, 1% yeast extract, 4% agar) and incubated at 37 ºC for 4 days, as described by Yarrow (1998).

Phospholipase and proteinase

The assays to detect the presence of the virulence factors phospholipase and proteinase were carried out as described by Mohan & Ballal (2008). All isolates that grew at 37 ºC were tested. Phospholipase was analyzed using a culture medium containing egg yolk. Isolates

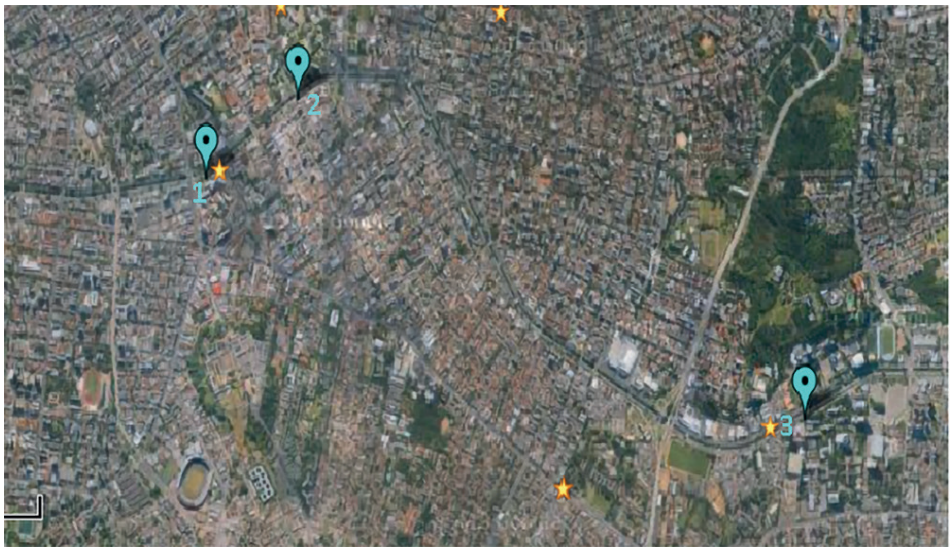

Figure 1. Location map along the Arroio Dilúvio in Porto Alegre / RS. Collection points: (1) Crossing of nearby Ipiranga Avenue with Érico Veríssimo Avenue; (2) Santana street with Ipiranga Avenue; (3) Ipiranga Avenue with Nelson Duarte Brochado Street. Source: Google Maps.

were inoculated by dot spot method. Isolates that used phospholipase produced a precipitation area around the colony and were considered positive.

Proteinase activity was evaluated using the same medium, supplemented with bovine serum albumin (BSA). Samples were inoculated in plates in the same manner as described above and kept at 37 °C for six days. After growth colonies were covered with a solution 0.1% starch black stain. A decolorization solution of distilled water with 0.1% acetic acid was used to remove the dye. The presence of a transparent halo around the colony was considered a positive result.

Susceptibility to antifungal agents

Susceptibility of isolates to antifungal agents was analyzed using the disk diffusion method and the minimum inhibitory concentration (MIC). Susceptibility was tested against fluconazole, voriconazole, amphotericin B, and nystatin according to the guidelines given by the Clinical and Laboratory Standards Institute (CLSI, M44-A2) (2009). Results were expressed as susceptible when the inhibition halo diameter (mm) was: >10 mm for amphotericin B and nystatin; ≥17 mm for voriconazole; ≥19 mm fluconazole; dose-dependent susceptible for 14-16 mm for voriconazole; 15-18 mm for fluconazole, and resistant ≤10 mm amphotericin B and nystatin; ≤13 mm voriconazole; ≤14 mm fluconazole. Interpretation criteria for fluconazole and voriconazole were based on CLSI M44-S3 (2012). For amphotericin and nystatin, interpretation criteria were developed using the manufacturer's instructions.

MIC assays were carried out following the standard M27-A3 of CLSI (2008). Sterile 96-well U-shaped microplates were used. The antifungal agents tested were fluconazole, amphotericin B, voriconazole, ketoconazole, and itraconazole. *Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as the control. Cutoff values for the results interpretation were based on MS27-S4 of CLSI (2012).

DNA extraction

Genomic DNA extraction of the isolates followed the protocol of Osorio-Cadavid et al. (2009). The cells were grown on Sabouraud broth for 16 h at 28 °C precipitated by centrifugation and resuspended in 400 µL of lysis buffer (0.15 M NaCl, 50 mM Tris-HCl, 10 mM EDTA, 2% SDS, pH 8.0) followed by incubation at 65 °C for 1 h. After that, 200 µL of 5 M potassium acetate (pH 4.8) was added, and the samples were homogenized for at least 30s and then placed in an ice bath for 30 min. Following this, the samples were centrifuged for five minutes at 14.000 rpm, and the supernatants were transferred to new tubes. Sample cleaning steps were as follows: one time extraction with chloroform; 1X phenol; 1X phenol/chloroform (1:1); and 1X chloroform/isoamyl alcohol (24:1). The DNA was precipitated with 0.1 volume of 3 M sodium acetate and 2.5 volumes of isopropyl alcohol and incubation at -20 °C for 30 min. Samples were centrifuged at 14.000 rpm for 10 min, the supernatant was discarded, and the pellet washed with 500 µL chilled 70% ethanol. The samples were again centrifuged and DNA was resuspended in 50 µL TE (10 mM Tris, 1 mM EDTA, pH 7.4).

DNA amplification and sequencing

Amplification of the ITS1-5.8S-ITS2 region was performed in a final volume of 25 µL. Reaction mixtures contained 10 pmol of each primer, ITS1 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3' (White et al. 1990), 2.5 µL of reaction buffer (10X), $MgCl_2$ 2 mM, 1 µL of each dNTP 2.5 mM, 1.0 U of Taq polymerase, 50 ng of genomic DNA and sterile deionized water. Amplification reactions were performed with initial denaturing at 94 °C for 5 min, followed by 35 cycles of 94 °C for 45 s, 53 °C for 45 s and 72 °C for 1 min, followed by a final extension of 72 °C for 5 min. Amplification products were purified with a PureLink® Quick Gel Extraction PCR Purification Kit Combo (Invitrogen) and sequenced on an ABI 3500 Genetic Analyzer with 50 cm capillaries and POP7 polymer (Applied Biosystems) at Ludwig Biotec Company. The sequences obtained were analyzed and aligned with sequences available in the database MycoBank.org (http://www.mycobank. org/) and GenBank database using BLAST (http:// www.ncbi.nlm.nih.gov).

Statistical analysis

The data obtained during collections were analyzed using the Tukey test (90% confidence level). Urease, DBB, thermotolerance at 37 °C, phospholipase, and proteinase results were evaluated using the two-way ANOVA (95% confidence level).

RESULTS

In total, 193 yeasts were isolated from water samples collected from Dilúvio Stream. Seventy-seven were isolated from samples collected in winter, and 116 from samples collected in summer. The number of isolates varied across the collection sites and seasons of the year. Site 1 showed the lower number of isolates in both collections (six isolates from winter collection and 28 from the summer). In site 2, the number of isolates remained constant in the two seasons (46). In site 3, 42 yeasts were isolated in the summer collection and 25 in the winter.

The physicochemical parameters showed an increase in BOD (46.5 mg/L) and COD (154.9 mg/L) values in winter, while the values recorded in summer were very low (<0.1 mg/L and <3 mg/L, respectively). Higher values of total nitrogen were also observed for samples collected in winter (Table 1).

In the urease and DBB analyses of the 77 isolates collected in winter and of the 116 isolates obtained from samples collected and in summer, 35% and 67.2% were negative in both assays, respectively. However, 49.3 % and 28.4% were positive for urease and negative for DBB, and only 15.7% and 7.8% were positive for both tests.

Table 1. Physicochemical analysis of Dilúvio Stream samples collected in different sites in winter and summer.

	Winter			Summer		
	Site 1	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**
BOD mg O_2/L	46.5	46.5	46.5	<0.1	<0.1	<0.1
COD mg O_2/L	154.9	154.9	154.9	<3	<3	<3
Total nitrogen mg/L	13.7	15.4	14.9	8.45	9.20	8.53

Collection sites: Site 1, Ipiranga Avenue and Erico Veríssimo Avenue crossroad; Site 2, Ipiranga Avenue and Santana Street crossroad; Site 3, Ipiranga Avenue and Nelson Duarte Brochado Street crossroad. Source: Google Maps.

Regarding the thermotolerance test at 37 °C, the number of positive yeasts for this assay was high (n = 97), when compared to the total number of isolates (n = 193). The summer collection had a higher number of positive isolates compared to winter collection 55 and 42 isolates respectively.

The phospholipase and proteinase activity for isolates that showed thermotolerance was tested. Thirty-nine isolates from samples collected in summer were positive for phospholipase and proteinase. In turn, among the yeasts isolated from winter samples, 20 were positive for the enzymes.

From the initial screening tests, urease, DBB, growth at 37 °C and virulence factors, 50 yeasts were selected to undergo the susceptibility tests to antifungal agents through the fungigrama technique and minimal inhibitory concentration. Of these samples, 17 were collected in winter and 33 in the summer. These isolates were chosen because they show relation to ascomycota potentially opportunistic and ability to present virulence factors.

In the antifungal susceptibility assay, it was observed that 18% (3/17) of the isolates were resistant to voriconazole and 23% (4/17) to fluconazole. For the antifungal agents' amphotericin B and nystatin, all yeasts showed susceptibility. Of the 33 isolates belonging to the summer collection, 6% (2/33) were resistant to voriconazole and fluconazole, 6% dose-dependent sensitivity to fluconazole and amphotericin B. All isolates were susceptible to nystatin.

The majority of the yeast in the MIC assay were susceptible to the antifungal agents tested, and only resistance to fluconazole and itraconazole was observed. Of the 50 isolates tested, 15 were resistant to fluconazole and 15 resistant to itraconazole (Table 2). For amphotericin B and ketoconazole, all yeasts tested showed sensitivity to these drugs. Similar results were observed for voriconazole, for which 82% of the isolates showed susceptibility and no case of resistance were found. However, 18% of the isolates presented dose-dependent sensitivity for voriconazole. Isolates that were dose-dependent sensitive to this drug were resistant to fluconazole and itraconazole. The exception was the I3C9 isolate that showed no resistance to fluconazole (Table 2).

The three isolates that showed the same profile of resistance in antifungal susceptibility assay and the CIM assay had their ITS-5.8 DNA fragment sequenced and were identified as *Candida glabrata* (I2B21), *Pichia cactophila* (I3B6) and *Candida parapsilosis* (V2B14). All three isolates showed resistance fluconazole.

DISCUSSION

Concern about water quality refers mainly to water for consumption and recreational activities, often disregarding environmental aspects. Microbiological indicators are used to ensure the safety of water quality for use since the presence of certain microorganisms suggests fecal contamination accompanied by other probable pathogens.

Yeasts are microorganisms that occur naturally in aquatic environments, whether permanently or transiently. Several studies indicate that increased yeast concentration accompanies increased pollution of these environments. However, little of this knowledge is employed to analyze and evaluate the quality of aquatic ecosystems (Hageskal *et al.* 2009).

The Dilúvio Stream continuously receives different kinds of waste. Oliveira *et al.* (2012) demonstrated that these waters were contaminated in the entire riverbed, though less intensity at the Riverhead.

In the present study, 193 yeasts were isolated from waters collected from a polluted watercourse in two seasons of the year (winter and summer). The number of yeasts isolated in winter was smaller than in summer, which may be associated with the increase in rainfall (273.7 mm) observed in September in the region when collections were made. This difference between the number of isolates obtained in a rainy and a dry season has been reported by Medeiros *et al.* (2008), in a study that correlated the drop in the number of yeasts isolated with increased rainfall. Moreover, the difference in the number of isolates obtained at distinct sites where collections were done may be explained in light of the oscillations in water quality, which in turn is affected by the inflow of several kinds of pollutants that directly influence microbial biodiversity in aquatic environments (Medeiros *et al.* 2008).

The physicochemical parameters analyzed in samples collected in Dilúvio Stream showed increased BOD values (Table 1) in water during the winter. Similar results were obtained by Oliveira et al. (2012), in a study that evaluated samples collected along the same watercourse, when high BOD values were observed in waters in September 2009 (35 mg/L). Besides, total nitrogen in waters

Table 2. Minimum inhibitory concentration values (CIM - µg / mL) and susceptibility profile for the 50 isolates tested against antifungal. Abbreviations: AMP-B, amphoterecin B; KCZ, ketoconazole; ICZ, itraconazole; VCZ, voriconazole; FCZ, fluconazole; S, susceptible; SDD, sensitive-dose-dependent; R, resistant.

Isolate	AMP-B		KCZ		ICZ		VCZ		FCZ	
	CIM	Profile	CIM	Profile	CIM	Profile	CIM	Profile	CIM	Profile
I1B1	0.125	S	0.062	S	0.125	S	0.125	S	4	S
I1B3	0.125	S	0.062	S	0.5	SDD	0.125	S	16	R
I2A8	0.125	S	0.125	S	0.062	S	0.062	S	4	S
I2A9	0.125	S	0.062	S	0.125	S	0.125	S	4	SDD
I2B4	0.031	S	0.062	S	0.062	S	0.125	S	2	S
I2B10	0.062	S	0.031	S	0.062	S	0.125	S	4	SDD
I2B11	0.031	S	0.031	S	0.031	S	0.031	S	0.5	S
I2B12	0.062	S	0.031	S	0.125	S	0.062	S	1	S
I2B18	0.031	S	0.062	S	1	R	0.25	SDD	8	R
I2B21	0.125	S	0.5	S	1	R	0.25	SDD	32	R
I2C5	0.5	S	0.125	S	2	R	0.25	SDD	8	R
I3A9	0.25	S	0.031	S	0.125	S	0.031	S	1	S
I3A11	0.5	S	0.062	S	1	R	0.125	S	8	R
I3B1	0.25	S	0.062	S	1	R	0.125	S	8	R
I3B5	0.125	S	0.062	S	1	R	0.125	S	8	R
I3C4	0.5	S	0.062	S	0.125	S	0.125	S	4	SDD
I3C9	0.5	S	0.062	S	1	R	0.25	S	4	SDD
V1A5	0.5	S	0.125	S	0.125	S	0.125	S	4	SDD
V1A9	0.031	S	0.062	S	0.125	S	0.125	S	4	SDD
V1A11	0.031	S	0.031	S	0.125	S	0,06	S	1	S
V1A12	0.25	S	0.25	S	1	R	0.5	SDD	64	R
V1B2	0.25	S	0.031	S	0.031	S	0.031	S	1	S
V1C2	0.031	S	0.031	S	1	R	0.062	S	8	R
V2A8	0.031	S	0.031	S	0.062	S	0.031	S	0.5	S
V2B5	0.5	S	0.031	S	0.125	S	0.031	S	0.5	S
V2B10	0.062	S	0.125	S	0.125	SDD	0.25	S	4	SDD
V2B14	0.031	S	0.0625	S	2	R	0.125	S	8	R
V2B15	0.125	S	0.031	S	0.25	SDD	0.062	S	4	SDD
V2B16	0.062	S	0.25	S	1	R	0.25	SDD	32	R
V2B17	0.5	S	0.031	S	0.125	S	0.031	S	4	SDD
V2C4	0.25	S	0.031	S	0.125	S	0.031	S	0.5	S
V2C6	0.25	S	0.25	S	2	R	0.5	SDD	16	R
V2C7	0.125	S	0.031	S	0.125	S	0.031	S	0.5	S
V2C8	0.125	S	0.25	S	1	R	0.5	SDD	32	R
V2C9	0.125	S	0.25	S	1	R	0.25	SDD	16	R
V2C11	0.062	S	0.062	S	0.125	S	0.125	S	4	SDD
V2C14	0.5	S	0.031	S	0.125	S	0.062	S	2	S
V3A1	0.031	S	0.062	S	0.125	S	0.031	S	0.25	S
V3A5	0.25	S	0.031	S	0.062	S	0.031	S	1	S
V3A6	0.125	S	0.031	S	0.062	S	0.031	S	0.25	S
V3A7	0.5	S	0.031	S	0.25	SDD	0.031	S	4	SDD
V3A8	0.25	S	0.031	S	0.125	S	0.031	S	0.5	S
V3A18	0.25	S	0.031	S	0.125	S	0.031	S	1	S
V3A21	0.25	S	0.031	S	0.125	S	0.031	S	1	S
V3A23	0.25	S	0.031	S	0.125	S	0.031	S	0.5	S
V3B3	0.031	S	0.062	S	2	R	0.062	S	8	R
V3B4	0.5	S	0.062	S	0.125	S	0.031	S	0.5	S
V3C1	0.25	S	0.031	S	0.062	S	0.125	S	1	S
V3C6	0.125	S	0.031	S	0.062	S	0.125	S	0.5	S
V3C9	0.062	S	0.031	S	0.062	S	0.0313	S	0.125	S

during winter was greater than 10 mg/L, which is the maximum acceptable level, as established by local water authorities (Portaria 05/89, Secretaria da Saúde e Meio Ambiente (SSMA) do Rio Grande do Sul). The difference in total nitrogen values may be linked to the different pollutants discharged in Dilúvio Stream (Jordão *et al.* 2007). Water quality may also be influenced by the season of the year, due to oscillations in weather parameters and volume of pollutants discharged in watercourses, which affect physicochemical variables (Bertoldo *et al.* 2004).

The initial evaluation assays showed that samples contained high numbers of urease and DBB-negative yeasts, which suggests a prevalence of yeasts classified as Ascomycota. De Almeida (2005) observed that most yeasts isolated from the waters of Tangus River in Portugal belonged to the phylum Ascomycota. The author also reported the correlation between season of the year and prevalence of Ascomycota.

The waters from Dilúvio Stream contained high numbers of yeasts that were thermotolerant at 37 °C. Numbers were especially high in summer. According to Arvanitidou *et al.* (2005), the diversity and the abundance of yeasts are influenced by temperature, with more consistent numbers being observed in regions with a tropical climate when compared to temperate zones. Moreover, the presence of yeasts thermotolerant at 37 °C is correlated with fecal pollution, which indicates that the primary source of contamination with these microorganisms is the untreated domestic sewage discharged in watercourses (Bradão *et al*. 2010, Coelho *et al.* 2010).

There is a lack of information about the expression of phospholipase and proteinase hydrolytic enzymes in yeasts isolated from the environment. In this study, only 13% of the isolates were positive for both enzymes. Studies using clinical isolates of *Candida* sp. showed that 73% of isolates exhibit the activity of proteinase and phospholipase (Mattei *et al*. 2013). Our results suggest that the yeast isolates potentially opportunistic present in the environment are less virulent. Mohan & Ballal (2008) indicate that virulence of opportunistic yeasts is not attributed to one factor alone, but to a combination of several factors. In this sense, the yeasts isolated from the waters of Dilúvio Stream may have other virulence factors.

Of the 193 isolates initially obtained from the waters of Dilúvio Stream, 50 potentially opportunistic were tested against antifungal agents. The disk-diffusion method revealed the susceptibility to amphotericin B and nystatin and resistance to voriconazole and fluconazole. Demitto *et al*. (2012) studying clinical isolates of *Candida* sp. observed susceptibility of these yeasts to amphotericin B and resistance to fluconazole and voriconazole using the disk diffusion method. And the resistance to these antifungals was confirmed in the MIC analysis. In our study, 15 isolates out of 50 were resistant to fluconazole, and the same number of isolates exhibited resistance to itraconazole in the MIC assay. Medeiros *et al.* (2012) observed the presence of opportunistic yeasts in samples collected in Doce River, Minas Gerais, Brazil. The strains isolated were susceptible to fluconazole and amphotericin B, although approximately 50% of these isolates were resistant to itraconazole. Brandão *et al.* (2010) analyzed the susceptibility profile of yeasts isolated from waters of Turvo Limpo River, Minas Gerais, Brazil, and observed that some were resistant to amphotericin B and itraconazole; however, some isolates were highly susceptible to fluconazole. The differences in susceptibility to antifungal agents may be associated with the geographical region where samples are collected and to the characteristics of the populations living in these areas (Silva *et al.* 2012).

In the present study, only three yeast isolates were resistant to one antifungal only (fluconazole) using the two techniques. Vasconcelos *et al.* (2012) compared the two methods using clinical isolates of *Candida* sp. and observed that some isolates were resistant to fluconazole in the disk diffusion method, but not in the MIC assay. However, Demitto *et al.* (2012) observed 100% correlation between the results obtained by MIC and disk diffusion, in the evaluation of resistance to fluconazole.

The results achieved in the present study demonstrate the variability in yeasts living in the waters of Dilúvio Stream. More studies are necessary to investigate the potential presence, in aquatic environments, of opportunistic microorganisms that can express resistance to antifungals.

ACKNOWLEDGMENTS

We would like to thanks, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship.

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