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Original article

Yeasts isolated from nosocomial urinary infections: Antifungal susceptibility and biofilm production

Alessandra Ribeiro de Freitas[a], Lilian Cristiane Baeza[a], Maria Graciela Iecher Faria[a], Kelen Fátima Dalben Dota[a], Patrício Godoy Martínez[b], Terezinha Inez Estivalet Svidzinski[a,*]

[a] Teaching and Research in Clinical Analysis Laboratory, Division of Medical Mycology, State University of Maringá, Maringá, Paraná, Brazil
[b] Instituto de Microbiología Clínica, Facultad de Medicina, Universidad Austral de Chile, Valdivia, XIV Región, Chile

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ABSTRACT

Background: Urinary *Candida* infections in the hospital environment are frequent and need to be better understood.
Aims: To compare the results of antifungal susceptibility profiles of yeasts isolated from patients with urinary infections obtained by broth microdilution method (BM) and by disk diffusion (DD), and also evaluate the capacity of these yeasts to form biofilms.
Methods: Only yeasts obtained from pure urine cultures with counts higher than 10^5 colony-forming units per milliliter, without bacteria development, of symptomatic patients were included. The isolates were identified by classical methods and the antifungal susceptibility tests were performed with the following drugs: amphotericin B, ketoconazole, fluconazole, itraconazole, voriconazole and caspofungin. The biofilm studies were carried out in polystyrene microtitration plates.
Results: Ninety-five yeasts isolates were analyzed, including 40 *Candida albicans*, 31 *Candida glabrata*, 24 *Candida tropicalis*. In general, the majority of the isolates were susceptible to the tested drugs but some resistance was observed, especially against fluconazole. Great variability in the antifungal susceptibility results was observed with the different tested drugs and a few discrepancies were observed between both methods. We suggest that in case of DD resistance this result should be confirmed by BM, the standard method. *C. tropicalis* isolates showed high biofilm production (91.7%) compared to *C. albicans* (82.5%) and *C. glabrata* (61.3%), with statistical significance ($p = 0.0129$).
Conclusions: Candiduria in critical patients requires major attention and a better control. The different susceptibility results obtained in this study showed the need to identify yeasts up to the species level, especially in patients with urinary tract infection. The development of techniques of antifungal susceptibility tests can help the clinicians in the empiric treatment of candiduria.

Levaduras aisladas de pacientes con infecciones urinarias nosocomiales: sensibilidad antifúngica y producción de biofilm

Palabras clave:
Candiduria
Biofilm
Microdilución en caldo
Difusión con disco
Infección urinaria

RESUMEN

Antecedentes: Las infecciones urinarias producidas por especies del género *Candida* en el ámbito hospitalario son frecuentes, por lo que se requieren mayores conocimientos.
Objetivos: Examinar los resultados de los perfiles de sensibilidad de las levaduras aisladas de pacientes con infección urinaria a los fármacos antimicóticos, comparar los resultados obtenidos con las técnicas de microdilución en caldo y difusión en agar con disco, y valorar la capacidad de estas levaduras para producir biofilm.
Métodos: Solo se incluyeron en el estudio las levaduras obtenidas a partir de urocultivos puros de pacientes sintomáticos con recuentos superiores a 10^5 unidades formadoras de colonias, sin el desarrollo de bacterias. Las levaduras se identificaron con técnicas clásicas y se realizaron pruebas de sensibilidad frente a los antimicóticos siguientes: anfotericina B, ketoconazol, fluconazol, itraconazol, voriconazol y caspofungina. Los exámenes de producción de biofilm se efectuaron en placas de microtitulación de poliestireno.

* Corresponding author.
E-mail addresses: terezinha.svidzinski@gmail.com, terezinha@email.com (T.I.E. Svidzinski).

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Resultados: Se analizaron 95 aislamientos de levaduras que incluían 40 *Candida albicans*, 31 *Candida glabrata* y 24 *Candida tropicalis*. En general, la mayoría de los aislamientos eran sensibles a los fármacos examinados, aunque se observaron algunas resistencias, en especial al fluconazol. Se observó una variabilidad considerable en los resultados de la sensibilidad a los diferentes antimicóticos examinados, detectándose algunas discrepancias entre ambos métodos de examen. Sugerimos que los casos valorados como resistentes por difusión con disco se confirmen mediante microdilución en caldo, que es el método de referencia. Los aislamientos de *C. tropicalis* mostraron una elevada producción de biofilm (91,7%) en comparación con *C. albicans* (82,5%) y *C. glabrata* (61,3%), siendo la diferencia estadísticamente significativa (p = 0,0129).

Conclusiones: Es preciso prestar mayor atención a la candiduria detectada en pacientes en estado crítico, al igual que un mejor control. Los diferentes resultados de sensibilidad a los antimicóticos obtenidos en el presente estudio demuestran la necesidad de identificar las especies de las levaduras aisladas de pacientes con infecciones del tracto urinario. El progreso de las técnicas de sensibilidad a los antimicóticos puede ayudar a los médicos en el tratamiento empírico de la candiduria.

Hospital infections are a major cause of mortality in developing countries, due to their high incidence and also the difficulty of early diagnosis.[4] Although fungi were rarely involved in hospital infections during the 1980s, nowadays they are one of the main causes. Most hospital fungal infections are caused by yeasts of the genus *Candida*, and urinary infection is one of the most common types.[2,5,25] Nevertheless, little is known about the characteristics of yeasts isolated from these infections.

No consensus exists as to whether yeasts disseminate from the urinary tract to the bloodstream; however, the association between fungal urinary-tract infections and high mortality rates is undeniable.[19] A finding of candiduria is difficult to interpret, since it may indicate only a simple colonization, or alternatively a severe infectious process.

The recent increase in candiduria has been attributed to several factors, such as the length of hospital stay, indiscriminate use of antimicrobial agents, high frequency of use of invasive devices, the patient's degree of susceptibility,[8,14,24,26,29] vaginal colonization can also be a risk to nosocomial candiduria.[12,41]

Urinary catheter use is the principal determining factor for the emergence of urinary yeast infections. The manipulation of this type of device by health professionals could facilitate the migration of yeasts to the bladder, contributing to the appearance of an infection.[21] In addition, catheters are substrates that support the formation of biofilms, which protect the microorganisms against phagocytosis and antimicrobial action.[10,16]

In some cases, candiduria may resolve spontaneously, depending on the correction of risk factors, such as rational use of antibiotics, diabetes control, and removal of catheters.[10,16,30,43] However, in some patients, the infectious condition persists, requiring treatment with antifungals, which is usually empirical. Antifungal therapy that is administered without previous determination of the agent incurs the risk of being ineffective, and could also contribute to the selection of resistant microorganisms.

Authors have shown an increase in the number of cases of candiduria due to non-*Candida albicans Candida* (NCAC) species,[12,43] and some of them are naturally resistant to azoles. This situation has made necessary to identify the yeasts involved, in order to determine not only the species, but also its susceptibility profile to the available antifungals.[4]

The objectives of this study were to compare the results of antifungal susceptibility profiles of yeasts isolated from patients with urinary infections obtained by the broth microdilution method and by disk diffusion, and also to evaluate the capacity of these yeasts to form biofilms.

Materials and methods

Organisms and culture conditions

A total of 95 yeast strains (40 samples of *C. albicans*, 31 *Candida glabrata*, and 24 *Candida tropicalis*), previously isolated from hospital patients with urinary infections in Maringá, Paraná, Brazil, were studied. After identification, the microorganisms were included in the pathogenic fungal collection (Medical Mycology Laboratory of State University of Maringá). They are maintained in Sabouraud Dextrose Broth (SDB) (Difco, Detroit, MI, USA) with glycerol at −80 °C.

Only urine samples from patients with proven urinary-tract infections, according to criteria proposed by the Hospital Infection Control Committee (HICC), were included. Criteria for inclusion were urine cultures from patients with at least one of the following signs or symptoms, without other recognized cause: fever (38 °C or higher, under antibiotics use), urinary urgency, increased urinary frequency, dysuria, or suprapubic tenderness.[15,43,46] Laboratory parameters included pure cultures for yeasts, without the development of bacteria, and with counts higher than 10^5 colony-forming units per milliliter (CFU/mL). Patients with a urinary catheter had their urine collected 24 h after the catheter was changed. Other markers utilized were detection of leukocyte esterase, nitrite-positive, and pyuria (10 or more leukocytes per mL).[43]

The yeasts were identified on the basis of their micromorphology in cornmeal supplemented with 1% Tween 80 agar and biochemical tests were performed with the commercial system ID 32C, bioMérieux Marcy l'Etoile, France.[47] To carry out the tests, the yeasts stored were inoculated in SDB and incubated at 25 °C for 48 h. Next, they were grown in Sabouraud Dextrose Agar (SDA) (Difco, Detroit, MI, USA) and in CHROMagar *Candida*™ (CHROMagar Microbiology, Paris, France) to verify the viability and purity of the colonies.

Susceptibility test

Broth microdilution "BM": The antifungals amphotericin B (Bristol-Meyers-Squibb), ketoconazole (Janssen), itraconazole (Janssen), fluconazole (Pfizer), voriconazole (Pfizer) and caspofungin (Merck) were used. Stock solutions were prepared in dimethyl sulfoxide (DMSO) or water according to the solubility of each antifungal, and then serial dilutions were carried out according to the document M27-A3.[7,31] The culture medium used was RPMI-1640 (Gibco, Detroit, Michigan, USA) buffered with morpholino propanesulfonic acid (Sigma, St. Louis, MO, USA) pH 7.0, supplemented with 2% glucose as proposed by EUCAST.[11] To prepare the inoculum, yeasts were suspended in saline solution