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Original article

Molecular characterization of *Candida* isolates from intensive care unit patients, Krakow, Poland

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ABSTRACT

Background: Over the last decades, *Candida* species have emerged as important pathogens in immunocompromised patients. Nosocomial infections are mainly of endogenous origin. Nevertheless, some cases of exogenous candidiasis have also been reported.
Aims: The aim of this study was to evaluate the genetic relatedness between *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei* and *Candida kefyr* isolates recovered from intensive care unit (ICU) patients.
Methods: A total of 132 *Candida* clinical isolates (62 *C. albicans*, 40 *C. glabrata*, 13 *C. tropicalis*, 11 *C. krusei*, 6 *C. kefyr*), obtained from specimens of endotracheal aspirate, urine and blood taken from patients of a tertiary hospital in Poland, were included in the study. Species identification was performed by PCR method and genetic relatedness was assessed by randomly amplified polymorphic DNA assay (RAPD) with five primers.
Results: The RAPD analysis revealed high genetic diversity among the studied *Candida* isolates, indicating that most of the strains were from endogenous sources. Only two clonal strains of *C. glabrata* isolated from different patients were observed, suggesting a possible cross-transmission of these pathogens.
Conclusions: Our study confirmed the high discriminatory power of the RAPD assay. This genotyping method can be applied to local epidemiological studies of *Candida* species.

Caracterización molecular de aislamientos de *Candida* de pacientes en cuidados intensivos en Cracovia, Polonia

Palabras clave:
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RESUMEN

Antecedentes: En las últimas décadas, el hongo *Candida* se ha convertido en un patógeno importante para los pacientes con trastornos del sistema inmune. Las infecciones nosocomiales son fundamentalmente de origen endógeno; sin embargo, también se han documentado algunos casos de candidiasis exógena.
Objetivos: El objetivo del estudio fue evaluar la relación genética entre las cepas de *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei* y *Candida kefyr* aisladas de pacientes en cuidados intensivos.
Métodos: Se estudiaron 132 aislamientos de *Candida* (62 *C. albicans*, 40 *C. glabrata*, 13 *C. tropicalis*, 11 *C. krusei*, 6 *C. kefyr*) obtenidos de muestras procedentes de aspirado endotraqueal, orina y sangre tomadas de pacientes de un hospital en Polonia. La identificación de las especies se realizó mediante PCR, y el estudio de la relación genética con el método de amplificación aleatoria de ADN polimórfico (RAPD) con cinco oligonucleótidos.
Resultados: El análisis de la amplificación por RAPD mostró una alta diversidad genética entre los aislamientos objeto de estudio, lo que indica que la mayoría de ellos tenían un origen endógeno. Solo se observaron dos cepas clonales de *C. glabrata* procedentes de diferentes pacientes, lo que evidencia una posible transmisión cruzada de estos patógenos.

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Conclusiones: Nuestro estudio confirma el alto poder discriminatorio de la técnica RAPD, lo que validaría este método de genotipificación para el estudio de la epidemiología local de especies de *Candida*.

In the last decades *Candida* species have emerged as important nosocomial pathogens in immunocompromised individuals with severe underlying diseases and comorbidities, such as patients treated in intensive care units (ICU).[11,23]

Candida resides as a commensal in the oral cavity, gastrointestinal tract and vagina of healthy humans. Due to prior colonization, nosocomial infections caused by these yeasts are considered mainly endogenous. Nevertheless, some cases of exogenous candidiasis, occurring as a result of dissemination of *Candida* species in the hospital environment, have also been reported.[3,5]

Candida albicans remains the predominant species recovered from clinical specimens worldwide. However, in the last two decades other species of this genus have become increasingly prevalent. The distribution of *Candida* varies according to the geographic areas. The most commonly observed non-*C. albicans Candida* species in studies from Central and Northern Europe is *Candida glabrata*.[2,8,15,26,34] The other species are observed less frequently, although it has been reported that infections due to *Candida tropicalis* have increased on a global scale.[20] Also the frequency of isolation of *Candida krusei* in Poland and other several Eastern European countries is much higher than in other geographic areas.[27] Furthermore, new emerging pathogenic *Candida* species have been described: *Candida dubliniensis* and *Candida africana*, phenotypically similar to *C. albicans*, as well as *Candida nivariensis* and *Candida bracarensis*, closely related to *C. glabrata*. These pathogens can be easily misidentified by conventional diagnostic methods, because there is only little evidence for differences between their phenotypes.[12]

In most cases, *Candida* identification to the species level allows to predict their drug susceptibility. There is no transfer of resistance genes between yeasts. The acquisition of resistance is observed mainly in restricted clinical settings, due to prolonged antifungal treatment. Different susceptibility patterns to antifungal agents among the species is of particular concern. The increase of non-*C. albicans Candida* species isolation is accompanied by a higher proportion of isolates, which are frequently resistant to fluconazole. *C. krusei* has an inherited resistance to fluconazole and has been recognized as a potentially multidrug-resistant (MDR) fungal pathogen, due to its decreased susceptibility to both 5-fluorocytosine and amphotericin B.[27] *C. glabrata* isolates are also more resistant to fluconazole.[14] Several identified strains of *C. nivariensis* and *C. bracarensis* were resistant to azoles or amphotericin B or exhibited a decreased susceptibility to these antimicrobials.[6,16]

Understanding the local epidemiology of *Candida* is of great relevance for the clinical management and treatment of candidiasis, especially in critically ill patients. Therefore, it is important to determine the prevalence and diversity of *Candida*, as well as the phenotypic and genotypic characteristics of these pathogens.[10]

From a variety of molecular techniques, PCR-based approaches for molecular typing are applied with most success. They are simple, as well as rapid and cost-effective. The above-mentioned advantages of PCR techniques and their accuracy make them suitable tools for epidemiological investigation of *Candida* species on both global and local levels.[1] The aim of this study was to evaluate the genetic relatedness among selected *Candida* isolates, recovered from the ICU patients of a tertiary hospital in Krakow, Poland over a 4-year period, by randomly amplified polymorphic DNA assay (RAPD).

Material and methods

Patients and strains

One hundred and twenty three patients with multi-organ failure were enrolled into the study. They were hospitalised between 2009 and 2012 at the Intensive Care Unit of Ludwik Rydygier Memorial Hospital in Krakow. The study population consisted of 74 (60.2%) males and 49 (39.8%) females with the average age of 64 years (range 18–93) from south-eastern Poland. Antifungal prophylaxis had not been administered to the patients included in this study.

The predominant fungal species isolated in the study period were *C. albicans* (52.6%) and *C. glabrata* (16.2%), followed by *C. tropicalis* (6.7%), *C. krusei* (5.3%) and *Candida kefyr* (3.1%), whereas other *Candida* species were isolated less frequently (<2%). For the genotyping analysis we selected approximately 40% of all *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. kefyr* isolates recovered from endotracheal aspirate (ETA), urine and blood from ICU patients of a Krakow hospital in the years 2009–2012 (Table 1).

A total of 132 clinical isolates (62 *C. albicans*, 40 *C. glabrata*, 13 *C. tropicalis*, 11 *C. krusei* and 6 *C. kefyr* isolates) were included in the study. One hundred and twenty *Candida* isolates (55 *C. albicans*, 36 *C. glabrata*, 13 *C. tropicalis*, 11 *C. krusei* and 5 *C. kefyr*) were obtained from ETA, while nine (6 *C. albicans* and 3 *C. glabrata*) were taken from urine. These isolates were cultured at a concentration $\geq 10^4$ CFU/ml, indicating that the fungal colonization was high. Furthermore, one isolate each of the species *C. albicans*, *C. glabrata* and *C. kefyr* were recovered from blood. Each isolate was obtained from different patients, with the following exceptions: *C. albicans* CA1 and CA3, as well as CA46 and CA47 strains, were acquired from ETA and urine of one subject. Both *C. albicans* and one isolate each of the species *C. glabrata*, *C tropicalis*, *C. krusei* and *C. kefyr* were cultured from seven of the studied individuals.

The study was approved by the Ethics Committee of Jagiellonian University Medical College (KBET/129/B/2011).

Phenotypic characterization of strains

Endotracheal aspirate and urine specimens were cultured by quantitative technique on Sabouraud glucose agar (SGA) (bioMérieux, Marcy l'Etoile, France) and incubated at 37 °C for 48 h. Fungal concentration of the samples were inferred between 10^4 CFU/ml and 10^6 CFU/ml after 48 h of incubation.[24] The yeasts recovered from blood were detected in cultures using the automated BacT/ALERT system (bioMérieux) as a routine hospital

Table 1
Distribution of the *Candida* species isolated from ICU patients of Ludwik Rydygier Memorial Hospital in Krakow from ETA and urine (at concentration $\geq 10^4$ CFU/ml) and blood samples, between 2009 and 2012.

Species	Total no. of strains isolated between 2009 and 2012	No. (%) of strains included into the molecular studies
C. albicans	169	62 (36.7)
C. glabrata	74	40 (54.1)
C. tropicalis	41	13 (31.7)
C. krusei	29	11 (37.9)
C. kefyr	15	6 (40.0)
Total	328	132 (40.2)