Candida and other yeasts of clinical importance in Aseer region, southern Saudi Arabia

Presentation of isolates from the routine laboratory setting

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ABSTRACT

الأهداف: عزل وتمييز وتحديد مدى انتشار المبيضات والخمائر الأخرى ذات الأهمية في منطقة عسير بالسعودية.

الطريقة: اشتملت هذه الدراسة المستعرضة تحليل 6100 من العينات المقدمة إلى مختبر الأحياء الدقيقة، مستشفى عسير المركزي، أبها، المملكة العربية السعودية خلال الفترة ما بين 2011و 2012 وعزل وتحديد 84 خميرة معزولة من مختلف العينات الاكلينيكية التي عرضت على مختبر الأحياء الدقيقة بين 2012م و 2013م باستخدام النظام الآلي فيتيك 2.

النتائج: أظهرت نتائج التحليل بالأثر الرجعي خلال الفترة ما بين (2011م– 2012م) أن من بين 6100 عينة مختلفة إلى مختبر علم الأحياء الدقيقة وجود 143 (2.35%)من أنواع المبيضات. توزيع المبيضات (عدد 143) وفقا للعينات الاكلينيكية كما يلي: البول 72%؛ البلغم 10.5%؛ أنبوب القصبة الهوائية 7%؛ الدم 4.2%؛ القسطرة 2.1%؛ مسحة الحلق 2.1%؛ مسحة العين 0.7%؛ إفرازات الجروح 0.7% والسائل النخاعي 0.7%. أشارت نتائج الدراسة خلال الفترة ما بين (2012م– 2013م) عزل الخمائر من 84 عينة وهي المبيضة البيضاء (28.6%)، تليها المبيضة المرطية21.4% والمبيضة المدارية 14.3% ، المبيضة اللوسيتانية 9.5% .

الخاتمة: كشفت الدراسة نسب عالية من المبيضة المرطية والمبيضة المدارية والمبيضة اللوسيتانية بالإضافة إلى المبيضة البيضاء الأكثر شيوعاً. تبين وجود العديد من أنواع المبيضات وبعض الخمائر الأخرى للمرة الأولى في المنطقة، هذا وقد وجد أن عينات البول هي المصدر الرئيسي للمبيضات التي كشف عنها في هذه الدراسة.

Objectives: To isolate, identify, and determine the prevalence of *Candida* and other yeasts of clinical importance in Aseer region, Saudi Arabia.

Methods: This is a cross-sectional study involving retrospective analysis of 6100 samples submitted to the Microbiology Laboratory, Aseer Central Hospital, Abha, Saudi Arabia between 2011 and 2012, and prospective isolation and identification of 84 isolates recovered from various clinical specimens presented to the Microbiology Laboratory between 2012 and 2013 using the classic morphological schemes and the Vitek 2 automated system.

Results: The results of the retrospective analysis (2011-2012) indicated that of the 6100 various clinical specimens submitted to the routine microbiology analysis, 143 (2.35%) revealed the presence of *Candida* spp. The distribution of the 143 *Candida* spp. according to specimens was as follows: urine 72%, sputum 10.5%, endotracheal tube 7%, blood 4.2%, catheter tip 2.1%, throat swab 2.1%, eye swab 0.7%, wound exudates 0.7%, and cerebrospinal fluid 0.7%. The results of the prospective study (2012-2013), which involved the identification of yeast recovered from 84 specimens indicated that *Candida albicans* 28.6% was the predominant species, followed by *Candida parapsilosis* 21.4%, *Candida tropicalis* 14.3%, and *Candida lusitaniae* 9.5%.

Conclusions: Along with the commonly encountered *Candida albicans*, *Candida parapsilosis, Candida tropicalis,* and *Candida lusitaniae* were detected with significant rates. Many other *Candida* species and some other pathogenic yeasts have been detected for the first time in the region. Urinary tract samples were the main source of *Candida* species.

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Fungal diseases notably those due to candida have become an increasing risk to human health. This is particularly true among patients with immune compromised systems.[1,2] *Candida* and *Aspergillus* species are the most common agents associated with invasive fungal infections.[3] *Candida* infections like other fungal infections are believed to be opportunistic in nature, since some aspects of the host's defense system is impaired in some way. On the contrary, *Candida* infections manifest in a variety of forms ranging from superficial skin conditions, onychomycosis, oral, vaginal infections to fatal invasive illnesses that involve vital body organs such as heart, lungs, and central nervous system.[1,2] Candidiasis, notably candidemia continues to be a major cause of morbidity and mortality in the health care settings. Moreover, the epidemiology of *Candida* infection is changing.[4,5] *Candida* species are frequently encountered as part of the human commensal flora. Colonization mostly paves the way to candidemia and is considered an independent risk factor for the development of candidemia.[1,4] The frequency of nosocomial bloodstream infections by *Candida* species has risen dramatically in the past 2 decades. It has been found that more than two-thirds of patients with invasive candidiasis in ICUs have candidemia. Of these isolates, the non-albicans *Candida* species constituted about half of the isolates and death from these invasive ICU infections was notable.[1] There is a lack of sufficient literature showing in a systematic way, the incidence of fungal infections in the Kingdom of Saudi Arabia. Available data indicated that fungal infections, generally, represent approximately 10% of reported laboratory diagnosed infections; whereas gram-positive organisms (10%), gram-negative organisms (32%), and the remaining 48% were polymicrobial.[6,7] The aims of this study were to isolate, identify (prospectively), and to determine the prevalence (retrospectively) of *Candida* infections and other yeasts of clinical importance in Aseer region, Saudi Arabia.

Methods. This is a cross-sectional study involving firstly, a retrospective analysis of 6100 samples submitted to the Microbiology Laboratory, Aseer Central Hospital, Abha, Saudi Arabia between February 2011 and January 2012. Clinical and microbiology data of positive cases were collected. Secondly, a prospective analysis was undertaken, which included the isolation and identification of strains presented to the laboratory from October 2012 to November 2013. This was carried out using initial phenotypic identification based on morphological and culture characteristics[8] followed by confirmation using the Vitek 2 automated system. Samples included in this study were the ones with complete clinical records, requests from the relevant wards, and samples that met the criteria of submission. Samples that did not meet the above mentioned criteria were excluded from the study.

Ethical approval. This research was approved by the Research Ethics Committee, College of Medicine, King Khalid University, Abha, Saudi Arabia.

Isolation of yeasts. Fungal cultures were carried out on sabouraud dextrose agar (SDA) and Brain Heart Infusion Agar + 5% sheep blood (BHIA) plates. Inoculated plates were incubated at 30°C and examined daily for up to 10 days for yeast growth.

Identification of yeasts. Identification of yeasts encountered during routine bacteriological cultures or from SDA and BHIA plates was performed using conventional growth and colonial morphology criteria.[8]

Confirmation of identification of Candida spp. by VITEK 2 system. The VITEK 2 automated system was used for confirming identities of Candida species following protocols described by the manufacturer (bioMérieux Inc., Durham, NC 27712, USA). The VITEK card consists of 64 wells that contain various fluorescent biochemical tests. Of these, 20 are carbohydrate assimilation; 4 are phosphatase, urea, nitrate, and actidione tests. When a test result is recorded as "low discrimination," this means that the result is doubtful. In such cases, supplementary tests were carried out manually to resolve such uncertain findings. These supplementary tests were: microscopic detection of blastospores or arthrospores, apiculated cells, capsule, carotenoid pigment, convoluted colony, hyphae or pseudohyphae, sporangia, growth at 37°C, and growth without oil.

The VITEK 2 device handled card automatically from filling, sealing then transferring them into the connected incubator (35°C). The cards are filled automatically every 15 min by a fluorescence system. Each output profile is decoded as per a specific algorithm. The obtained results were equated to the ID-YST (identification of yeasts) database. This, in most of the known yeast with clear cut profile, led to a correct identification of the unknown yeast.

Results. *Retrospective analysis of 6100 samples.* Out of the 6100 various clinical specimens, 143

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(2.35%) revealed the presence of *Candida* spp. When the observed *Candida* infections proportion was 2.35%, the confidence intervals for the sample size was ±0.38. The distribution of the 143 *Candida* spp. recovered from the 6100 clinical specimens are shown in **Figure 2**.

Isolation and conventional identification of yeasts. Eighty-four yeasts were isolated in pure forms using SDA and BHIA. Yeasts were tentatively recognized on the basis of their morphologies, which are: colonies with white to cream colored, smooth, glabrous, and yeast-like in appearance (**Figure 1A**). Microscopically, they exhibit spherical to subspherical large yeast-like cells with budding, blastoconidia, and pseudohyphae, or both (**Figure 1B**). These were considered for confirmatory identification.

Identification of yeasts using Vitek 2. Eighty-one of the totally isolated 84 yeasts were successfully identified using the Vitek 2 system. The results of the identification system using the Vitek 2 system were as follows: *C. albicans* was the predominant species (28.6%), *C. parapsilosis* (21.4%), *C. tropicalis* (14.3%), and *C. lusitaniae* (9.5%). Other species encountered are shown in **Figure 3**.

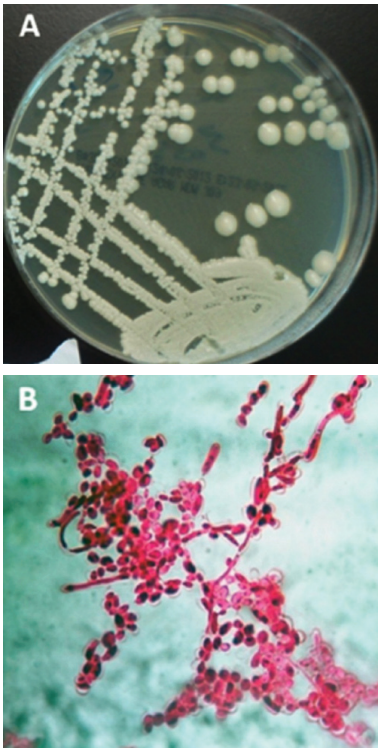

Figure 1 - Growth of *Candida albicans* on sabouraud dextrose agar **A)** Colonies with white to cream colored, smooth, glabrous, and yeast-like in appearance. **B)** Microscopic appearance of *Candida albicans* showing spherical to subspherical budding yeast-like cells or blastoconidia (Safranin stain x 100).

Discussion. *Candida* is an important opportunistic fungus, once barriers are broken down, infection and dissemination may occur with fatal consequences. The clinical signs and symptoms are nonspecific, and the routine laboratory methods are insufficient. This insufficiency is at least true when it comes to identifying the unknown "species level". As we have seen this in 143 isolated strains (**Figure 1**), all isolates were designated as "*Candida* spp." Our current recognition rate is lower (2.35%) than rates published from other parts of Saudi Arabia[7] or from other countries.[9] The incidence of candidemia is growing with the increasing complexity of surgical procedures, the existence of populations at higher risk of infections, and the changes in patient demographic characteristics. Approximately 80% of fungal infections in health care settings are due to *Candida* spp. Nosocomial represents approximately 93.6% and the community acquired 6.4% with 5-71% mortalities, mainly from invasive candidemia.[1,7,9] The overall incidence of candidiasis has a 5-fold increase in the past 10-years, and *Candida* spp. are currently between the fourth and the sixth most common nosocomial bloodstream isolates according to American[10] and European studies.[11] The present study recorded a prevalence of 2.35% for candidiasis in Aseer Central Hospital. This figure, though lower, it can be compared with the rates in other countries: Turkey 6.6%,[9] 10% in Scotland and Wales,[5] 1.7-10 episodes per 100,000 inhabitants in Spain,[11] and 5.79% in India.[12] In the present study, *C. albicans* was the predominant species (28.6%) (**Figure 3**). The prevalence of *Candida* spp. according to previous studies: Kingdom of Saudi Arabia,[13] Spain,[14] Brazil,[15] and France[16] observed variably high rates of *C. albicans* followed by *C. parapsilosis, C. glabrata, C. tropicalis, C. krusei, C. pelliculosa, C. guilliermondii*, and other yeasts.

The distribution of *Candida* spp. according to specimen types are shown in **Figure 2**. When data collected from Aseer Central hospital (2011-2012) was compared with the study from India[17] with the same period, we observed that there are some variations. Urine was the main source of *Candida* infections (72%) in Aseer, followed by the respiratory tract (4.2%) compared with 10.8% (urine) and 30.6% (respiratory tract) in India.[17] The respiratory tract (44.1%) followed by blood (30.6%) represented the highest body sites

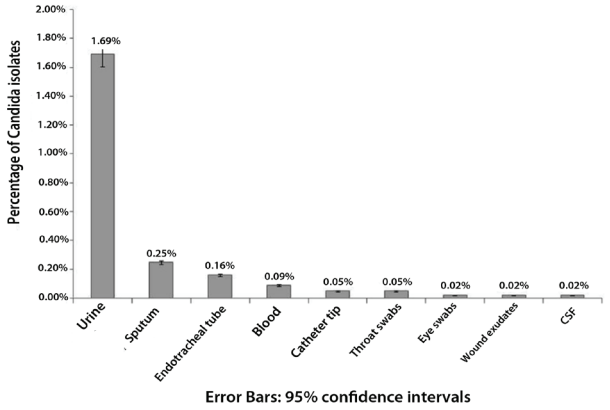

Figure 2 - Distribution of the 143 *Candida* spp. recovered from 6100 various clinical specimens analyzed retrospectively between 2011 and 2012 among patients from Aseer Central Hospital, Abha, Kingdom of Saudi Arabia.

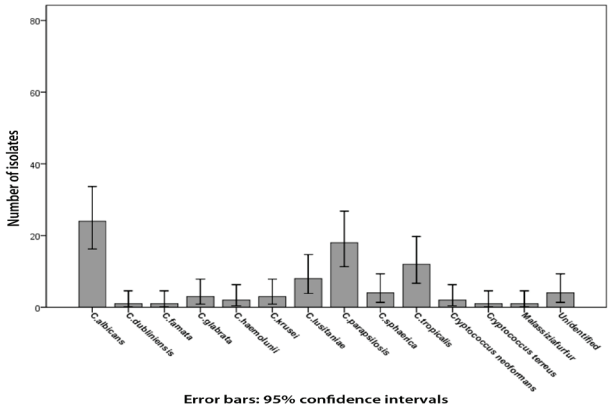

Figure 3 - Distribution of *Candida* spp. and other yeasts recovered from 84 clinical specimens among patients from Aseer Central Hospital, Abha, Kingdom of Saudi Arabia between October 2012 and November 2013 using the Vitek 2 automated system.

from which *Candida* was isolated in the Indian study compared with 17.5% and 4.2% in Aseer region in the present study.

The limitations of this study are represented by the fact that neither risk factors nor the *in vitro* sensitivity profiles of the isolates have been determined. However, in the retrospective study, the isolation of 143 *Candida* species was successful, but obviously our routine microscopic and colony morphology characterization practice were not sufficient to reveal species names other than *C. albicans*. This deficiency has been averted when the Vitek 2 identification system was applied in the prospective analysis of the 84 samples. The latter enabled us to uncover many species of the genus *Candida* and other yeasts as well.

The present study showed the implication of yeasts notably *Candida* in causing clinical diseases in different body systems especially the urinary tract. Future microbiological screening studies should consider these yeasts, the need for their early diagnosis, and determining their *in vitro* antimicrobial sensitivities to facilitate correct treatment. Clinicians are informed to consider empiric treatment in risk groups notably among those with the prolonged antibiotic (bacterial) therapy, frequent surgical interventions, frequent instrumentation, the immune-compromised patients, and the extensive use of intensive care facilities.[1,6] In all these patients at risk, cultures should be rationally performed.

In conclusion, many *Candida* species; namely: *C. parapsilosis, C. tropicalis, C. lusitaniae, C. sphaerica, C. glabrata, C. krusei, C. haemolunii, C. dubliniensis, C. famata*; and some other yeasts such as *Cryptococcus terreus* have been recorded from patients admitted to Aseer Central Hospital. Unlike other studies, urine and not blood samples were the main source of *Candida* species. Aside from *C. albicans,* other species encountered with significant rates were: *C. parapsilosis, C. tropicalis,* and *C. lusitaniae*.

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