Candidemia and the susceptibility pattern of *Candida* isolates in blood

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ABSTRACT

Objective: *Candida* species has become one of the most common blood isolates as well as one of the leading causes of nosocomial bloodstream infections. The purpose of our study was to determine the prevalence of *Candida* species among our bloodstream infecting organisms and the susceptibility pattern of the *Candida* isolates to antifungal agents.

Methods: A prospective study was carried out in the Division of Microbiology, King Khalid National Guard Hospital, Jeddah, Kingdom of Saudi Arabia of all positive blood cultures for *Candida* species. The study took place from 1st January 1998 to March 2002. Identification and susceptibility pattern of isolates were determined by the Candifast technique to amphotericin B, fluconazole, nystatin, Flucytosine, econazole, ketoconazole and miconazole.

Results: Over a 2-year period, 17,916 blood cultures were performed in our hospital. There were 2,972 positive cultures, of which 83 (2.8%) patients had *Candida* species isolated from their bloodstream. Of these, 38 (46%) were *Candida albicans (C.albicans)*. The remaining 45 strains were made up of *Candida tropicalis* 9 (10.8%); *Candida parapsilosis* 9 (10.8%); *Candida species* 9 (10.8%); *Candida guilliermondi* 6 (7.2%); *Candida krusei* 5 (6%); *Candida glabrata* 4 (4.8%); *Candida pseudotropicalis* 2 (2.4%) and *Trichosporon species* 1 (1.2%). All *Candida* species were susceptible to amphotericin B. However, only 18 (47%) out of 38 *C.albicans* were susceptible to fluconazole, while only 8 (17.7%) of 45 non-*C.albicans* strains were susceptible to this drug.

Conclusions: The susceptibility of *C.albicans* to fluconazole in our hospital using the Candifast method is very low (47%). These results need to be confirmed by carrying out the Etest or the NCCLS M27-A method to confirm the true susceptibilities of *Candida* strains in our locality.

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The emergence of *Candida* species as important nosocomial pathogens in the Intensive Care (ICU) and Oncology Units has been a dramatic recent development affecting critical care medicine.[1,2] During the past decade, the number of such infections, especially in non-neutropenic ICU and Oncology Unit patients has risen significantly. In many hospitals, *Candida* is the fourth most common blood isolate, the fourth leading cause of nosocomial bloodstream infections, and the fourth most commonly cultured organism from all sites in the ICU.[3,4] Overall, *Candida* species have been reported to account for approximately 7% of nosocomial bloodstream infections.[5,6] The increased incidence of *Candida* infection is directly attributable to widespread colonization of hospitalized patients and represents a serious concern in the ICU. The recent European Prevalence of Infection in Intensive Care (EPIC) study found that fungal isolates were reported with a surprisingly high frequency and accounted for 17% of pathogens isolated from ICU patients.[7] In the United States, the National Nosocomial Infections Surveillance System surveyed 115 hospitals from January 1980 to December 1990. The data collected was used to determine trends in fungal infections and to describe the epidemiology of nosocomial infections. Nosocomial fungal infections were defined as all infections from which *Candida albicans (C.albicans)*, other species of *Candida*

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Aspergillus and *Torulopsis*, or other fungal pathogens were recovered.[8] This survey confirmed an increase in the proportion of nosocomial fungal infections reported by all hospitals, from 6% in 1980 to 10.4% by 1990 at all major sites of infection (surgical wound, lung, urinary tract and blood stream).[8] As far as we are aware there has been only one publication on fungal blood stream infections in the Kingdom of Saudi Arabia (KSA), but no susceptibility pattern of the isolates were described.[9] The purpose of our study was to determine the prevalence of *Candida* species and other fungal organisms among our bloodstream infecting pathogens and the susceptibility pattern of the yeast isolates to common antifungal agents.

Methods. A prospective study was carried out in the Microbiology Department of King Khalid National Guard Hospital, Jeddah, KSA of all positive blood cultures for *Candida* species and fungi between 1st January 1998 and March 2002. The King Khalid National Guard Hospital is a 400-bed tertiary medical institution, which caters for the National Guardsmen and their families as well as some Saudi citizens in the Jeddah-Makkah area of KSA. Blood cultures submitted to our laboratory are processed in a BacT/Alert cabinet (Organon Teknika, Durham, NC.) Blood culture bottles flagged positive by the BacT/Alert cabinet are subjected to gram stain of the material and then plated on sheep blood agar, chocolate, and MacConkey agar and incubated overnight. Yeast isolates identified by the gram stain are subcultured on to Sabouraud's agar for the germ tube test. Identification and susceptibility pattern of *Candida* and fungal isolates were determined by the Candifast kit (International Microbio. Parc d'activites, Allee d'Athenes, 83870, Signes, France) to amphotericin B, fluconazole, nystatin, flucytosine, econazole, ketoconazole and miconazole. All the tests were performed according to manufacturer's instructions and results interpreted as indicated in the product package insert. Basically, the Candifast test tray consists of 2 8-well rows. The first row (the identification row) contains 7 different sugars, the fermentation of which produces a color change of the phenol red indicator. The first well of this row contains phenol red, acti-dione and glucose. The second row is the susceptibility row. The first well of this row is a growth control well and contains glucose. Wells 2 to 8 contain glucose and each with an antifungal agent as in **Table 1**. The wells are inoculated with standardized inocula and covered with 2 drops of paraffin oil. The tray is incubated at 37°C and the results read at 24 and 48 hours. The identification result of the Candifast was compared with the College of American Pathologists (CAP) Mycology Quality Control surveys and they were found accurate and correct. As this survey does not contain antimicrobial susceptibility determinations, the accuracy of the susceptibility test could not be compared with the CAP survey. However, Gundes et al[10] in a comparative performance of Fungichrom I, Candifast and API 20C Aux systems in the identification of clinically significant yeasts found the performance of the Candifast system to be 82.7%; 96 out of 116 yeast isolates were correctly identified.[10]

Results. Between 1st January 1998 and March 2002, 17,916 blood cultures were performed in our hospital. There were 2,972 (16.6%) positive cultures. Of the positive isolates, 83 (2.8%) patients had *Candida* species isolated from the bloodstream. Duplicate isolates from the same patient were excluded from the analysis. Of the 83 positive yeast cultures, 38 (46%) were *C.albicans*. The remaining 45 strains were made up of *Candida tropicalis (C.tropicalis)* 9 (10.8%); *Candida parapsilosis (C.parapsilosis)* 9 (10.8%); *Candida species* 9 (10.8%); *Candida guilliermondi (C.guilliermondi)* 6 (7.2%); *Candida krusei (C.krusei)* 5 (6%); *Candida glabrata (C.glabrata)* 4 (4.8%); *Candida pseudotropicalis (C.pseudotropicalis)* 2 (2.4%) and *Trichosporon species* 1 (1.2%). All *Candida* species were susceptible to

Table 1 - Susceptibility pattern of *Candida* isolates to common antifungal agents.

Species/antifungals	Number of strains susceptible							
	AB	**NY**	**FCT**	**EC**	**KTZ**	**MCZ**	**FCZ**	**Total no. of Strains**
C.albicans	38	37	31	4	25	14	18	**38**
C.guilliermondi	6	6	3	0	2	0	0	**6**
C.krusei	5	5	5	0	4	1	1	**5**
C.parapsilosis	9	9	9	3	9	6	6	**9**
C.pseudotropicalis	2	2	2	0	1	0	0	**2**
C.glabrata	4	4	4	0	0	0	0	**4**
C.tropicalis	9	9	9	1	5	1	1	**9**
Candida spp.	9	9	4	0	3	0	0	**9**
Trichosporon	1	1	0	1	0	0	0	**1**
Total	**83**	**82**	**67**	**9**	**49**	**22**	**26**	**83**
% Susceptible	**(100)**	**(99)**	**(81)**	**(11)**	**(59)**	**(27)**	**(31)**	

C. - Candida, AB - Amphotericin, NY- Nystatin, FCT - Flucytosine, EC - Econazole, KTZ - Ketoconazole, MCZ - Miconazole, FCZ - Fluconazole

amphotericin B. However, only 18 (47%) out of 38 *C.albicans* were susceptible to fluconazole, while only 8 (17.7%) of 45 non-*C.albicans* strains were susceptible to this drug (**Table 1**). The susceptibility of all strains tested to the various antifungal agents is shown in **Table 1.**

Discussion. Modern medical technology has made it possible to treat patients at the extreme of life. The rise in nosocomial fungal infections has been attributed to advances in medical techniques and the increased use of immunosuppressive drugs. The increasing use of broad-spectrum antibiotics, total parenteral nutrition, intravenous cannulae, prolonged stay in the ICU, abdominal surgery, compromised immune status and immunosuppressive drugs have led to a marked increase in the incidence of invasive fungal infections.[2,11] Candidal species normally reside on the skin and on the mucosa. They are normal commensals in the gastrointestinal tract and alterations in the other members of the bowel flora by antibiotics may predispose; otherwise, normal hosts to infection across the bowel wall into the systemic circulation. A common iatrogenic route of infection is via intravenous cannulae; particularly central lines inserted for parenteral nutrition. Disorders in cell-mediated immunity are associated with severe or recurrent mucocutaneous candidosis, whilst neutropenia or impaired neutrophil function is associated with invasive candidosis. Poorly controlled diabetics are predisposed to *Candida* infections because of increased carriage and reversible neutrophil dysfunction. The major risk factors for the development of systemic fungal infections are multi-organ failure, hemodialysis, colonization of *Candida* species at more than one site, central venous catheters used commonly in ICUs, neutropenia, severe underlying disease, and multiple antibiotic therapy leaving many patients susceptible to fungal superinfection.[2,6]

In the present study, *C.albicans* was the most common species isolated accounting for 46% (38/83) of the 83 isolates. This is similar to the findings of Richet et al,[12] who found 53% of their isolates were *C.albicans*. Similarly in the surveillance program (SENTRY) of blood stream yeast infections in the United States of America, Canada, Latin and Europe it was found that overall 55% of fungemia was due to *C.albicans*, followed by *C.glabrata* and *C.parapsilosis* (15%) and *C.tropicalis* (9%) and miscellaneous *Candida* species (6%).[13] Most studies worldwide found that *C.albicans* was the most commonly isolated species in blood stream infections.[14] However the only study of Candidemia in the Kingdom from the Eastern Province, found that *C.parapsilosis* was the most frequently isolated species (44%) followed by *C.tropicalis* (25%) and only 19% with *C.albicans*.[9] It is probable that peculiar local factors may be responsible for this high frequency of isolation of *C.parapsilosis* in the Eastern Province. Some reports have suggested that geographical differences in species distribution of Candida do occur.[5]

The rapid detection and identification of *Candida* species in clinical laboratories are extremely important for the prompt treatment of patients with hematogenous candidiasis. The blood culture systems commonly used in many laboratories (Bactec series, BacT/Alert and so forth) such as ours takes 24-48 hours to flag a positive blood culture. Regrettably, it has been estimated that 40-60% of patients with proven hematogenously disseminated candidiasis have negative blood cultures.[5] The biochemical method for species identification and susceptibility testing takes another 48-72 hours.[14,15] Consequently, many patients with candidemia might have died by the time laboratory diagnosis is made.[16] This provides the justification of starting empiric anti-candida therapy in high-risk patients suspected of candidemia. Presumptive anti-fungal therapy in high-risk patients should be initiated as soon as possible if fatality is to be prevented.[16] Nucleic acid amplification assay, and antigen detection methods may improve the detection rate and decrease the time needed for the detection and identification of *Candida* species.[15,17]

This study confirms the view that in ICUs and Oncology Units, high-risk patients should have optimum surveillance, prevention of colonization and appropriate treatment of these life-threatening infections.[12] Candidemia with septic shock is not common in non-immunocompromised patients but has a high mortality rate, a high likelihood of associated multiple organ failure and possibly a delayed recovery from multiple organ failure.[6] The susceptibility of *Candida* species to antifungals can be determined by a number of methods, of which the reference method is the broth microdilution method (NCCLS M-27-A method). However, it is complex, laborious and time-consuming for a clinical laboratory. Other methods that have been used in clinical laboratories are the Etest strip, disk diffusion method, Sensititre Yeast one, Candifast, and Integral System Yeast.[18] There has been considerable variation in the concordance of these tests with the gold standard with regards to the susceptibility testing of fluconazole, ranging from 27.4% for Candifast[19] to 83%.[20,21] The Candifast method used in this study is rapid and less laborious with a short hands-on time. After inoculating the corvettes of the kit with a suspension of the isolate, it is incubated for 24 hours and the identification of the isolate is read. The kit is further incubated for another 24 hours, when the susceptibility can now be read. However, this kit does not allow for controls being incorporated into the test system, although it has its own controls.

Forty-six percent (38/83) of the Candida strains causing blood stream infections were *C.albicans*. Although *C.albicans* is the most frequently isolated species in our center, non-albicans species (45/83; 54%) are emerging as a significant cause of candidemia in our Institution, as in many centers. There has been considerable variation in the reported susceptibility of *C.albicans* from blood stream infections to fluconazole

in various studies ranging from around 62.8%.[14,22-24] Geographical variation has been observed with regards to the susceptibility pattern of *C.albicans* isolated from different locations. The susceptibility of *C.albicans* to fluconazole in our hospital using the Candifast method is very low (47%), which does not support its use for empirical therapy, while identification and susceptibility results are being awaited. Furthermore, the majority of non-*C.albicans* isolates were not susceptible to fluconazole. Several reports attest to the fact that attributable mortality was significantly higher in patients infected with fluconazole resistant yeasts.[24] Consequently, it is important that the susceptibility of *Candida* species causing blood stream infections must be determined. Larger institutions in the Kingdom should complete identification and susceptibility determinations of their local strains to have an informed judgment for empiric antifungal therapy. Our results need to be confirmed by carrying out the minimum inhibitory concentration studies on these strains, by the NCCLS M27-A method or the Etest (which requires detail attention to procedural technique) to confirm the true susceptibilities of these strains in our locality.[25]

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