CHROMagar Candida in identification of four Candida species and detection of its antifungal resistance in immunocompromised patients.

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Presented by

Ahmed Gad Taha Mahmoud
M.B.B.ch
Faculty of Medicine, CairoUniversity
2004

Under the supervision of

Prof. Manal Mohamed Yassin
Professor of Microbiology & Immunology
Faculty of Medicine, Ain Shams University

Dr. Mona Hamed El -Shokry
Assistant Professor of Microbiology & Immunology
Faculty of Medicine, Ain Shams University

Dr. Ghada Metwally Tawfick El-Gohary
Assistant Professor of Internal Medicine
Faculty of Medicine, Ain Shams University

Ain Shams University
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Dedication to

The sole of my father

My loving& caring Mother

My Dear sisters

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Faculty of Medicine, Ain Shams University
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Abbreviations

ABC	atp-binding cassette.
ALS	agglutinin-like sequence
AMB	amphotericin B.
BDG	(1-3)-β-D-glucan.
BIGGY	Bismuth sulphit glucose glycine yeast.
BSA	Bovine serum albumin agar.
CAGT	C.albicans germ tube.
CLSI	Clinical and laboratory Standards Institute.
CMI	Cell mediated immunity .
CsLm	Confocal scanning laser Microscopy .
Cv	Crystal violet.
ELISA	Enzyme Linked immunosorbent assay.
FC	Flucytosine.
Fish	Fluoresent in-situ hybridization.
Ft-IRM	Fourier transform – infrared microspectroscopy.
HIV	human immunodeficiency virus.
IL	Interleukin.
Mab	Monoclonal antibody.
MDR	Multidrug resistance gene.
MH-GMB	Mueller-Hinton agar supplemented with glucose and methylene blue.
MICs	Minimal inhibitory concentrations.
MIS	Microbial identification system.
NAC	Non albicans candida.
NCCLS	National committee for Clinical laboratory Standards.
NPV	Negative predictive values.
PCR	polymerase chain reaction
PL	phosoholipases.
PPV	Positive predictive values.
REA	Restriction enzyme analysis.
S-DD	Susceptible-dose dependent.
SEM	Scanning electron Microscope.
SAPs	Secreted aspartic protienases.

Introduction
&
Aim of the work

I-Introduction

Candida species (spp.) are important nosocomial pathogens in critically ill patients and are associated with substantial mortality and prolonged hospitalization in the intensive care unit (ICU). Candida albicans accounts for the majority of cases with candidemia, but an increasing number of infections due to non-albicans candida. have been reported. The most commonly isolated (NAC) were C. glabrata followed by C.tropicalis, C. parapsilosis, C. krusei, and other Candida spp ***(Bassetti et al, 2008).***

Longer duration of parenteral nutrition, greater number of hemodialysis days, and bacteremia with enteric pathogens were identified as risk factors for blood stream infections with either C. albicans or non-albicans Candida spp. ***(Glück, 2008).***

Greater number of transfusions, major surgery before ICU admission, and gastrointestinal procedures were additional independent risk factors for blood stream infection with non-albicans Candida spp., whereas major surgery during the ICU stay was an additional risk factor for blood stream infections with C. albicans ***(Glück, 2008).***

Candida spp. are the fourth most common pathogens isolated from blood cultures and the numbers of (NAC) with decreased susceptibility to anti-fungal agents are also increasing. Rapid identification of yeast isolates to the spp. level is essential in order to optimize the antifungal treatment ***(Pfaller et al, 2003).***

In clinical laboratories, isolation of Candida spp. is generally based on the culture of specimens on Sabouraud dextrose agar medium. This strategy does not allow spp. identification on primary culture and makes it difficult to detect mixed cultures. Chromogenic media contain substrates that react specifically with different Candida spp. partly overcome these difficulties ***(Gaschet et al, 2008).***

CHROMagar Candida is a selective nutritive medium for the isolation and presumptive identification of yeast and differentiation of C. albicans, C. tropicalis, C.glabrata and C. krusei. Due to the differences in morphology and colors of the yeast colonies, this medium facilitates the detection of mixed yeast cultures in specimens. It may also be used as a selective isolation medium instead of Sabouraud Dextrose Agar or similar media. Hence allow rapid treatment and decrease mortality ***(Eraso et al, 2006).***

Testing Candida spp. against azole antifungal agents has provided valuable information for treatment of patients with invasive yeast infections. For instance, while C. albicans is susceptible to fluconazole, C. glabrata isolates are frequently resistant to this antifungal agent ***(Krcmery et al, 2002).***

Aim of the work

- To use the CHROMagar media as a method for isolation & identification of four Candida species (Candida albicans, C.glabrata, C. tropicalis and C. krusei) from blood cultures.

- To detect the resistance of these four species to Antifungal agents.

Review of literature

A-Genus Candida

Taxonomy of Candida

Lagenbeck, 1839 was the first to demonstrate yeast like fungus. The fungus was named Odium albicans by **Robin, 1853**. ***Berkhout, 1923***. Proposed the generic name Candida to include these fungi which develop a pseudomycelium and reproduce by budding. There are 166 species in the genus Candida, but only a small proportion of these are found in man ***(Barnett et al, 1990).***

Candida is classified into C. albicans which is the most common opportunistic yeast and NAC as Candida krusei, Candida tropicalis, Candida parapsilosis and Candida guilliermondii ***(Odds, 1988).***

Several non-albicans Candida species are known to be pathogenic and are responsible for disease in man ***(Barnett et al, 1991).*** Candida grows as typical 4 to 6 µm, budding, round or oval yeast cells under most conditions and at most temperatures. Under certain conditions including those found in infections they can form hyphae ***(Ryan, 1994).***

The genus candida belongs to the order Saccharomycetals within the class Hemiscomycetes into phylum Ascomycota. The genus contains approximately 200 species ***(Diezmann et al, 2004).***

There are characteristics that distinguish Candida species as an ascomycetous. For example, they are urease negative,

encapsulated fermentative, non inositol assimilative, make B-gluans in their cell wall and do not produce starch or carotinoid pigments ***(calderone, 2002).***

The genus Candida is composed of an extremely heterogeneous group of organism that grows as yeasts. Most members of the genus also produce a filamentous type of growth (pseudohyphae). C. albicans and C.dubliniensis form true hyphae (germ tubes) and thick-walled cells referred to as chlamydosopores ***(Jabra-Rizk et al, 2004).***

Habitat and ecology

Candida species can be present in clinical specimens as a result of environmental contamination of the urine specimen or colonization of the lower urinary tract or indicative of true invasive infection of the upper and/or lower urinary tract ***(Carvalhi et al, 2001).***

All areas of gastrointestinal tract can have Candida, from which the commonly isolated species is C.albicans (50-70%), followed by C.tropicalis, C.parapsilosis and C.glabrat ***(warren and hazen, 1999; spicer, 2000).***

C.albicans is part of normal microbial flora that colonizes mucocutaneous surfaces of the oral cavity, gastrointestinal tract and vagina of the healthy human host ***(Newman et al, 2005).***

C.albicans infection or colonization are mostly endogenously acquired while C.parapasilosis infection has environmental origin

and C.tropicalis infection can be either endogenous or environmental ***(Vrioni et al, 1999).***

C.glabrata occurs as a saprophyte on the human body and colonizes multiple sites preceding infection. The portals of entry include the respiratory tract, the genitourinary tract and wounds ***(Peltroche-liaeshanga et al, 1999).***

Virulence Factors of Candida

Candida possesses an array of virulence traits, which may contribute to the severity of symptomatic infections in hosts with impaired defense mechanisms and may account for the development of symptomatic episodes in healthy individuals ***(Tavanti et al, 2004).***

Analysis of candida virulence factors facilitate the clarification of critical aspects of the host pathogen interaction, which may result in development of better options for the therapeutic and/or diagnostic interventions ***(Kamran et al, 2004).***

A number of recognized virulence factors have been suggested in the enhancement of candida pathogenesis, these include adhesion factors, yeast to hyphal form transition, phenotype switching and the secretion of hydrolytic enzymes, such as aspartyl proteinases and phospholipases ***(Cheng et al, 2005).***

I- Adhesion factors

Adherence to a host and the subsequent aggregation of the infected cells serve as initial and critical steps in establishment of C.albicans as a commensal inhabitant or pathogen ***(Verstrepen and klis, 2006).***