Ain Shams University
Faculty of Pharmacy
Microbiology & Immunology dept.

In Vitro Appraisal of Potential Association between Some Virulence Factors and Resistance to Antifungal Agents in *Candida albicans* Recovered from Different Clinical Specimens

A Thesis

Submitted in Partial Fulfillment of the Requirements for the

Master's Degree

In Pharmaceutical Sciences

(Microbiology and Immunology)

By

Houdaii Housam El-Houssaini Ahmed Khalil

Bachelor of Pharmaceutical Sciences,

Faculty of Pharmacy, Misr International University, 2011

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Houdaii H.

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LIST OF ABBREVIATIONS

5- FC	5- Flucytosine
ABC	ATP-binding cassette
Als	Agglutinin- like sequence
AMB	Amphotericin B
ATCC	American Type Culture Collection
BG	β-Glucan
BMD	Broth microdilution
BSA	Bovine serum albumin
C. albicans	*Candida albicans*
C. glabrata	*Candida glabrata*
C. krusei	*Candida krusei*
C. parapsilosis	*Candida parapsilosis*
C. tropicalis	*Candida tropicalis*
CDR	*Candida* drug resistance
CLSI	Clinical and laboratory standards institute
CLT	Clotrimazole
Co.	Company
CSH	Cell surface hydrophobicity
DD	Disk diffusion
DMSO	Di methyl sulfoxide
ECM	Extracellular matrix
ELISA	Enzyme- linked immunosorbent assay

Eno1p	Enolase1
ESCMID	The European Society of Clinical Microbiology and Infectious Diseases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FLU	Fluconazole
g	Gravity
h	hours
Hsp	Heat shock protein
Hwp1	Hyphal wall protein1
Hz	Haemolysin index
I	Intermediate
Ig	Immunoglobulin
LIP	Lipase
MALDI - TOF MS	Matrix-assisted laser desorption/ionization-time of flight mass spectrometry
MATH	Microbial adhesion to hydrocarbons
MCF	Micafungin
MDR	Multidrug resistance
MFS	Major facilitator superfamily
MICs	Minimum inhibitory concentrations
Mn/A-Mn	Mannan Ag/anti-mannan Ab
MOPS	Morpholinepropanesulfonic acid
NYS	Nystatin
OD	Optical Density
ODc	OD cut-off value
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction

PL	Phospholipase
Prz	Protease index
Pz	Phospholipase index
R	Resistant
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RPMI	Roswell Park memorial Institute
Saps	Secreted aspartyl proteases
spp.	Species
r_s	Spearman correlation coefficient
RTqPCR	Real-time reverse transcriptase quantitative PCR
S	Sensitive/ Susceptible
SDA	Sabouraud's dextrose agar
SDB	Sabouraud's dextrose broth
SDD	Susceptible dose- dependent
SEM	Scanning electron microscopy
SOD	Superoxide dismutase
TCA	Trichloro acetic acid
UK	United Kingdom
USA	United states of America
VOR	Voriconazole
YEPD	Yeast extract peptone dextrose
+ve	Positive
-ve	Negative
°C	Degree Celsius

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ABSTRACT

Background: *Candida albicans* remains the most frequent pathogen of nosocomial fungal infections due to several virulence determinants involved in its pathogenesis. Moreover, antifungal resistance has increased dramatically, narrowing the few available therapeutic options due to their potential toxicity. Nevertheless, correlation between resistance profiles and virulence patterns of *C. albicans* is not very well- investigated, in addition to the impact of antifungal therapy on such virulence attributes.

Objectives: Based on this information, the present study was carried out to explore the potential associations between resistance profiles and virulence patterns of *C. albicans* clinical isolates, as well as their potential correlation with the source of clinical specimens. Moreover, this study addressed the effect of subinhibitory concentrations of selected antifungal agents on some virulence factors of *C. albicans* clinical isolates, since common antifungal agents may disturb the production of secreted hydrolases as well as biofilm formation.

Methods: *Candida* spp. isolates (n= 107) were recovered from different clinical specimens (vaginal swabs, urine, sputum and others) and identified to the species level using standard phenotypic methods. Antifungal susceptibilities of isolates were performed against amphotericin B, nystatin, clotrimazole, fluconazole, voriconazole, and micafungin according to Clinical and Laboratory Standards Institute M27-A3guidelines. Virulence patterns including secreted hydrolases: (phospholipase, aspartyl protease, and haemolysin), cell surface hydrophobicity and biofilm formation were evaluated. Correlations between resistance profiles and virulence patterns of tested *C. albicans* isolates, in addition to their potential association with the source of clinical specimens were analyzed. Phenotypic virulence patterns including secreted hydrolases (phospholipase, aspartyl protease and haemolysin) and biofilm formation were evaluated in the presence of subinhibitory concentrations of nystatin, fluconazole and micafungin.

Results: Ninety isolates (84%) of the *Candida* spp. collected were confirmed to be *C. albicans.* All tested *C. albicans* isolates (100%) were sensitive to amphotericin B and nystatin and 98.9% of them were micafungin susceptible. On the other hand, high resistance rates were detected against clotrimazole, fluconazole and voriconazole as estimated to be 87.8, 95.6 and 93.3%, respectively. Phospholipase, aspartyl protease, and haemolysin activities were detected in almost 58, 57 and 100% of the tested isolates, respectively. Moreover, *C. albicans* isolates recovered from urine samples showed the highest phospholipase and aspartyl protease production in comparison to other groups. Haemolytic activity was evident in all tested isolates regardless of the clinical specimen source. Cell surface hydrophobicity and biofilm formation were shown in approximately 13 and 11% of the isolates, respectively. *C. albicans* isolates recovered from the miscellaneous followed by sputum groups showed the highest hydrophobicity levels as well as biofilm forming capacities as compared to other groups.

There are significant (p <0.05) negative correlations between fluconazole resistance and aspartyl protease, cell surface hydrophobicity and biofilm formation. Moreover, there are significant (p <0.05) negative correlations between voriconazole resistance and aspartyl protease as well as cell surface hydrophobicity. In addition, source of clinical isolates showed significant (p <0.05) influence on some resistance and virulence patterns. Nystatin and clotrimazole resistance profiles are significantly (p < 0.05) different across different sources groups with the highest resistance rates detected in *C. albicans* isolates recovered from sputum, as compared to the other source groups. In addition, aspartyl protease activity is significantly (p < 0.05) different across different sources groups with *C. albicans* isolates recovered from urine samples having the highest levels of aspartyl protease production as compared to the other source groups.

Furthermore, treatment of clinical *C. albicans* isolates with subinhibitory nystatin, fluconazole and micafungin concentrations significantly (p <0.05) decreased production of extracellular hydrolases. Nystatin had the greatest effect on suppressing phospholipase and aspartyl protease activities. However, micafungin showed the highest effect on decreasing the hemolytic activity of treated clinical isolates. Moreover, nystatin and micafungin, but not fluconazole, had a significant (p <0.05) impact on inhibiting biofilm formation of *C. albicans* clinical isolates.