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Detection of Molecular Genetic Variation of *Candida albicans* in Diyala Governorate

A Thesis

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By

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بِسمِ اللهِ الرَّحمَنِ الرَّحِيم

"قُلْ لَا أَمْلِكُ لِنَفْسِي نَفْعًا وَلَا ضَرًّا إِلَّا مَا شَاءَ اللَّهُ ۚ وَلَوْ كُنْتُ أَعْلَمُ الْغَيْبَ لَاسْتَكْثَرْتُ مِنَ الْخَيْرِ وَمَامَسَّنِيَ السُّوءُ ۚ إِنْ أَنَا إِلَّا نَذِيرٌ وَبَشِيرٌ لِقَوْمٍ يُؤْمِنُونَ" ﴿١٨٨﴾

صدق الله العظيم

سورة الأعراف آية (١٨٨)

Supervisor Certification

I certify that this thesis entitled **(Detection of Molecular Genetic Variation of *Candida albicans* in Diyala Governorate)** was prepared by **(Sherihan Kanaan Taher)** under my supervision at the Department of Medical Microbiology/ College of Medicine/ University of Diyala, as a partial fulfillment of the requirements for the Master degree in Medical Microbiology.

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Committee Certification

We, the examining committee, certify that we have read this thesis entitled "**Detection of Molecular Genetic Variation of *Candida albicans* in Diyala Governorate** **”** was prepared by " **Sherihan Kanaan Taher** " and examined the student in its contents and that in our opinion; it is adequate for awarding Degree of Master of Science in medical microbiology**.**

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Dedication

To ....

The lord of mercy ..... Allah

The prophet Muhammad .... Blessings and peace be upon him

My Mother...my happiness

My father..... my backing in life

My husband .... the kindhearted

My lovely son ... my sweet heart

My brothers and sisters... all love and gratitude

All my family .....who encouraged me all time

I dedicate this humble work

Sherihan

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Summary

Candida species appeared as successful pathogen that causes various infections in immunocompromised patients. Recently, *Candida* species showed increasing resistance to antifungal agents, and this had contributed to high morbidity and mortality among the patients. The aim of this study is to detect *Candida* species and *C. albicans* genotypes isolated from vagina of women with signs and symptoms of vaginal candidiasis in Diyala Governorate.

One hundred vaginal swabs were taken from the period of 1st August to 30th October 2016; the samples were collected from women infected by vaginal candidiasis in Diyala Governorate. Patients were divided into groups according to their ages, vulvovaginal candidiasis symptoms such as (intense vulval pruritus, erythema, burning, and dyspareunia), and predisposing factors such as (pregnancy and contraception using). *Candida* isolates were identified morphologically in kalar hospital laboratory by culturing on Sabouraud Dextrose Agar (SDA) medium and sub cultured on CHROM agar which is selective media to identify *Candida* species. Germ tube test was done to identify *C. albicans*. Finally, DNAs of *C.albicans* isolates were extracted in Diyala medical college laboratory for polymerase chain reaction (PCR) assay by targeting 25S rDNA of transposable intron I region and analyzed by using gel image patterns of bands based on ultraviolet transilluminator band software.

The sensitivity of *Candida albicans* against Fluconazole, Ketoconazole, Miconazole, and Nystatin were determined by disc diffusion method on Muller Hinton agar media. The obtained data showed that *Candida albicans* is the predominant species (39.6 %), followed by *C.glabrata* (26.4 %), *C.tropicalis* (20.8 %), and *C. krusie* (13.2 %).The results of polymerase chain reaction study showed that *C. albicans*

genotype A with amplification product (450 bp) was the predominant (66.7%), followed by genotype B (19 %) with amplification product (840 bp), and genotype C (14.3%) with amplification product (450 and 840 bp).

The results of sensitivity test showed that all *C.albicans* genotypes (100%) were sensitive to Miconazole. In the case of Nystatin; (95.2%) of isolates were sensitive and (4.8 %) were resistant. For ketoconazole; (71.4 %) of isolates were sensitive, (23.8%) were resistant, and (4.8%) showed dose dependent sensitivity. Finally, (66.7%) of isolates showed sensitivity to Fluconazole, (28.6%) were resistant, and (4.7%) were dose dependent.

The results of this study showed that CHROM agar had a great advantage in rapid identification of *Candida* species, and Polymerase Chain Reaction assay was more sensitive in genotyping study. This study also showed that detection of *C.albicans* at the genotype level, and studying antifungal sensitivity will be very useful in the epidemiological study, treatment of infection, and preventing resistance development against antifungal drugs.

Abbreviation	Meaning
AIDS	Acquired Immune Deficiency Syndrom
bp	basepair
CAC	CHROM Agar *Candida*
CC	Cutaneous Candidiasis
CLSI	Clinical & Laboratory Standards Institute
CSH	Cell Surface Hydrophobicity
COCP	Combined Oral Contraceptive Pill
cTAB	Cetyl trimethylammonium bromide
DNA	Deoxyribo Nucleic Acid
DNTPs	DeoxyriboNucleotides Phosphates
D.W.	Distilled Water
EDTA	Ethylene Diamine Tetra Acetic Acid
GIT	Gastrointestinal tract
GTT	Germ Tube Test
HIV	Human Immunodeficiency Virus
IUCD	Intra Uterine Contraceptive Device
mg .	milligram
µg .	microgram
Min.	minutes
ml.	milliliter
µl.	microliter
mm	millimeter
mM	milliMolar
ng.	nanogram
O.D	Optical Density
PCR	Polymerase chain reaction
pg	picogram
pH	Power of Hydrogen
SDAM	Sabouraud Dextrose Agar Medium
spp.	Species
STD	Sexually Transmitted Disease
TBE	Tris/Borate/EDTA
TE	Tris EDTA
UV	Ultraviolet
VVC	Vulvovaginal Candidiasis
YPD	Yeast Peptone Dextrose

Chapter One

Introduction

Introduction

The genus *Candida* is responsible for fungal infections in immunocompromised patients and hospitalized patients with underlying disease (Kumamoto, 2011; Brenda *et al*., 2014). *Candida* species usually colonize the skin and mucous membranes of human and animals (Ruhnke *et al*., 2011; Udayalaxmi *et al*., 2014). There are about 350 diverse *Candida* species, but a few cause human diseases (Ruhnke *et al*., 2011; Williams *et al*., 2011).

Candida albicans causes the majority of infections. However, non-*albicans* caused an increase in the rate of infections in many parts of the world (Nishikaku *et al*., 2010; Brandit and Lockhart, 2012; Quindos, 2014), among them *C.glabrata* and *C.parapsilosis* which appeared as important opportunistic pathogens that infect human (Gizachew *et al*., 2013; Daiichi *et al*., 2014; Zahra *et al*., 2014). Other non-*albicans* species were less likely found in clinical samples although many reports described infections caused by these uncommon *Candida* species (Brandt and Lockhart, 2012).

Vulvovaginal candidiasis (VVC) is a common presentation of candidal infection; 50% of women may be infected at least once throughout their life, and about 40-50% of them will show further episodes (Foxman *et al.,* 2000; Erdem *et al.,* 2003). Isolation of *Candida* spp. from vaginal samples showed that they can be presented in 20-50% of healthy women during their reproductive period, but they showed a marked decrease after the menopause age (Sobel, 1997; Ferrer, 2000). *C.albicans* normally inhabits in the vagina. It coexists with the beneficial bacteria such as *Lactobacillus* spp. which interfere with the adherence of *C.albicans* to vaginal epithelial cells (Witkin and Giraldo, 2000).

It was recorded that *C. albicans* constitutes about 80-90% of the isolated yeast species from women with VVC (Ben-Haroush *et al.*, 2004;

Boselli *et al.,* 2004). In some conditions, such as prolonged administration of antibiotic therapy, reduced immunity, use of steroids, pregnancy, use of contraceptives, malnutrition, diabetes, and obesity; *C.albicans* becomes pathogenic and causes infection (Okungbowa *et al.*,2003; Grigoriou *et al.*, 2006).

Clinical symptoms of vulvovaginal candidiasis include itching, erythema and edema of the vulva, white discharge from the vagina, and pain during sexual intercourse (Paulitssch *et al.* 2006). Vaginal candidiasis can be diagnosed by routine techniques such as conventional culture, and biochemical tests, but these are time-consuming and may not give accurate results (Hospenthal *et al*., 2006).

Molecular techniques such as (PCR) are more rapid and have high sensitivity and specificity than conventional techniques (Goswami *et al*., 2006). Detection of *C. albicans* genotypes is important in epidemiological studies. For this objective McCullough and his colleagues (1999) evolved a polymerase chain reaction (PCR) method by using a primer pair prepared especially to target 25S rRNA gene, the site of the transposable intron (group I). In this method *C. albicans* was classified into five genotypes according to the size of amplified PCR products: genotype A (450) bp, genotype B (840) bp, genotype C (450 and 840) bp, genotype D (1,080) bp, and genotype E (1,400) bp (McCullough *et al*., 1999; Tamura *et al*., 2001; Bii *et al*., 2009). Studying *Candida* species and *C.albicans* genotypes is important for correct treatment because there is a difference in response to same antifungal treatment (Shivanand *et al*., 2011; Haddadi *et al*., 2014).

It was founded that there was a significant increase in the incidence of yeast infections in humans (Themistoklis *et al.,* 2011). The increase of infection rate by *Candida* species and the excessive using of antifungal drugs have led to resistance to these drugs over the past few decades

(Pappas *et al*., 2009). Ultimately, this led to increasing in the morbidity and mortality (Shivanand *et al*., 2011)

This study aimed at:

1. Isolation and identification of *Candida* species from the vagina of women with VVC.
2. Studying the distribution of *Candida* species in different age groups, contraceptive (users and non –users), and in different VVC symptoms.
3. Detecting of *C. albicans* genotypes causing VVC by conventional PCR method, and studying their distribution among various patients groups such as age group, contraception use, and VVC conditions.
4. Determining of *C.albicans* genotypes′ susceptibility to commonly used antifungal drugs.