**An-Najah National University**
**Faculty of Graduate Studies**

# Genotype Comparisons of Strains of *Candida albicans* from Patients with Vaginal Candidiasis

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**This thesis was defended successfully on 9/5/2012 and approved by**

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## *Dedication*

to My dear father, mother, sisters and  my brother for their patience and encouragement, with love and respect

# *A CKNOLEDGEMENTS*

*I would like to express my sincere special thanks and gratitude to my supervisor Professor Dr. Mohammad S. Ali-Shtayeh for his encouragement, guidance, patience, and help throughout this study.*

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**إقرار**

**أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:**

# Genotype Comparisons of Strains of *Candida albicans* from Patients with Vaginal Candidiasis

# مقارنة الطرز الجينية لسلالات من فطر *Candida albicans* من مرضى مصابون ب Candidiasis المهبلي

**اقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وان هذه الرسالة ككل أي جزء منها لم يقدم من قبل لنيل أية درجة أو لقب علمي أو بحث لدى أي مؤسسة تعليمية أو بحثية أخرى.**

## Declaration

**The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.**

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**Date: .....التاريخ:**

# List of Contents

# List of Tables

# List of Figures

# List of Abbreviations

| | |
|---|---|
| **SDA** | Sabouraud Dextrose Ager |
| **YPD** | Yeast peptone dextrose |
| **GTT** | Germ tube test |
| **Kac** | Putasiun acetate |
| **EDTA** | Ethylenediaminetetraacetic acid |
| **SDS** | Sodium dodecyl sulfate |
| **EtOH** | Ethanol |
| **TE** | Tris –EDTA |
| **TBE** | *Tris/Borate/EDTA* |
| **ICU** | Intensive Care Unit |
| **RPS** | Repetitive Sequences |
| **ALT** | Alternative lengthening of telomerase |
| **PCR** | Polymerase chain reaction |
| **DNTPs** | Deoxyribonucleotides phosphates |
| **CC** | Cutaneous candidiasis |
| **VVC** | Vulvovaginal candidiasis |
| **CAC** | CHROMagar *Candida* |
| **PFGE** | Pulsed field gel electrophoresis |
| **RAPD** | Random amplification of polymorphic DNA |
| **SDW** | Sterile distilled water |

# Genotype Comparisons of Strains of *Candida albicans* from Patients with Vaginal Candidiasis

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## Abstract

**Background** *Candida albicans* is one of the most important etiological agents causing vaginal candidiasis in pregnant womem. The aim of this study was to investigate whether there are significant relations between *C. albican*s genotypes and vulvovaginal candidiasis (VVC) conditions such as ( intense vulval pruritus, erythema, burning and dyspareunia associated with a creamy discharge age), residence and predisposing factors such as ( presence of vaginal candidiasis at pregnancy, prolonged administration of antibiotics, tight clothes, and no predisposing factor).

**Methods** This study was conducted during the period from May 2010 to November 2010, The subjects of the study were pregnant women with vaginal candidiasis from Jenin area. A total of 104 isolates of *C. albicans* were isolated from 104 pregnant women with vaginal candidiasis. Patients were divided into groups according to the VVC conditions such as (intense vulval pruritus, erythema, burning and dyspareunia associated with a creamy discharge age), age, place of residence and predisposing factors. Genotypes were identified using polymerase chain reactions (PCR) targeting 25S rDNA and ALT repeat sequences of the RPS.

**Results** Fourteen genotypes were detected. Significant relation was found between genotypes and the VVC conditions such as ( intense vulval pruritus, erythema, burning and dyspareunia associated with a creamy discharge age), and patients age. However, no significant relation was found between genotypes and place of residence or predisposing factors. We found that genotype A (62.5%) had the highest frequency on the basis of amplification of 25SrDNA, while genotype IV (42.3%) had the highest frequency on the basis of amplification of RPS , and genotype A-IV (26%) had the highest frequency on the basis of amplification of 25S rDNA and RPS.

**Conclusion** A significant association exists between genotypes of VVC strains and VVC conditions such as (intense vulval pruritus, erythema, burning and dyspareunia associated with a creamy discharge age), and patients age groups in this study. On the others hand, the genotypes of VVC strains were not related to predisposing factors or patients residence. Differences in genotype distribution between our results and other studies, may be attributed to differences in study populations, demographic characteristics and geographical locations.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Epidemiology of *Candida albicans*

### 1.1.1 Introduction:

The colonization of *Candida spp.* could be endogenous or exogenous (Pittet *et al.,* 1991; Voss *et al.,* 1994; Miranda *et al*., 2009). The infection could be arised from invasion by the patient's own endogenous colonizing flora, or from the exogenous acquisition of the infecting yeast strains as reported in several outbreaks. Several studies have documented that 60-70% of patients in Intensive Care Units (ICUs) are colonized with *Candida spp.* (Pittet *et al.,* 1991; Voss *et al.,* 1994; Miranda *et al*., 2009).

Candidiasis is a primary or secondary mycotic infection caused by members of the genus candida (Anaissie *et al*., 2003). The clinical manifestations may be acute, sub acute or chronic to episodic. The infection may be localized in mouth, throat, skin, scalp, vagina, nails, bronchi, lungs or in the gastrointestinal tract or become systemic as in septicemia, endocarditis and meningitis (Rippon, 1988).

Vulvovaginal candidiasis (VVC), often referred to as a yeast infection, is a common gynecologic disease, affecting 3 out of 4 women in their lifetimes (Das *et al*., 2008). More than 40% of affected women will have 2 or more VVC episodes (Ferrer, 2000; Eschenbach, 2004; Das *et al*., 2008) and infection occurs more frequently in pregnant women. It is believed that higher estrogen levels and higher glycogen content in vaginal secretions during pregnancy increase a woman’s risk of developing VVC (Monif & Baker, 2003).

childbearing years, during their As VVC is so common in women pregnancy it is important to understand the pathology of this disease as well as the safety or risks of drugs used to treat it during pregnancy. Vulvovaginal candidiasis is caused by overabundant growth of yeast, belonging to the *Candida* species in the vaginal mucosa (Baron *et al*., 1993).

Cell mediated immunity (CMI) by T helper (Th) 1 type responses is generally considered to be associated with resistance to mucosal candidiasis, whereas Th2 type responses are associated with susceptibility to infection (Cenci *et al*., 1995; Romani *et al*., 1995; Romani *et al*., 1996).

At the vaginal mucosa, local rather than systemic immunity is critical for protection against infection, although confirmed protective roles for CMI or humoral immunity have not been established (Zakikhany *et al*., 2007).

*Candida albicans* is a commensal of the normal human microflora but can also cause a variety of infections in superficial mucosal infections including vagina (Zakikhany *et al*., 2007). *C. albicans* has to cross physical barriers such as epithelial cell layers by active penetration and/or induced endocytosis (Zakikhany *et al*., 2007). During the different stages of a *C.*

*albicans* infection and within different host tissues environments, the fungus has to express general as well as stage- and tissue-specific virulence or fitness factors (Hube, 2004).

The first step for successful colonisation of mucosal surfaces or any other tissue by *C. albicans* is adhesion. Some factors involved in adhesion also have additional roles in tissue invasion by *C. albicans* is the ability to switch between ovoid yeast and filamentous hyphal growth forms. It has been proposed that yeast cells are more suitable for dissemination whereas hyphal forms play a key role during invasion (Gow, 2002).

### 1.1.2 Etiological agents and source of infection with *Candida:*

*Candida* is a part of human flora. It becomes pathogenic when certain conditions are present and becomes opportunistic infection (Kwon-Chung & Bennett, 1992; Eloy *et al*., 2006). The major etiological agent is *Candida albicans* , whereas different *Candida* species can cause a variety of infections (Bodey, 1984; Kamiya *et al*., 2005), including *C. tropicalis, C. parapsilosis, C. krusei, C. guillermondii, C. glabrata* , and *C. kefyer* which represent many clinical forms of candidiasis. Some of these species are encountered as secondary infection to another species, for example; *C. parapsilosis* is second infection only to *C. albicans* as a cause of *Candida* endocarditis (Hickey *et al.,* 1983). Still other species of *Candida* have been occasionally isolated from clinical specimen like *C. catenulata, C. intermedia, C. lambica,* and *C. zeylanoides* so these species are therefore

not considered as agents of opportunistic infections (Crozier *et al.,* 1977; Odds, 1988).

### 1.1.3 Incidence and prevalence:

In recent years, the incidence of life-threatening mycoses caused by opportunistic fungal pathogens has increased dramatically (Barnett *et al.,* 1990; Kamiya *et al*., 2005). Many studies have showed that the prevalence of infection increased with age (Heihkila *et al.,* 1995; Murray *et al.,* 2000).

According to earlier reports, *C. albicans* was the cause of 80-95% cases of symptomatic fungal vulvovaginitis, whereas other candida species such as *C. glabrata, C. parapsilosis*., and *C. tropicalis* constitute the remaining cases (Vincent *et al*., 1995; Nolla-Sallas *et al*., 1997; Kamiya *et al*., 2005).

### 1.1.4 Predisposing factors:

Invasive candidiasis is a life threatening infection in immuno-compromised hosts such as bone marrow and organ transplant recipients, in patients receiving intensive chemotherapy treatment and in AIDS patients (Lyles *et al.,* 1999; Pfaller *et al*., 2007; Perlroth *et al*., 2007). Moreover, systemic candida infections are observed in patients with extensive surgery or burns, intensive antibiotic therapy, indwelling catheters, patients with diabetes mellitus, oral contraception, pregnancy, local warmth and moisture, skin irritance, trauma, recurrent disease and in elderly patients (Wenzel, 1995; Dean *et al.,* 1996; Richardson *et al*., 2008).

The significance of candida in the vagina of a symptomatic women between episodes of recurrent vulvovaginal candidiasis is not clear. Prevalence studies indicates that 10-55% of healthy women who are completely asymptomatic, have vaginal cultures positive for *C. albicans* (Linden *et al*., 1978; Sobel *et al*., 1993). The finding of this organism during the symptom free period could indicate previous inadequate treatment, resistance of the organism to complete eradication by drugs, insufficient use of antifungal medication or recolonization.

It requires host dysfunction to become pathogenic such as the defects caused by administration of broad spectrum antibiotics, or in the cases of neutropenia, disruption of protective barriers including catheterisation and taking advantage of impaired immunity in a debilitated patient to establish the disease (Davis *et al.,* 2000).

**1.1.5 Pathogenesis :**

The life cycle of candida is characterized by budding in which the parent noncapsulated oval blastophore gives rise to filamentous mycelium (Friedrich, 1988). The mycelium is composed of non- branching hyphae whose growth is initiated by germ tube formation. Germ tube formation is associated with adherence of Candida to epithelial cells and occurs optimally at pH less than 5.5 and at a temperature greater than 33 C° (Friedrich, 1988).

At least 18 different strains of *Candida albicans* have been identified, but there is no significant difference in pathogenesity has been found between these strains (Friedrich, 1988).

It is important to study the phenotypic variations which can switch back and forth at high frequency (Soll *et al.,* 1987).

**1.2 Clinical manifestations:**

In healthy individuals, candidiasis occurs as a result of dysfunction in epithelial barrier of normal flora. The clinical manifestations can be acute, sub acute, chronic to episodic. The location of infection is usually localized to the mouth, throat, skin, scalp, vagina, finger, nails, bronchi, lungs, or the gastrointestinal tract, or becomes more complicated in systemic septicemia, endocarditis and meningitis (Murray *et al.,* 2000).

**1.2.1: Vulvovaginal candidiasis**

The vagina is muscular passageway from external opening of the vagina to the uterus. A normal function of the vaginal walls and the cervix (opening of the uterus into the vagina) is to produce secretions that are typically watery, mucousy or milky white. These secretions help to maintain healthy conditions inside the vagina and provide lubrication during sexual intercourse, These secretions produced by the vagina and cervix can sometimes to noticed outside of the vagina (this is referred to as vaginal discharge) (Workowski et al., 2010; Sena et al., 2007).

Normal vaginal secretions and discharge change from time to time; sometimes clear, almost like water, and at other times, mucousy and whitish in color, sometimes scant and other times a larger in amount. Normal vaginal secretions and discharge contain *C. albicans* (Workowski et al., 2010).

Abnormal vaginal secretion and discharge may be caused by a number of conditions, including bacterial infections. Abnormal vaginal secretion and discharge have yellowish, grayish, or greenish as opposed to clear and white (Workowski et al., 2010; Sena et al., 2007).

*Candida albicans* is normally present in vaginal secretions and discharge if there is balance between bacteria and *C. albicans.* However under unbalanced conditions, candida growth can become excessive. This is reffered to candida or yeast infection (Murray *et al.*, 2000).

**1.3 Symptoms of VVC**

Symptoms of VVC include external dysuria, vulval pruritus, swelling, or redness. Signs include vulval oedema, fissures, excoriation, or thick curdy discharge. The vaginal pH is usually normal (Eckert *et al*., 1998; Sonnex *et a*l., 1999).

*Candida albicans* infection occurs in the vast majority (80% to 90%) of

diagnosed VVC cases (Baron *et al*.,1993). There is also a population of women (5–10%) that suffer from recurrent VVC (RVVC)(Cenci *et al*., 1995; Romani *et al*., 1995; Romani *et al*., 1996).

*Candida albicans* and other opportunistic fungal pathogens are frequent colonizers of human mucosal surfaces. They are often harmless commensals in immunocompetent individuals but may be associated with minor infections such as thrush in babies and vaginal infections in women. In immunocompromised patients *C. albicans* can cause systemic infections with high mortality rates (Pappas *et al*., 2003).

**1.4 Taxonomy of *C.albicans*:**

Kingdom: *Fungi*, Phylm: *Ascomycota,* Subphylm: *Saccharomycotina*, class: *Saccharomycetes*, order: *Saccharomycetales*, family: *Saccharomyceteae*, genus: *Candida*, species: *C. albican*s, yeasts belonging to the genus candida have emerged as major opportunistic pathogens, mainly due to the increase of immunocompromised patients (Pfaller *et al*., 1995; Wenzel, 1995; Dean *et al*., 1996; Hajjeh *et al*., 2004).

Although *Candida albicans* is the most frequently isolated species, other species, such as *C. tropicalis, C. guilliermondii, C. krusei, C. parapsilosis*, and *C.glabrata*, have increasingly been recognized as pathogens with a wide distribution(Hazen, 1995; Fidel *et al*., 1999).

**1.5 Microbiological Characteristics of *C. albicans*:** Macroscopically, colonies of candida spp. are cream- colored to yellowish. Depending on the species, their texture may be pasty, smooth or dry, wrinkled, and dull. Microscopic features show important species-related variations. All species produce blastoconidia, which may be round or elongated. Most produce pseudohyphae that are long, branched, or curved. In addition, true hyphae and chlamydospores are produced by some candida strains. Although members of the same genus, the various species present a degree of unique behaviour with respect to their colony texture, microscopic morphology on cornmeal Tween 80 agar at 25°C (Dalmau method), and fermentation or assimilation profiles in biochemical tests that help to differentiate candida from other yeasts. Commercial kits are available for rapid identification (Khan'& Gyanchandani, 1998).

### 1.5.1 Cell wall biology of *C. albicans*:

The cell wall of *C.albicans* comprises -glucans, mannoproteins, and chitin (Sullivan *et al*., 1983). The main component is carbohydrate and the wall also contains lipid and protein. Similar amounts of these polymers are found in yeast cells, germ tubes, and hyphal elements but change during morphogenesis. The chitin component of the cell wall increases notably and mycelial cells contain three times more chum as contrast to yeast cells (Shepard, 1987).

Cell wall biology is an important area of discovery. It is in or on the cell wall that antigenic factors reside, it is a site of adhesion and colonization, and a location for secretion of potential virulence factors (e.g., toxic extracellular products). It offers a target(s) for new antifungal agents exhibiting selective toxicity against fungal structures such as glucans and chitin, which are present in mammalian cells. The cell wall appears to be made up of five distinct layers (commening from plasma membrane outward) mannoprotein, 3-glucan/chitin, i-glucan, manrioprotein, and a fibrillar layer (Cassone *et al*., 1973).

In *C. albicans* here is an outer "fuzzy coat" containing the fibrillar layer. This fuzzy coat is believed to be important in overall virulence by affecting adherence and phagocytosis (Douglas, 1987).

The major structural elements of the cell wall are the β-glucans. with covalentely linked chitin leading to a secondary wall structure (Shepard, 1990).

The major antigenic component is mannoprotein (serotypes A and B of *C. albicans*). Another important feature of the *C. albicans* cell surface is the presence of receptors for the complement fragment C3iC3b, which, by being bound non-covalently to human polymorphonuclear leukocytes, impairs phagocytic uptake and enhances yeast virulence (Shepard, 1990).

### 1.5.2 Switching of *Candida albicans* and Colonial Morphology:

One additional area that has engendered significant interest in candidal biology has been termed “Switching”. *C. albicans* exhibits various colonial forms when grown in vitro. A smooth colony-forming yeast may form a proportion of colonies with rough topography when inoculated into an agar surface (Shepard, 1990).

This phenomenon of switching was first described in 1952 (Di Meena, 1952; Odds, 1988). It is a well known fact that switching may be triggered by ultraviolet irradiation. *C. albicans* once triggered into high frequency of switching mode demonstrates high rates of alteration in colonial morphology (Slusky *et al*., 1985).

The substantially high frequency and reversibility of switching, the distinct phenotypes in the two switching systems, the differences in hyphal formation, and the differences in susceptibility to antifungal drugs suggest that switching over phenomenon has a definite role in pathogenesis of candidiasis (Slusky *et al*., 1985; Soll *et al*., 1987; Shepard, 1990).

Switching may potentiate invasion and proliferation in entirely different body locations/environments, eluding immune defenses by alterations in surface antigenicity, and escaping the effects of antifungal therapy. Switching also enhances adhesion of candidal organisms to mucosal surfaces, tissue penetration, and secretion of enzymes such as proteinases and phospholipases, which may be virulent in some forms of *Candida* (Khan & Gyanchandani, 1998).

## 1.6 Identification of *Candida albicans:*

Identification to the species level of yeasts isolated from clinical isolates is often problematic for diagnostic laboratories, but it has become increasingly necessary (Koehler, 1999).

### 1.6.1 Microbiological Tests:

#### 1.6.1.1 Germ tube test (GGT):

Since the germ tube (GT) is a characteristic morphology observed only in *C. albicans,* confirmation of GT is available as a rapid method for identifying *C. albicans*(kwon-chung & Bennett, 1992; Ha *et al*., 2011).

*C .albicans* can be reliably identified in 2.5 – 3 hours hours using a germ tube test (Ha *et al*., 2011).

Traditionally, the preliminary identification of *C. albicans* is made through the use of a germ tube test (GTT) performed on a sub- cultured colony grown on SDA agar (Donald *et al*., 2008), yeast cells transforming into germ tubes in human serum at 37°C for 2.5 – 3 hours (Pappas *et al*., 2004).

#### 1.6.1.2 CHROMagar candida:

Several brands of chromogenic media have been developed to produce rapid yeast identification. These media contain chromogenic substrates that react with enzymes secreted by microorganisms producing colonies with

various pigmentations. These enzymes are species specific, allowing organisms to be identified to the species level by their color and colony characteristics. CHROMagar candida has been shown to allow differentiation of candidal yeasts by color and morphology (Odds *et al*.**,** 1994; Adam *et al*., 2010; Ha *et al*., 2011**)**.

The manufacturer currently advertises its product as able to detect and differentiate three species of candida. Per the package insert for CHROMagar candida, the product identifies *C. albicans* by growth as light to medium green colonies, *C. tropicalis* by growth as steel blue colonies accompanied by purple pigment diffusion into surrounding agar, and *C. krusei* by growth as large, fuzzy, rose-colored colonies with white edges, all after incubation for 48 h at 37°C. This media has been demonstrated to identify *C. albicans*, *C. krusei*, and *C. tropicalis* in several studies (Odds *et al*., 1994; Baumgartner *et al*., 1996**;** Bernal *et al*., 1996; Adam *et al*., 2010; Ha *et al*; 2011).

Independent groups have reported success in differentiating *C. dubliniensis* from *C. albicans*(Duane *et al*., 2006).

### 1.6.2 PCR - based techniques used for identification of *Candida albicans*:

PCR-based techniques have been adapted as tools for clinical diagnosis of candidiasis (Posteraro *et al*., 2000; Luo &Mitchell, 2002; kanbe *et al*., 2003; Kanbe *et al*., 2005).

Targeting the DNA topoisomerase II gene was suitable as a target gene not only for study of the phylogenetic relationship among candida species, but also for PCR-based identification of pathogenic fungi in species such as candida, aspergillus and dermatophytes (Kato *et al*., 2001).

It was demonstrated that PCR using a primer mix specific for the DNA topoisomerase II gene, which was identified on the basis of the numbers and the sizes of PCR products, was useful for rapid identification of the major species of pathogenic candida such as *C. albicans, C. tropicalis, C. parapsilosis* and *C. glabrata* (Kanbe *et al*., 2003; Leon *et al*., 2006).

*Candida albicans* strains have been subdivided into different biological groups based upon genetic subtypes (Tamura *et al*., 2001; Millar *et al*., 2002; Hattori *et al*., 2006; Iwata *et al*., 2006).

Several studies have supported the concept that genotypic differences among *C. albicans* isolates might be correlated with their invasive environments or different body sites (Soll *et al*., 1991; Lockhart *et al*., 1996; Lian *et al*., 2004; Hattori *et al*., 2006).

Polymerase chain reaction (PCR) amplification (Howell *et al*., 1996; Lian *et al*., 2004; Ergon & Gulay, 2005; Willinger, 2006), and restriction enzyme digestion analysis (Bart-Delabesse *et al*., 1995; Pujol *et al*., 2002; Lian *et al*., 2004) are two of the most frequently used techniques in

establishing the genotyping of *C. albicans* (Mehta *et al*., 1999; Pujol *et al*., 2002; Lian *et al*., 2004).

PCR targeting 25S rDNA, which has frequently been used for genotype analyses of *C. albicans*, allows *C. albicans* to be grouped into five genotypes A, B, C, D and E (McCullough *et al*., 1999; Tamura *et al*., 2001; Millar *et al*., 2002; Hattori *et al*., 2006; Iwata *et al*., 2006).

It has been accepted that *C. albicans* chromosomes contain characteristic repetitive sequences, each of which contains a tandem short repeating unit of 172 bp, designated ALT (Iwaguchi *et al*., 1992). The numbers of ALT repeats in the RPS vary in each chromosome, thereby leading to variation in the molecular sizes of RPSs, and these molecular characteristics of the different sizes and copy numbers of the ALT sequence are attractive for the genotyping of *C. albicans* Hattori *et al*. (2006). Recently, it has been reported that a PCR system targeting the RPS region containing the inner ALT repeat sequences was quite powerful for distinguishing *C. albicans* from its related species *C. stellatoidea* and *C. dubliniensis* (Kanbe et al., 2005). Furthermore, that a combined PCR system targeting 25S rDNA and RPS produced a high performance as a tool for *C. albicans* genotyping (Iwata et al., 2006).

## 1.7 Previous work:

Mercure (1993) reported that PCR amplification of the 25S rRNA gene of *C. albicans* from patients with candidiasis demonstrated that

genotype A was the most predominant (62.5%) of all clinical isolates in canada.

Iwata *et al* (2006) reported that PCR amplification of the 25S rRNA gene of *C. albicans* from patients with mucocutaneous candidiasis demonstrated that genotype A which comprise the majority (51.4%) of the clinical isolates followed by genotype B (27.3%) while genotype C (21.2%) was found as the third group in Japan.

Hattori *et al* (2006) reported that PCR amplification of the 25S rRNA gene of *C. albicans* from patients with superficial candidiasis demonstrated that genotype A was the most predominant (75.6%) followed by genotypes B (14.6 %), and genotype C (9.8%).

Bii *et al* (2009) reported that PCR amplification of the 25S rRNA gene of *C. albicans* from clinical sources blood, sputum, swabs, urine and catheters tips revealed that genotype A was the most predominant genotype (60%) followed by genotypes B, C and D, respectively in Kenya.

**1.8 Aims of this study**

This study was aimed to determine and compare genotypes of *Candida albicans* strains causing different conditions of vulvovaginal candidiasis (VVC) in Palestinian women in the Jenin area, and to investigate whether there are significant associations between strain

genotypes and VVC conditions, patients age, residence, and predisposing factors.

# CHAPTER TWO

# Materials and Methods

## 2.1:Study population and clinical isolates:

One hundred and four patients (104 females, aged 17 to 44 years) with vaginal candidiasis from five gynecologist clinics of primary health care in Jenin area were the subject of this study during the period of May 2010 to November 2010 .

Most patients were in non-immunocompromized status and a few of them were in imunocompromized status due to different disease.

All suspected cases were interviewed and data was recorded using specially designed questionnaires included demographic data on name, age, medical history as present disease, symptoms and predisposing factors (Appendix A).

Vaginal swaps were immediately placed in a sterile culture tubes filled with yeast peptone Dextrose (YPD) liquid medium amended with chloramphenicol (50 µg/ml). The clinical isolates were transported in ice bags to the laboratory. Sterile culture tubes with YPD broth were incubated at 37 ˚C (Shaking incubator, Human lab. Co, Korea) for 24-48 hours (APPENDIX C).

**2. 2 Isolation and Identification of *C. albicans*:**

The identification was based on physiological and morphological characteristics. Yeast cells from YPD broth medium were streaked out on Sabouraud dextrose agar plates (SDA, Oxoid, Ltd, Basingstoke UK) amended with Chloramphenicol (0.05 mg/L ) and incubated for 24-48 hours at 37 ˚C (Kown & Bennett, 1992).

Isolates with a creamy to yellowish colonies were accepted and considered as positive specimens for candida spp., while isolates with different colors colonies were rejected and considered as negative specimens for *Candida albicans* (Figure2.1).

**Figure 2.1: *C. albican* growth on SDA agar.**

Yeast cells were picked up from YPD broth medium, spread onto chromogenic agar plates and cultured for 24-48 hours at 37˚C (Oxoid, Ltd, Basingstoke, UK).

The appearance of light green colonies indicates the presence of *Candida albicans* (Figure 2.2).

**Figure 2.2:*C.albicans* growth on chromogenic agar.**

Germ tube test (GTT) was done for identification of *Candida albicans* (Kown & Bennett, 1992).

The GTT was carried out as follows. Cells of *Candida albicans* were picked up by hocking a pure colony from SDA ager lightly with a sterile swab. These cells were suspended in human serum 0.5 ml at room temperature and the loop was rubbed against the wall of the tube. Serum cultures were incubated at 37 ˚C for 2.5-3 hours.

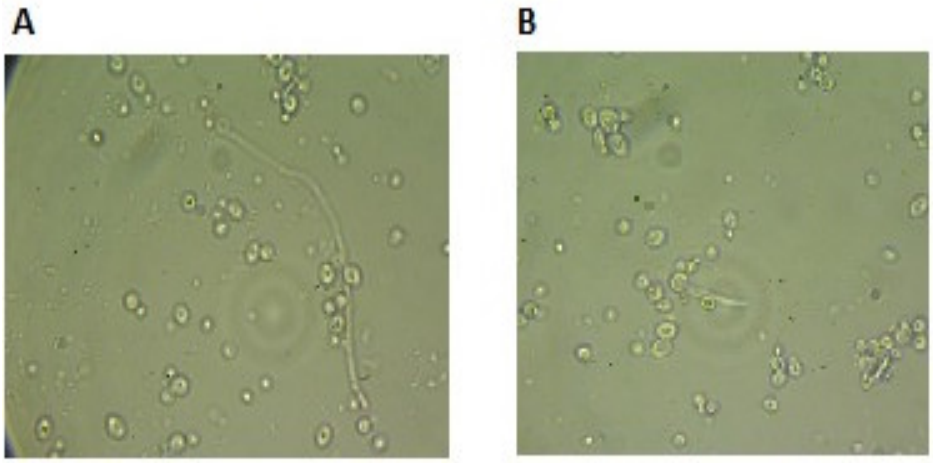


**Figure 2.3 Germ tube formation by *C. albicans.***

A drop of the serum culture was placed on a clean slide and examined under the microscope using low and high powers. Formation of germ tubes were observed in positive isolates .

Single light green colonies from each plate were inoculated to 800µl of YPD broth and 200 µl of 86 % glycerol and stored in freezer at -80 ˚C

**Total Genomic DNA Extraction from Yeast Cells: 2.3**

Total genomic DNA extraction from yeast cells was carried out as described by sambrook (2001).

One single colony of yeast from chromogenic agar was inoculated in tubes containing 5 ml YPD medium and the tubes were than incubated at 30 ˚C overnight. The tubes were Centrifuged at 3000 rpm for 5 minutes (variable speed refrigerating multiple rotor, scientific Ltd, UK). One ml of sorbitol (1 M) was transferred to 1.5ml tube. Lyticase 5u/µl 20 µl was added and incubated at 30 ˚C for 30 minutes. The tubes were centrifuged at 15000 rpm for 10 sec and the supernatant was discarded. The pellet was resuspended in 0.5 ml EDTA-SDS ( 50mM EDTA and 0.2% SDS) and incubated at 70 ˚C for 15 min.

Fifty µl of 5 M potassium acetate were added. The mixture was put on ice for 30 min and then centrifuged. The supernatant was transferred to a new 2 ml tube, and extracted with phenol -chlorophorm 2 times (vortex shortly), then centrifuged for 5 min. The supernatant was extracted1-2 times with chlorophorm.

The supernatant was transferred into a new 2 ml tube and 1 volume 100% EtOH was added. The tube was centrifuged 10 sec and the supernatant was discarded. The pellet was washed with 500 µl 70% EtOHmixedincubated at room temp for 5 min, centrifuged for 10 sec and the supernatant was discarded. Pellet was dried and resuspended in 100ul TE buffer (Tris-EDTA) for 1 hour or overnight.

Gel electrophoresis was used to check the quality of the extracted DNA samples. For DNA check, 0.8% TBE agarose gel was used. Agarose suspension was heated in a microwave oven to enable agarose melting and then cooled to 50-60°C. For visualization of nucleic acids, 50 ng/ml of EtBr were added to agarose suspension. Agarose solutions were poured into a horizontal gel trays carrying a comb for formation of wells. After gel solidification, combs were removed and gels were placed in horizontal gel chamber (Electrophoresis- Midi- horizontal, Electrophoresis unit, Jencons., UK) filled with 1x TBE buffer.

Two µl of DNA samples were mixed with 10x DNA loading dye (Fermentas) and loaded into the wells from the cathode side. Electrophoresis was carried out at 120 V using Electrophoresis power supply (Jencons, UK). Electrophoresis was stopped after 1 hour and nucleic acids were visualized on a transilluminator under UV light (TL-2000 Ultraviolet Translinker, UVP, USA) and photographed (Nikon, Thailand). One Kb DNA Ladder Mix was used as a size marker.

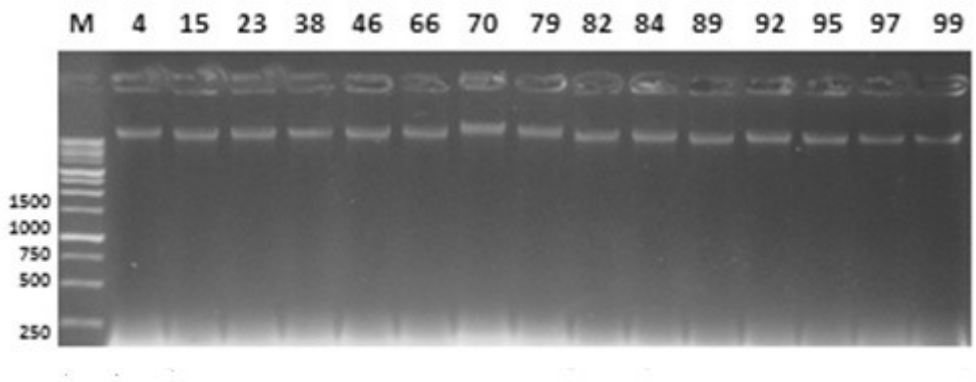


**Figure 2.4 DNA extracted from various isolates from patients with VVC. M indicates DNA ladder 1kb**

## 2.4 PCR primers :

For genotype determination of *C. albicans* on the basis of 25S rDNA, primers CA-INT-L and CA-INT-R were used (Tamura et al., 2001). The primer set CA-INT-L/CA-INT-R is referred to as P-I in this study. *C. albicans* was grouped into five genotypes on the basis of the sizes of PCR products.

Genotype D *C. albicans* corresponds to *C. dubliniensis* (McCullough *et al*.,1999; Tamura *et al*.,2001).

For typing of *C. albicans* on the basis of ALT repeats, two further primers designed on the basis of the nucleotide sequences of *C. albicans* RPS, and designated as ASDcF and pCSCR were used in this study (Chibana *et al*.,1994). The primer set ASDcF/ pCSCR is referred to as P-II in this study.

**Table 2.1: List of PCR primers and expected sizes of PCR products**

| Primer | Nucleotide sequence (5'—3') | Expected band size (bp) and 25S rDNA type |
|---|---|---|
| CA-INT-L (a) | ATAAGGGAAGTCGGCAAAATAGATCCGTAA | 450 A<br>840 B<br>450,840 C<br>1040 D<br>1080 E |
| CA-INT-R (a) | CCTTGGCTGTGGTTTCGCTAGATAGTAGAT | |
| Primer | Nucleotide sequence (5'—3') | Expected band size (bp) and ALT repeat number (b) |
| ASDcF (c) | TGATGAACCACATGTGCTACAAAG | 526 1<br>698 2<br>870 3<br>1042 4<br>1214 5<br>1386 6 |
| pCSCR (c) | CGCCTCTATTGGTCGAGCAGTAGTC | |

a Primer set CA-INT-L/CA-INT-R was specific for 25S rDNA and referred to as P-I.

b Repeat numbers of ALT sequence in PCR products were estimated to the sequences of the RPS published by Chibana et al.(1994).

The numbers of ALT repeats were used for RPS-based genotyping in this study .

c Candida Primer set ASDcF/pCSCR was specific for RPS sequences and referred to as P-II. (Iwata *et al*., 2006).

**Table 2. 2 Classification of *C. albicans* genotypes according to P-II**

| Type (RPS) | Patterns (intense band) | Band size |
|---|---|---|
| I | 1 | 526 |

| | | |
|---|---|---|
| II | 2 | 698 |
| III | 3 | 870 |
| IV | 2/3 | 698/870 |
| V | 2/3/4 | 698/870/1042 |
| VI | 3/4 | 870/1042 |

**2.5 PCR Conditions and agarose-Gel Electrophoresis:**

Genomic DNAs were amplified in a reaction mixture (25µl) containing 1 µl genomic DNA, 2.5 µl 10X buffer, 2.5 µl MgSo4, 0.5 µl dNTPs (10Mm) (2.5 U/ml; Hylab, Ltd., Israel), one µl forward primer of P-I or P-II (10mM) (Hylab, Ltd., Israel), 1µl reverse primer of P-or P-II (10mM) (Hylab, Ltd., Israel) and 0.2 µl Taq DNA polymerase (2.5 U/ µl) (Hylab, Ltd., Israel) and 16.3 µl sterile D.W (Hattori *et al*., 2006; Iwata *et al*., 2006).

All reaction mixtures were vortexed and spinned down except genomic DNA and kept on ice.

PCR cycle parameters were as follows: Preheating at 96 °C for 120 s; then 35 cycles of 96 °C for 30 s, annealing temperature 65 °C (P-I) or 60 °C (P-II) for 30s, elongation at 72 °C for 1 minute, and final extension for 5 minutes. All reaction mixtures were amplified using a thermal cycler(TC-Plus, Techne, UK).

PCR products were electrophoresed on a 1.2 % agarose gel for identification and genotyping of *C. albicans* on the basis of 25S rDNA and RPS. (Voltage 120 V for 30 minutes).

For 1.2% agarose gel preparation, 100 ml TBE buffer were added to 1.2 gm agarose, boiled in microwave, and poured in the rack of the electrophoresis chamber. The comb was put and wait until the gel was solidified the rack of the electrophoresis chamber was transferred, TBE buffer as a running buffer was added.

PCR products were loaded in the well by micropipette as follow: 3µl DNA , 2µl loading dye10x commercially available and 5 µl sterile distilled water. The first well was loaded with 5 µl 1 Kb DNA ladder.

Agarose gels were stained with 0.5 mg/ml ethidium bromide in distilled water at 21—25 °C for 20 min, and then destained in distilled water at 21—25 °C for 20 min. DNA bands were visualized with a UV transilluminator (TL-2000 ultraviolet Translinker, UVP., USA) and photographed(Camera, Nikon., thiland).

**2.6 Statistical analysis**

All statistical analyses were conducted using SPSS. 17 statistical software. The chi-square test was performed to determine the differences between the *C. albicans* genotypes and other variables such as symptoms, age, residence and predisposing factors. A $P$ value $< 0.05$ was considered statistically significant.

# CHAPTER THREE

# RESULTS

### 3.1 Study population

One hundred and four pregnant women patients with vaginal candidasis (VVC) were recruited into the study. The patients, aged 17-44 years comprised four subgroups based on the underling conditions of VVC frequency of 25S rDNA (A, B, C) genotypes among conditions (Table 3.1).

### 3.2 Strain number and *Candida* species

A total of one hundred and four isolates of candida species were obtained from pregnant women patients with VVC. Based on phenotypic identification, all isolates from patients were *C. albicans*.

### 3.3 Genotypic identification for all *C. albicans* strains by PCRs targeting 25S rDNA and the RPS from pregnant patients with VVC :

The genomic DNAs of the *C. albicans* isolates obtained from the above clinical specimens were amplified for PCR using P-I and P-II to determine the genotypes based on variations in the 25S rDNA and RPS.The PCR profiles amplified with P-I defined DNA products of 450 bp for genotype A, 840 bp for genotype B and both 450 bp and 840 bp for genotype C *C. albicans* (Figure 3.3 lanes 1, 10, 27, 67, 98, genotype A, lanes 11, 31, 60, 84, 96 genotype B, lanes 48, 91 genotype C). None of the PCR products in our study were the 1040 bp long that corresponds to *C. dubliniensis*. Of the 104 *C. albicans* isolates, 65 (62.5%), 27 (26 %) and 12 (11.5%) isolates were recognized as genotypes A, B and C, respectively (Table 3.1).

**Table. 3 . 1 Genotype variation of *C. albicans* isolated from pregnant women patients with VVC on the basis of 25S rDNA and**

| RPS | Intense Vulval pruritus (%) | | | | Burning (%) | | | | Erythemia (%) | | | | Dyspareunia (%) | | | | Totals | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | Total | A | B | C | Total | A | B | C | Total | A | B | C | Total | A % | B % | C % | Total % |
| I | 3 (18.8) | | | 3 (13.6) | | | | | | | | | | | | | 3 (4.6) | | | 3 (2.9) |
| II | 2 (18.5) | | | 2 (9.1) | | | | | | | 2 (100 | 2 (9.5) | 4 (13. | 3 (23) | | 7 (15. | 6 (9.3) | 3 (11.1) | 2 (16.7 | 11 (10.6) |
| III | | | | | | 3 (42. 8) | | 3 (18.8) | 5 (31.3) | | | 5 (23.8) | 8 (27. 6) | 2 (15. 4) | 2 (66. 7) | 12 (26. 7) | 13 (20) | 5 (18.5) | 2 (16.7 ) | 20 (19.2) |
| IV | 6 (37.5) | 1 (25) | | 7 (31.8) | | 3 (43. 8) | 4 (80) | 7 (43.8) | 11 (68.7) | | | 11 (52.4) | 10 (34. 5) | 8 (61. 5) | 1 (33. 3) | 19 (42) | 27 (41. 5) | 12 (44.4) | 5 (41.6 ) | 44 (42.3) |
| V | | 3 (75) | | 3 (13.6) | 4 (100 ) | 1 (14. 3) | | 5 (31.3) | | 3 (100 ) | | 3 (14.3) | 3 (10. 3) | | | 3 (6.7 ) | 7 (10. 8) | 7 (26) | | 14 (13.5) |
| VI | 5 (31.3) | | 2 (10 0) | 7 (31.8) | | | 1 (20) | 1 (6.3) | | | | | 4 (13. 8) | | | 4 (8.9 ) | 9 (13. 8) | | 3 (25) | 12 (11.5) |
| Total | 16 (72.5) | 4 (25) | 2 (12 .5) | 22 (100) | 4 (25) | 7 (43. 8) | 5 (31. 2) | 16 (100) | 16 (76) | 3 (14. 3) | 2 (9.7) | 21 (100) | 29 (64. 4) | 13 (28. 8) | 3 (6.7 ) | 45 (100 ) | 65 (62. 5) | 27 (26) | 12 (11.5 ) | 104 (100) |

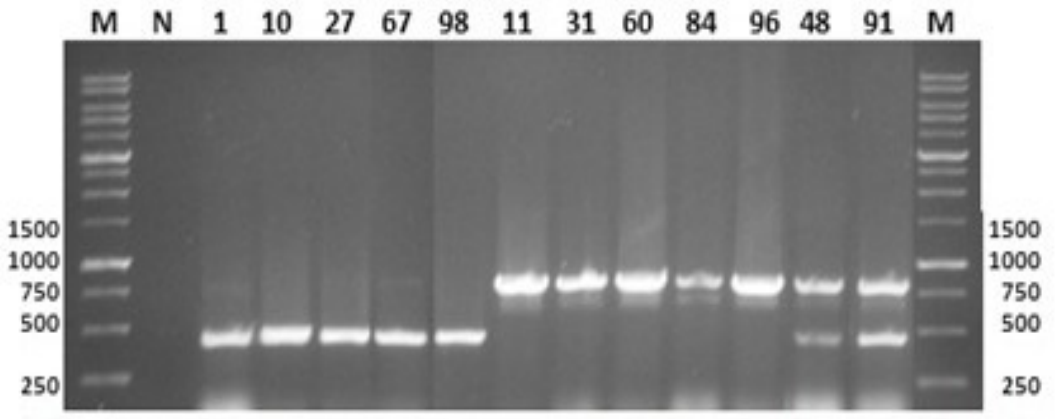


**Figure 3.1 Amplification patterns and genotyping of *C. albicans* by PCR targeting 25S rDNA. Genomic DNAs were amplified by P-I for25S rDNA - based genotyping. P-I amplifies DNA products of 450 bp for genotype A lanes (1, 10, 27, 67, 98), 840 bp for genotype B lanes (11, 31, 60, 84, 96), both 450 bp and 840 bp for genotype C lanes (48, 91). M indicates the lane containing 1 kb DNA ladder. The molecular sizes (bp) of the DNA marker are shown on the left and right sides of the panels. N refers to negative control.**

On the other hand, six genotypes were found named genotypes I, II, III, IV, V and VI based on PCR amplification of RPS profiles generated by primer pair P-II (Figure 3.2).

Table 3.1 shows the genotype variation of *C. albicans* isolated from pregnant women patients with VVC on the basis of 25 SrDNA and RPS, the highest rates of genotype A-IV in the erythema, intense vulval pruritus and dyspareunia associated with a creamy discharge 68.7%, 37.5% and 34.5%, respectively. On the other hand A-V had its highest rate in burning (100%); genotype B-III in burning (42.8%); and B-IV in dyspareunia associated with a creamy discharge (61.5%). Also B-V had its highest frequency in the erythemia (100%), followed by intense vulval pruritus (75%), and C-II had its

highest frequency in the erythema (100%), C-III in the dyspareunia associated with a creamy discharge (66.7%), and C-VI in the intense vulval pruritus (100%).

Genotype IV and VI were found to be the most frequent in intense vulval pruritus (31.8%), genotype IV was found to be the most frequent in erythema (52.4%), burning (43.8%), and dyspareunia associated with a creamy discharge (42%). Genotype IV comprised the highest frequency of all RPS types (42.3%) followed by III, V, VI, II and I with(19.2%), (13.5%), (11.5%), (10.6%) and (2.9%), respectively (Table3.1).

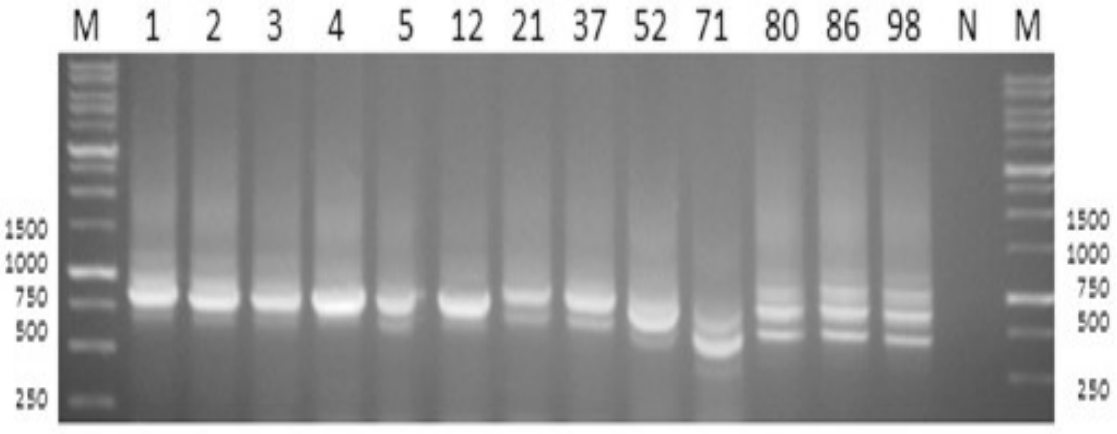


**Figure 3.2 Amplification patterns and genotyping of *C. albicans* by P-II targeting (RPS). Numbers on the upper part of the figure refer to clinical isolates that gave 450 bp with P-I. lanes (1, 2, 3, 4, 12) were classified as A-III, (5, 21, 37) as A-VI, (71,80, 86, 98) as A-V. M indicates the lane containing 1 kb DNA ladder. The molecular sizes (bp) of the DNA marker are shown on the left and right sides of the panels. N refers to negative control.**

The 25S rDNA and RPS-based PCR products showed that the *C. albicans* isolates with similar genotypes at the 25S rDNA gene often had a different genotype at the RPS regions (Figure 3.2).

When genotype information was combined from the two markers, a total of 14 different genotypes were identified in this study (Table 3.2).

Genotypic distribution based on the two markers (P-I and P-II) of the isolates isolated from different conditions of VVC is presented in Table 3.1 and 3.2.

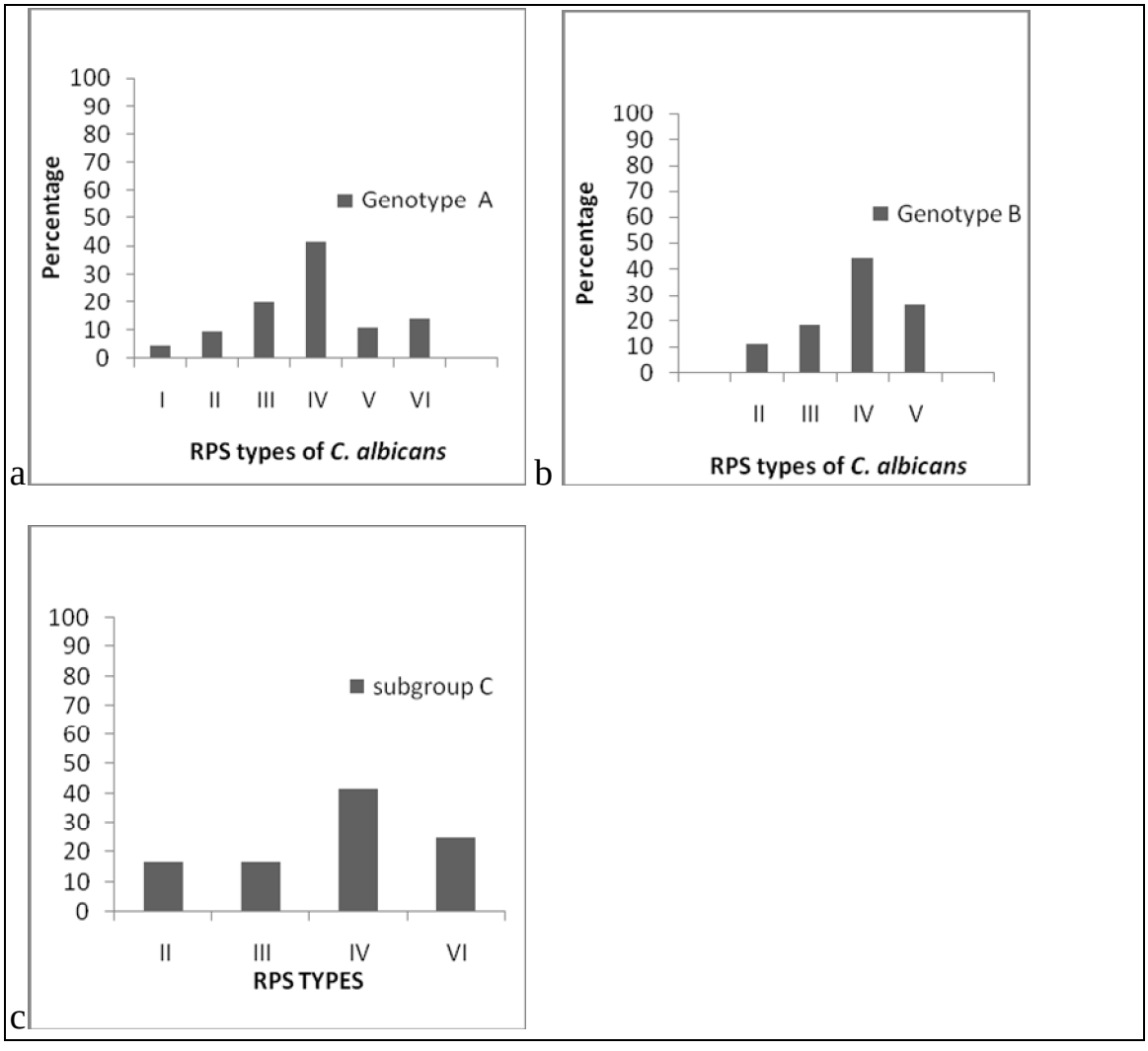


**Figure 3.3 shows that genotype IV was the most frequent genotype within all P-I genotypes (A, B and C) (Figure 3.3) .**

Figure 3.3 Distribution of RPS types within the 25S rDNA genotypes. a, genotype A; b, genotype B; c, genotype C.

* **Frequency and distribution of genotypes among VVC conditions.** There was not a subgroup that contained only one genotype. Analysis of genotypes distribution of *C. albicans* in every subgroup of VVC conditions did not show any obvious association between the isolates with a certain genotype colonizing a specific VVC condition. However, isolates of genotype IV seems to predominate (44/104) (42.3%) in all VVC conditions.

**Table 3.2 Distribution of the genotypes of C. albicans on the basis of P-I and P-II from women with VVC.**

| RPS(a) type and Genotype 25srDNA type | Total & % |
|---|---|
| A I | 3 (2.9%) |
| A II | 6 (5.8%) |
| A III | 13 (12.5%) |
| A IV | 27 (26%) |
| A V | 7 (6.7%) |
| A VI | 9 (8.7%) |
| B II | 3 (2.9%) |
| B III | 5 (4.8%) |
| B IV | 12 (11.5%) |
| B V | 7 (6.7%) |
| C II | 2 (1.9%) |
| C III | 2 (1.9%) |
| C IV | 5 (4.8%) |
| C VI | 3 (2.9%) |
| **Total** | **104 (100%)** |

The results showed that genotype A-IV comprised the highest rates of all isolates (26%) followed by A-III and B-IV, 12.5% and 11.5%, respectively(Table 3.2, Figure 3.3).

## 3.4 Patterns of *C. albicans* genotypes distribution among VVC pregnant women patients in relation to different VVC conditions and demographic characteristics of study population

Table 3.3 and Table 3.4 summarize *C. albicans* 25S rDNA genotypes distribution among VVC patients in relation to conditions and demographic characteristics of study population. There was not a condition that contained only one genotype. Analysis of genotypes distribution of *C. albicans* 25S rDNA genotypes (A, B, C), showed that these genotypes differed significally between VVC patients in relation to VVC conditions ($\chi_2$=14.481, df = 6 ,*P*= 0.022), with genotype A showing the highest frequency in erythemia (76.2%), intense vulval pruritus (72.7%) and dyspareunia (64.4%) followed by genotype B in burning condition(43.8%).

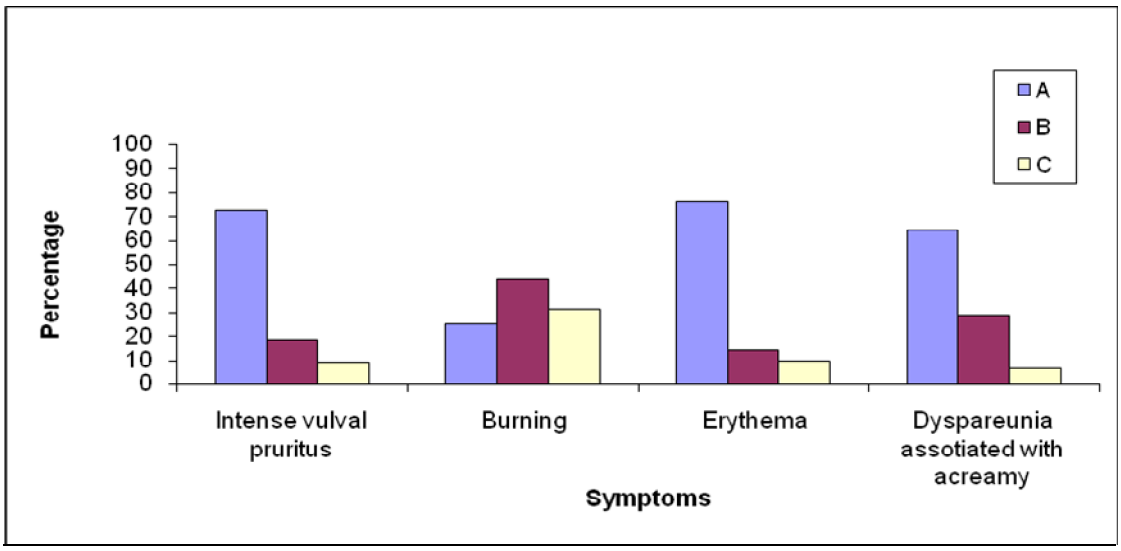


**Figure 3.4 P-I Genotype distribution among VVC isolates in relation to VVC conditions**

**Table 3.3 Frequency of *C. albicans* P-I genotypes by VVCs conditions, patients age, residence and predisposing factors.**

| Variable | Genotype | | | p Value* |
|---|---|---|---|---|
| | A<br>Num (%) | B<br>Num (%) | C<br>Num (%) | |
| **Age** | | | | |
| 17-23 | 24 (85.7%) | 3 (10.7%) | 1 (3.6%) | 0.0045* |
| 24-30 | 38 (61.3%) | 15 (24.2%) | 9 (14.5%) | |
| 31-37 | 2 (18.2%) | 8 (72.7%) | 1 (9.1%) | |
| 38-45 | 1 (33.5) | 1 (33.5%) | 1 (33%) | |
| **Residence** | | | | |
| Jenin | 24 (61.5%) | 14 (35.9%) | 1 (2.6%) | 0.130 |
| Tubas | 21 (70%) | 4 (13.3%) | 5 (16.7%) | |
| Tamon | 9 (50%) | 4 (22.2%) | 5 (27.8%) | |
| Aqaba | 8 (61.5%) | 4 (30.8%) | 1 (7.7%) | |
| Tayaser | 3 (75%) | 1 (25%) | 0 (0.00%) | |
| **Predisposing factors** | | | | |
| Tight clothes | 13 (86.7%) | 2 (13.3%) | 0 (0.00%) | 0.208 |
| Prolonged administration of antibiotics | 16 (72.7%) | 5 (22.7%) | 1 (4.5%) | |
| Presence of vaginal candidiasis at pregnancy | 31 (55.4%) | 16 (28.6%) | 9 (16.1%) | |
| No predisposing factor | 5 (45.5%) | 4 (36.4%) | 2 (18.2%) | |
| **Conditions (Symptoms)** | | | | |
| Intense vulval pruritis | 16 (72.7%) | 4 (18.2%) | 2 (9.1%) | 0.022 |
| Burning | 4 (25%) | 7 (43.8%) | 5 (31.3%) | |
| Erythema | 16 (76.2%) | 3 (14.3%) | 2 (9.5%) | |
| Dyspareunia | 29 (64.4%) | 13 (28.9%) | 3 (6.7%) | |

The distribution of genotypes also different significantly among patients at different age groups ($\chi^2$= 21.173, df = 6, *P*= 0.0045), with genotype A showing the highest frequency in age group (17-23) (85.7%) and(24-30) (61.3%) and genotype B showing highest frequency in age group 31-37 (72.7%) (Table 3.3, and Figure 3.5).

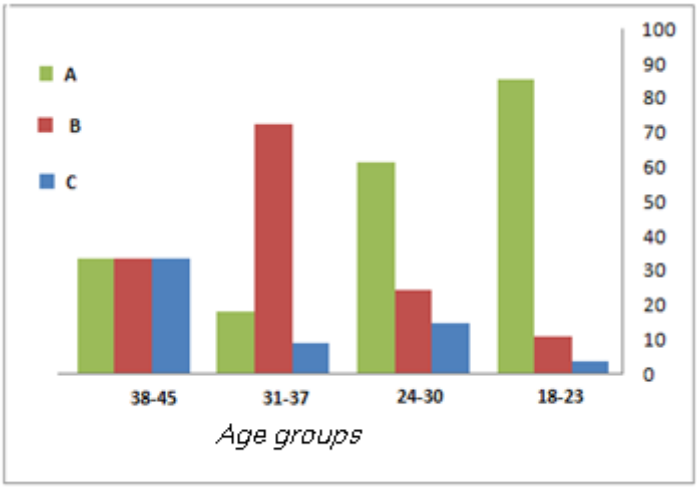


**Figure 3.5 Distribution of VVC isolates P-I genotypes in relation to age groups.**

However, no statistically significant association was identified between genotype distribution in patients in relation to other demographic, characteristics including residence ($\chi 2$= 12.516, df = 10, *P*= 0.130) and predisposing factors ($\chi 2$=8.435, df = 8 *P*= 0.208), with genotype A seems to be predominant in all residence locations especially in Tayaser (75%), Tubas (61.5%), and B in Jenin (35.9%), (Table 3.3 and Figure 3.6, and 3.7).

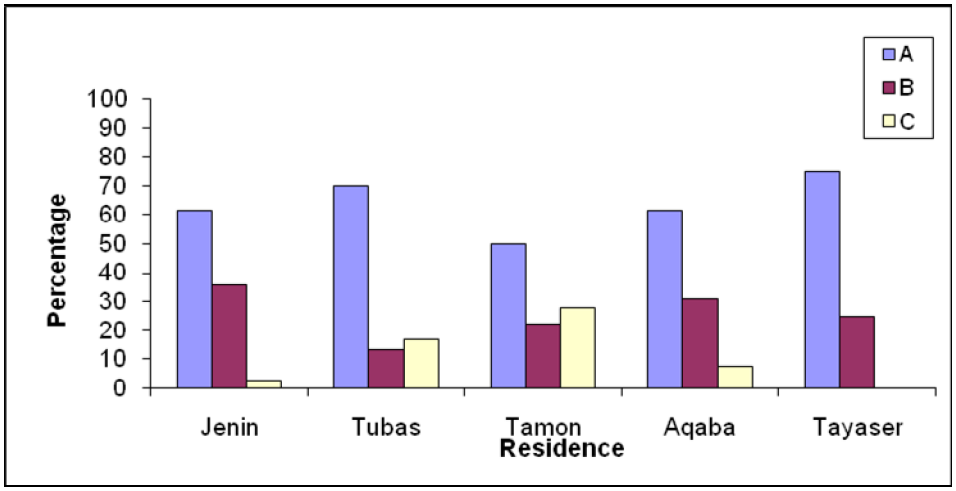


**Figure 3.6 Distribution of VVC isolates P-I genotypes in relation to residence**

genotype A seems to predominate in all predisposing factors especially in patients wearing, tight clothes (86.7%) (Table 3.3 and Figure 3.7).

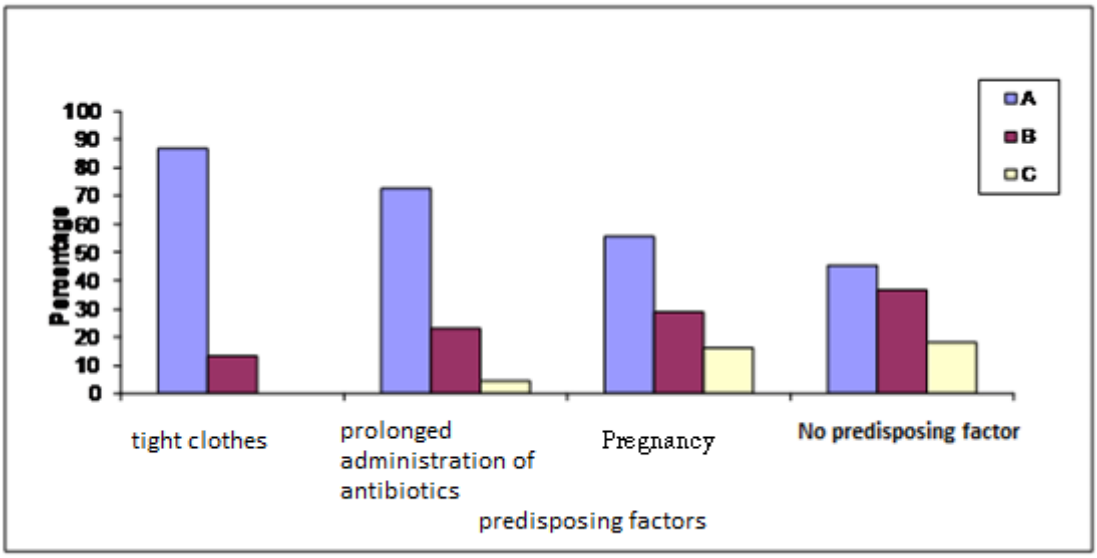


**Figure 3.7 genotype distribution due to predisposing factors and P-I genotype.**

Genotype A was highest frequency during Dyspareunia assotaited with a creamy discharge (44.6%), genotype B Dyspareunia assotaited with a creamy discharge (48.1%), genotype C in erythema(41.7%). The highest frequency of genotype A in Jenin (36.9%), during pregnancy (48%) and in aged group (24-30) (58.5%), genotype B in Jenin (51.8%), during pregnancy (59.3%), and in aged group (24-30) (55.5%) and genotype C during pregnancy(75%), in both Tamon and Tubas (41.6%), and in aged group (24-30) (75%).( Table 3.4).

**Table 3.4 P-I genotypes of VVC isolates distribution in relation between genotype of *C. albicans* and VVC conditions, Pateints age, residence and predisposing factors.**

| Title | Subtitle | A no & % | B no & % | C no & % | Total no |
|---|---|---|---|---|---|
| Age Interval | | | | | |
| | (17-23) | 24(37%) | 3(11.1%) | 1(8.3%) | 28 |
| | (24-30) | 38(58%) | 15(55.5%) | 9(75%) | 62 |
| | (31-37) | 2(3%) | 8(29.6%) | 1(8.33%) | 11 |
| | (38-44) | 1(1.5%) | 1 (3.7%) | 1(8.333%) | 3 |
| | Total no & % | 65(100%) | 27(100%) | 12(100%) | 104 |
| Residence | | | | | |
| | Jenin | 24(36.9%) | 14(51.8%) | 1(8.33%) | 39 |
| | Tubas | 21(32.3%) | 4(14.8%) | 5(41.67%) | 30 |
| | Tamon | 9(13.8%) | 4(14.8%) | 5(41.67%) | 18 |
| | Aqaba | 8(12.3%) | 4(14.8%) | 1(8.33%) | 13 |
| | Tayaser | 3(4.61%) | 1(3.7%) | 0(0%) | 4 |
| | Total no & % | 65(100%) | 27(100%) | 12(100%) | 104 |
| Predisposing factors | | | | | |
| | Tight clothes | 13(20%) | 2(7.4%) | 0(0%) | 15 |
| | Prolonged administration of antibiotics | 16(24.6%) | 5(18.5%) | 1(8.4%) | 22 |
| | Pregnancy | 31(48%) | 16(59.3%) | 9(75%) | 56 |
| | No predisposing factor | 5(7.7%) | 4(14.8%) | 2(16.7%) | 11 |
| | Total no & % | 65(100%) | 27(100%) | 12(100%) | 104 |
| Symptoms | | | | | |
| | Intense vulval pruritus | 16(24.6%) | 4(14.8%) | 2(6.7%) | 22 |
| | Erythema | 4(6.2%) | 7(25.9%) | 5(41.7%) | 16 |
| | Burning | 16(24.6%) | 3(11.2%) | 2(16.7%) | 21 |
| | Dyspareunia associated with a creamy discharge | 29(44.6%) | 13(48.1%) | 3(25%) | 45 |
| | Total no & % | 65(100%) | 27(100%) | 12(100%) | 104 |

# CHAPTER FOUR

# DISCUSSION

In this study, vulvovaginal candidiasis was caused by a single *candida* species namely *C. albicans*. Furthermore, similar to previous studies from Turkey (Karahan *et al*., 2004) and China (Xiao-dong *et al*.,2008), to my clinical isolates was could not detect any *C. dublinansis*. To my knowledge, no *C. dublinansis* has been reported from Palestinian patients yet, although it is possible that *C. dublinansis* may exist in a low frequency in the Palestinian population (McCullough *et al*., 2004) .

My results in therefore in agreement with those of a previous epidemiological study on vulvovaginal candidiasis (VVC) in pregnant women from Nablus (Aslan, 2004), where no *C. dubliniensis* was detected.

PCR can detect very small quantities of DNA and cause earlier detection of *C. albicans* and subsequently allow early diagnosis and hence may improve chances of survival in immunocompromized patients . These methods can directly detect the presence of fungi with high degree of specificity and sensitivity (Mirhendi, & Makimura, 2003). *C. albicans* isolates have been subdivided into different biological groups based upon genetic subtypes (Tamura *et al*., 2001; Millar *et al*., 2002; Hattori *et al*., 2006; Iwata *et al*., 2006). Several studies have supported the concept that genotypic differences among *C. albicans* isolates might be correlated to their invasive environments or different body sites (Soll *et al*., 1991; Lockhart *et al*., 1996; Lian *et al*., 2004; Hattori *et al*., 2006).

Previous studies have also indicated that one genotype defined by length polymorphisms at the 25S rDNA was associated with 5-

fluorocytosine susceptibility (Mercure et al., 1993). It is therefore necessary to discriminate the pathogen at the strain level (Hattori *et al*., 2009).

To classify strain types isolated from 104 pregnant patients with VVC we used Iwata et al. (2006) genotyping method PCR targeting 25SrDNA (P-I) and RPS (P-II). This method has been confirmed as a specific and reproducible method of genotype analysis of *C. albicans* (Hattori *et al*., 2006).

PCR targeting 25S rDNA, which has frequently been used for genotype analysis of *C. albicans*, allows *C. albicans* to be grouped into five genotypes A, B, C, D and E (McCullough *et al*., 1999; Tamura *et al*., 2001; Millar *et al*., 2002; Hattori *et al*., 2006; Iwata *et al*., 2006).

In the PCR amplification using P-I, it was revealed in our results revealed that genotype A *C. albicans* constituted the majority (62.5%) of the VVC isolates in all of the 25S rDNA based genotypes, followed by genotype B *C. albicans* (26%) and genotype C *C. albicans* (11.5%).

The frequency and distribution of genotypes originating from our isolates(62.5%, 26%, and 11.5%) for genotype A, B, and C of *C. albicans* in agreement with the results of previous researches that have been carried out on VVC isolates in China by Xiao-dong *et al* (2008),also found genotype A to constitute the majority of VVC isolates with 92.7% followed by genotype B (5.5%) and genotype B (1.8%). The results of this research

were also similar to those observed in a previous study on VVC isolates in Turkey (71.4%, 14.3% and 14.3%, for genotypes A,B, and C respectively) (Karahan & Akar, 2005). However our results disagreed with those of Emmanuel *et al* (2012), who recognized all *C.albicans* isolates isolated from patients with VVC in Nigeria as being of genotype A.

It has been shown by several researchers that the frequency of genotype A is higher than genotypes B and C (Xiao-dong *et al.,* 2008; Zhu *et al.,* 2003; Karahan & Akar, 2005; Emmanuel *et al.,* 2012). Our results are in accordance of these results where it was shown that genotype A was represented with the highest frequency (87%) followed by genotype B and C (8.7, and 4.3, respectively). It has been shown that genotypes A is significantly resistant to Fluconazol and flucytosine, than genotypes B and C (McCullough *et al.,* 1999; Tamura *et al*., 2001; Emmanuel *et al.,* 2012). Thus identifying *C. albicans* at the genotype level help in prescribing the suitable antifungal drugs by specialists, and help in the control of *C. albicans*.

For typing of *C. albicans* on the basis of ALT repeats, it has been reported that *C. albicans* chromosomes contain characteristic repetitive sequences (RPSs), each of which contains a tandem short repeating unit of 172 bp, designated ALT (Iwaguchi *et al*., 1992; Chindamporn *et al.,* 1995).The numbers of ALT repeats in the RPS varies in each chromosome, thereby leading to variation in number and size of PCR

products (Chibana *et al*., 1994., Doi *et al.*, 1994). Kanbe *et al.* (2005) reported that these molecular characteristics of the different sizes and copy numbers of the ALT sequence are attractive for the genotyping of *C. albicans*. Thus, it is expected that several DNA products of different sizes and intensities should be generated when the ALT regions are amplified by PCR using appropriate primers. If *C. stellatoidea* and *C. dubliniensis* have unique sequences similar to the *C. albicans* RPSs, it should be possible to create a system that can distinguish between *C. albicans* and these related two species sequences similar to the *C. albicans* RPSs.

For typing of *C. albicans* on the basis of ALT repeats, two further primers were a previous designed on the basis of the nucleotide sequences of *C. albicans* RPS, and were designated as ASDcF and pCSCR (Chibana *et al*.,1994). The primer set ASDcF/ pCSCR is referred to as P-II in this study.

The ALT repeats in the RPS sequences has been confirmed as a specific and reproducible method of genotype analysis of *C. albicans* (Hattori *et al*., 2006).

PCR targeting RPS, which has frequently been used for genotype analyses of *C. albicans*, allows *C. albicans* to be grouped into genotypes I, II, III, IV, V and IV, and to differentiate *C. albicans* from non- albicans

(Iwaguchi *et al*., 1992; Kanbe et al., 2005; Hattori *et al*., 2006; Iwata *et al*., 2006).

*Candida albicans* isolates from specimen in Jenin area from pregnant women with vulvovaginal candidiasis six genotypes where identified namely, I, II, III, IV, V and VI on based on the number of the intense strong bands.

In the PCR amplification using P-II, the results revealed that genotype IV *C.albicans* constituted the majority (42.3%) of the VVC isolates in all of the RPS based genotypes, followed by genotype III *C.albicans* (19.2%), genotype V *C.albicans* (13.5%), genotype VI *C.albicans*(11.5%), genotype II *C.albicans* (10.6%) and genotype I *C.albicans* (2.9%). present results disagreed from those of a previous study (Xia-dong et al., 2008) on VVC isolates where genotype I *C.albicans* constituted the majority (74.6%) of the isolates, followed by genotype II *C.albicans* (16.4%) and genotype III *C.albicans* (9.1%).

These variations of genotypes of *C. albicans* among these studies may be attributed to differences in geographic locations (Clemons et al., 1997).

In the PCR amplification using P-I and P-II, the results revealed that genotype A-IV *C. albicans* constituted the majority (26%) of the VVC isolates in all of the 25SrDNA and the RPS based genotypes. present results disagreed from those of a previous study (Iwata et al., 2006) on

scale, nail, vaginal secretion, sputum, and other clinical specimens, where genotype A-III *C. albicans* constituted the majority (34%) of the isolates.

These variations of genotypes of *C. albicans* among these studies may be attributed to differences in geographic locations (Clemons et al., 1997).

This is the first report of genotypic analysis of vaginal candidiasis isolates in the Palestinian authority territories that also examine associations between genotype and conditions of VVC such as (intense vulval pruritus, erythema, burning and dyspareunia associated with a creamy discharge age), age of patients, residence, and predisposing factors.

After analyzing the genotypes in the VVC isolates it was demonstrated that there were significant associations between genotypes of *C. albicans* and their various conditions of vaginal infection, and age of patients. This finding may be attributed to the hypothesis that genotypic differences among *C. albicans* might be correlated with their invasive environment (Lian, 2004), weakness of immune system , and release of estrogen hormone of pregnant women (Guzel *et al*., 2011).

The results of this study, however, disagreed from those of Xia-dong et al. (2008) who found no noticeable differences in genotypes of isolates from various VVC conditions such as (intense vulval pruritus, erythema, burning and dyspareunia associated with a creamy discharge age).

On the other hand, no significant association between genotypes of *C. albicans* and predisposing factor, and residence detected in the study. The

influences of residence and predisposing factors were minor and could not influence the genotype of strains.

In conclusion, the research showed that the genotypes of isolates from various VVC conditions such as (intense vulval pruritus, erythema, burning and dyspareunia associated with a creamy discharge age), and patients age groups were significantly different. However, the genotypes of VVC strains were not related to predisposing factors or patients residence.

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(Chin) 36: 446-448

# APPENDIXES

# Appendix A

***Msc.project form***

***Genotype Comparisons of Strains of Candida Albicans from Patients with Vaginal Candidiasis***

---

Number of Sample: Date of collection of Sample:

| Sampling | Vaginal channel |
|---|---|

***Health institution and person in-charge :***

Patient name : age :

**Medical history :**

| Previous diseases (before 2 months ) : type | Duration |
|---|---|
| | |
| Present disease | Initiation date |
| | |
| Drugs used | Treatment period |
| | |
| **Symptoms:** | |

**Predisposing Factors**

| | |
|---|---|
| Tight clothes (Yes , No ) | NO predisposing factor (Yes , No ) |
| Intravenous catheters (Yes , No ) | Cytoreductive chemotherapy (Yes , No ) |
| Prolonged administration of antibiotics (Yes , No ) | Hematologic malignant diseases (Yes , No ) |
| Burns (Yes , No ) | Neutropenia (Yes , No ) |
| Presence of vaginal candidiasis at pregnancy (Yes , No ) | Latrogenic immunosuppresion (Yes , No ) |

# Appendix B

Chemical Solution preparations

-20% KOH:

2 gm of KOH was dissolved in 10 ml D.W.

- 50 mM EDTA _ 0.2 % SDS:

1.46 g of EDTA were dissolved in 100 ml of Sterile DW at PH 8

by using NaOH 1 M and then add of 0.2 g of SDS to solution .

Total volume of solution equal 100 ml and store in glass flask in refrigerator at 8°C ( used glass flask until don't get oxidation ) .

- 5M KAC:

4.9075 g of potassium acetate were dissolved in 10 ml of Sterile D.W ,then put solution in sterile tube and then store in refrigerator at 8°C.

-Sorbitol:

Weight of 9.15 g of sorbitol were dissolved in 50 ml of sterile D.W then

put solution in sterile cup and store in refrigerator at 8°C.

- TE buffer:

1.21g of EDTA were dissolved in 100 ml of sterile D.W at PH 8 by NaoH

1M and then dissolved of 2.92 g of Tric _ HCL to solution .

Total volume of solution equal 100 ml and store in sterile cup in refrigerator at 8°C .

- 5u/µl 20 µl Lyticase:

261.6 µl of sterile D.W to prepare of 1 mg (1 bottle) of lyticase ,

2001.24 µl sterile D.W were added to the components 7.65

mg, 1308 unit/ 1mg contains in 261.6 µl /mg and stored at -20 °C .

-TBE buffer:

-1 liter of 5x TBE buffer:

2.922g EDTA were dissolved in 20 ml of sterile D.W at PH 8 by NaoH 1M, then dissolved of 54 g Tric _ base , 27.5 g boric acid in1 liter of D.W , then 20 ml of EDTA to solution , Pour of solution in glass bottle and store in refrigerator at 8°C.

-1 liter of 1x TBE buffer:

Add 100 ml of TBE buffer to 400 ml of D.W in glass bottle.

-86% glycerol:

14 ml of D.W were added to 86 ml of pure glycerol and autoclaved.

-70% Ethanol:

30 ml D.W were added to 70 ml of pure ethanol(100%).

# Appendix C

Media Preparation

- YPD Broth:

5 gm yeast extract 10 gm peptone 20gm dextrose were dissolved in 1litre distilled water then autoclaved.

Add chloramphenicol ( 50 µg /ml ) in 2 ml of absolute alcohol to SDA.

-SDA agar:

65 grams of SDA agar were dissolved in1 liter of D.W then autoclaved then chloramphenicol ( 50µg /ml ) was dissolved in 2 ml of absolute alcohol.

-Chromogenic agar:

15.5 grams of chromogenic agar were Dissolved in ½ liter of D.W , boiling with shaking , and do not autoclave.

# Appendix D

Molecular Method Preparation

- Forward primer I (10 MM Ca-INT-L-F):

590 µl sterile D.W were added to 59 n mol of the primer the molarity should be 100 MM then 10ML of 100 MM primer were added to 90 µl sterile D.W to prepare 10 MM of the primer**.**

-Reverse primer I (10 MM Ca-INT-R-R):

636 µl sterile D.W were added to 63.6 n mol of the primer the molarity should be 100 MM then 10 µl of 100MM primer were added to 90 µl sterile D.W to prepare 10 MM of the primer**.**

-Forward primer II (10 MM ASDcFc-F):

686 µl sterile D.W were added to 68.6 n mol of the primer the molarity should be 100 MM then 10 µl of 100 MM primer were added to 90 µl sterile D.W to prepare 10 MM of the primer.

-Reverse primer II (10 MM pCSCRc-R):

824 µl sterile D.W were added to 82.4 n mol of the primer the molarity should be 100 MM then 10 µl of 100 MM primer were added

to 90 µl sterile D.W to prepare 10 MM of the primer.

- DNTPs:

Thowing of 4 vails of dATP ,dTTP,dCTP,dGTP on ice , then vortex and spin down , then mix of all vials together, then divided into several PCR tubes and then stored in freezer at -20 °C .

- Agarose:

0.8 g or 1.2 g of agarose were dissolved in 100 ml of 1x-TBE buffer , then add of E.P and then pour of agarose in tray.

## Appendix E

Table: List of specimen number, age ,gender, residence, infected lesion and *Candida albicans* subgroups according to amplification of P-I isolated from the patients:

| Specimen No. | Age / Gender | Residence | Infected lesion | *C. albicans* Subgroups (P-I) |
|---|---|---|---|---|
| 1 | 21/F | Tubas | Vaginal tract | A |
| 2 | 22/F | Tubas | Vaginal tract | A |
| 3 | 26/F | Tubas | Vaginal tract | A |
| 4 | 17/F | Tubas | Vaginal tract | A |
| 5 | 27/F | Tubas | Vaginal tract | A |
| 6 | 23/F | Tamon | Vaginal tract | A |
| 7 | 27/F | Tamon | Vaginal tract | C |
| 8 | 30/F | Tayaser | Vaginal tract | A |
| 9 | 22/F | Tubas | Vaginal tract | A |
| 10 | 19/F | Aqaba | Vaginal tract | A |
| 11 | 32/F | Aqaba | Vaginal tract | B |
| 12 | 26/F | Tamon | Vaginal tract | A |
| 13 | 21/F | Tamon | Vaginal tract | A |
| 14 | 30/F | Tamon | Vaginal tract | A |
| 15 | 17/F | Tayaser | Vaginal tract | A |
| 16 | 21/F | Tubas | Vaginal tract | A |
| 17 | 22/F | Tubas | Vaginal tract | A |
| 18 | 29/F | Tubas | Vaginal tract | A |
| 19 | 28/F | Tubas | Vaginal tract | B |
| 20 | 25/F | Aqaba | Vaginal tract | A |
| 21 | 24/F | Aqaba | Vaginal tract | A |
| 22 | 20/F | Tubas | Vaginal tract | A |
| 23 | 27/F | Tubas | Vaginal tract | A |
| 24 | 37/F | Tubas | Vaginal tract | B |
| 25 | 35/F | Tamon | Vaginal tract | B |
| 26 | 22/F | Tamon | Vaginal tract | C |
| 27 | 23/F | Tamon | Vaginal tract | A |
| 28 | 25/F | Tamon | Vaginal tract | B |
| 29 | 29/F | Tubas | Vaginal tract | A |
| 30 | 18/F | Tubas | Vaginal tract | A |

| | | | | |
|---|---|---|---|---|
| 31 | 26/F | Aqaba | Vaginal tract | B |
| 32 | 44/F | Aqaba | Vaginal tract | C |
| 33 | 24/F | Aqaba | Vaginal tract | B |
| 34 | 31/F | Tayaser | Vaginal tract | B |
| 35 | 26/F | Tubas | Vaginal tract | C |
| 36 | 19/F | Tubas | Vaginal tract | A |
| 37 | 27/F | Tubas | Vaginal tract | A |
| 38 | 30/F | Tubas | Vaginal tract | A |
| 39 | 25/F | Tubas | Vaginal tract | B |
| 40 | 22/F | Tayaser | Vaginal tract | A |
| 41 | 24/F | Tamon | Vaginal tract | C |
| 42 | 36/F | Tamon | Vaginal tract | B |
| 43 | 28/F | Tamon | Vaginal tract | A |
| 44 | 35/F | Aqaba | Vaginal tract | B |
| 45 | 30/F | Aqaba | Vaginal tract | A |
| 46 | 26/F | Aqaba | Vaginal tract | A |
| 47 | 21/F | Tubas | Vaginal tract | A |
| 48 | 29/F | Tubas | Vaginal tract | C |
| 49 | 27/F | Tubas | Vaginal tract | A |
| 50 | 30/F | Tubas | Vaginal tract | A |
| 51 | 24/F | Tubas | Vaginal tract | B |
| 52 | 24/F | Tubas | Vaginal tract | A |
| 53 | 29/F | Tubas | Vaginal tract | A |
| 54 | 27/F | Tamon | Vaginal tract | A |
| 55 | 22/F | Tamon | Vaginal tract | A |
| 56 | 24/F | Aqaba | Vaginal tract | A |
| 57 | 26/F | Aqaba | Vaginal tract | A |
| 58 | 29/F | Aqaba | Vaginal tract | A |
| 59 | 32/F | Tamon | Vaginal tract | C |
| 60 | 30/F | Tamon | Vaginal tract | B |
| 61 | 26/F | Tubas | Vaginal tract | C |
| 62 | 29/F | Tamon | Vaginal tract | C |
| 63 | 28/F | Tamon | Vaginal tract | A |
| 64 | 29/F | Tubas | Vaginal tract | C |
| 65 | 30/F | Tubas | Vaginal tract | C |
| 66 | 26/F | Jenin | Vaginal tract | B |
| 67 | 24/F | Jenin | Vaginal tract | A |
| 68 | 28/F | Jenin | Vaginal tract | A |
| 69 | 29/F | Jenin | Vaginal tract | A |
| 70 | 30/F | Jenin | Vaginal tract | A |
| 71 | 26/F | Jenin | Vaginal tract | A |

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| 72 | 19/F | Jenin | Vaginal tract | A |
| 73 | 25/F | Jenin | Vaginal tract | A |
| 74 | 28/F | Jenin | Vaginal tract | B |
| 75 | 27/F | Jenin | Vaginal tract | B |
| 76 | 28/F | Jenin | Vaginal tract | A |
| 77 | 17/F | Jenin | Vaginal tract | A |
| 78 | 21/F | Jenin | Vaginal tract | A |
| 79 | 24/F | Jenin | Vaginal tract | A |
| 80 | 26/F | Jenin | Vaginal tract | A |
| 81 | 32/F | Jenin | Vaginal tract | A |
| 82 | 20/F | Jenin | Vaginal tract | A |
| 83 | 25/F | Jenin | Vaginal tract | A |
| 84 | 34/F | Jenin | Vaginal tract | B |
| 85 | 30/F | Jenin | Vaginal tract | A |
| 86 | 28/F | Jenin | Vaginal tract | A |
| 87 | 44/F | Jenin | Vaginal tract | A |
| 88 | 26/F | Jenin | Vaginal tract | A |
| 89 | 22/F | Jenin | Vaginal tract | A |
| 90 | 35/F | Jenin | Vaginal tract | A |
| 91 | 24/F | Jenin | Vaginal tract | C |
| 92 | 37/F | Jenin | Vaginal tract | B |
| 93 | 21/F | Jenin | Vaginal tract | B |
| 94 | 29/F | Jenin | Vaginal tract | B |
| 95 | 27/F | Jenin | Vaginal tract | B |
| 96 | 41/F | Jenin | Vaginal tract | B |
| 97 | 30/F | Jenin | Vaginal tract | B |
| 98 | 19/F | Jenin | Vaginal tract | A |
| 99 | 22/F | Jenin | Vaginal tract | B |
| 100 | 18/F | Jenin | Vaginal tract | B |
| 101 | 23/F | Jenin | Vaginal tract | A |
| 102 | 27/F | Jenin | Vaginal tract | B |
| 103 | 25/F | Jenin | Vaginal tract | B |
| 104 | 27/F | Jenin | Vaginal tract | A |

# Appendix F

**Sequence of 25S rDNA gene in *Candida albicans*:**

(caaccaagcgcgggtaaacggcgggagtaactatgactctcaacctataagggaggcaaaagtagg
gacgccatggtttccagaaatgggccgcggtgtttttgacctgctagtcgatctggccagacgtatctgtg
ggtggccagcggcgacataacctggtacggggaaggcctcgaagcagtgttcaccttgggagtgcgc
aagcacaaagaggtgagtggtgtatggggttaatcccgtggcgagccgtcagggcgcgagttctggca
gtggccgtcgtagagcacggaaaggtatgggctggctctctgagtcggcttaaggtacgtgccgtccca
cacgatgaaaagtgtgcgtgcagaatagttcccacagaacgaagctgcgccggagaaagcgatttctt
ggagcaatgc ttaaggtagccaaatgcctcgtcatctaattagtgacgc )

Length: 459 base pair , A Count:110, C Count:104, G Count:152, T Count:93, Others Count:0 exon(1…40 ) & (420…459) intron (41…419 ) (Lemay, 1993; Mercure, 1993).

# Appendix G

## Nucleotide sequence of ALT repeats (172 bp) of *C. albicans*

5'GAATTTGCGGTGATGTCCGTTGAAGACTGCGCGATGAAAAATA
ACGCTACAAAAATCAAACTAGTGCCGATTTATACCTTTTTCTTAT
GAGTGCTAACCATGCAAGAACTGTTAGAAACGAAATACAACTG
CTATCTGTGGAACAAAAAAGGCCGTTTTGGCCATAGTTAAG3'

(Hattori *et al*., 2009)

# Appendix H

Tables

TABLE OF SYMPTOMS

| PATEINT NO | intense vulval pruritus | | | burning | | | erythema | | | dyspareunia associated with a creamy-white, curd-like discharge | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
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| 102 | | | | | | | | | | | + | |
| 103 | | | | | | | | | | | + | |
| 104 | | | | | | | + | | | | | |
| Total | 17 | 4 | 3 | 4 | 7 | 4 | 15 | 3 | 2 | 29 | 13 | 3 |

# TABLE OF RESIDENCE

| PATEINT NO | JENIN | | | TUBAS | | | TAMON | | | AQABA | | | TAYASER | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sup A | Sup B | Sup C | Sup A | Sup B | Sup C | Sup A | Sup B | Sup C | Sup A | Sup B | Sup C | Sup A | Sup B | Sup C |
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| 78 | + | | | | | | | | | | | | | | |
| 79 | + | | | | | | | | | | | | | | |
| 80 | + | | | | | | | | | | | | | | |
| 81 | + | | | | | | | | | | | | | | |
| 82 | + | | | | | | | | | | | | | | |
| 83 | + | | | | | | | | | | | | | | |
| 84 | | + | | | | | | | | | | | | | |
| 85 | + | | | | | | | | | | | | | | |
| 86 | + | | | | | | | | | | | | | | |

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| 87 | + | | | | | | | | | | | | | | |
| 88 | + | | | | | | | | | | | | | | |
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| 90 | + | | | | | | | | | | | | | | |
| 91 | | | + | | | | | | | | | | | | |
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| 93 | | + | | | | | | | | | | | | | |
| 94 | | + | | | | | | | | | | | | | |
| 95 | | + | | | | | | | | | | | | | |
| 96 | | + | | | | | | | | | | | | | |
| 97 | | + | | | | | | | | | | | | | |
| 98 | + | | | | | | | | | | | | | | |
| 99 | | + | | | | | | | | | | | | | |
| 100 | | + | | | | | | | | | | | | | |
| 101 | + | | | | | | | | | | | | | | |
| 102 | | + | | | | | | | | | | | | | |
| 103 | | + | | | | | | | | | | | | | |
| 104 | + | | | | | | | | | | | | | | |
| Total | 24 | 14 | 1 | 21 | 4 | 5 | 9 | 4 | 5 | 8 | 4 | 1 | 3 | 1 | 0 |

# TABLE OF PREDISPOSING FACTOR

| PATEINT NO | Tight clothes | | | Prolonged administration of antibiotics | | | Presence of vaginal candidiasis at pregnancy | | | Non Predisposing factor | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sup A | Sup B | Sup C | Sup A | Sup B | Sup C | Sup A | Sup B | Sup C | Sup A | Sup B | Sup C |
| 1 | | | | + | | | | | | | | |
| 2 | | | | + | | | | | | | | |
| 3 | | | | + | | | | | | | | |
| 4 | | | | | | | + | | | | | |
| 5 | | | | | | | + | | | | | |
| 6 | | | | | | | | | | + | | |
| 7 | | | | | | | | | + | | | |
| 8 | | | | | | | + | | | | | |
| 9 | + | | | | | | | | | | | |
| 10 | | | | + | | | | | | | | |
| 11 | | | | | + | | | | | | | |
| 12 | | | | | | | + | | | | | |
| 13 | | | | | | | + | | | | | |
| 14 | | | | + | | | | | | | | |
| 15 | | | | + | | | | | | | | |
| 16 | | | | | | | | | | + | | |
| 17 | | | | | | | + | | | | | |
| 18 | | | | | | | + | | | | | |
| 19 | | | | | | | | | | | + | |
| 20 | | | | | | | + | | | | | |
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| 37 | | | | | | | + | | | | | |
| 38 | | | | | | | + | | | | | |
| 39 | | | | | | | | + | | | | |

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| 40 | | | | + | | | | | | | | |
| 41 | | | | | | | | | + | | | |
| 42 | | | | | + | | | | | | | |
| 43 | | | | | | | + | | | | | |
| 44 | | | | | | | | + | | | | |
| 45 | + | | | | | | | | | | | |
| 46 | + | | | | | | | | | | | |
| 47 | | | | | | | + | | | | | |
| 48 | | | | | | + | | | | | | |
| 49 | + | | | | | | | | | | | |
| 50 | | | | | | | + | | | | | |
| 51 | | | | | | | | | | | + | |
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| 56 | | | | | | | | | | + | | |
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| 58 | | | | + | | | | | | | | |
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| 66 | | | | | | | | + | | | | |
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| 68 | | | | | | | + | | | | | |
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| 73 | + | | | | | | | | | | | |
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| 76 | + | | | | | | | | | | | |
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| 78 | + | | | | | | | | | | | |
| 79 | | | | | | | | | | + | | |
| 80 | | | | + | | | | | | | | |
| 81 | + | | | | | | | | | | | |
| 82 | + | | | | | | | | | | | |
| 83 | | | | | | | + | | | | | |
| 84 | | | | | | | | + | | | | |
| 85 | + | | | | | | | | | | | |
| 86 | | | | + | | | | | | | | |
| 87 | | | | | | | + | | | | | |

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| 88 | | | | | | | + | | | | | |
| 89 | | | | | | | + | | | | | |
| 90 | + | | | | | | | | | | | |
| 91 | | | | | | | | | | | | + |
| 92 | | | | | | | | + | | | | |
| 93 | | | | | | | | + | | | | |
| 94 | | | | | | | | + | | | | |
| 95 | | | | | | | | + | | | | |
| 96 | | | | | | | | + | | | | |
| 97 | | | | | | | | + | | | | |
| 98 | + | | | | | | | | | | | |
| 99 | | | | | | | | + | | | | |
| 100 | | | | | + | | | | | | | |
| 101 | | | | | | | + | | | | | |
| 102 | | | | | | | | | | | + | |
| 103 | | | | | | | | + | | | | |
| 104 | | | | | | | + | | | | | |
| Total | 13 | 2 | 0 | 15 | 5 | 1 | 32 | 16 | 9 | 5 | 4 | 2 |

Table of relation between age, residence ,predisposing factor and symptom and all sup groups of c.albicans:

| Variable type | | *C. albicans* genotypes | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | | B | | C | |
| | | No. | % | No. | % | No. | % |
| Age | 17-23 | 24 | 37 | 3 | 11 | 1 | 8 |
| | 24-30 | 38 | 58 | 15 | 55 | 9 | 75 |
| | 31-37 | 2 | 3 | 8 | 30 | 1 | 8 |
| | 38-44 | 1 | 1.5 | 1 | 4 | 1 | 8 |
| Residence | Jenin | 24 | 61.5 | 14 | 35.9 | 1 | 2.60 |
| | Tubas | 21 | 70.0 | 4 | 13.3 | 5 | 16.7 |
| | Tamon | 9 | 50.0 | 4 | 22.2 | 5 | 27.8 |
| | Aqaba | 8 | 61.5 | 4 | 30.8 | 1 | 7.70 |
| | Tayaser | 3 | 75.0 | 1 | 25.0 | 0 | 0.00 |
| Predisposing factor | Tight clothes | 13 | 86.7 | 2 | 13.3 | 0 | 0.00 |
| | Prolonged administration of antibiotics | 16 | 72.7 | 5 | 22.7 | 1 | 4.5 |
| | Presence of vaginal candidiasis at pregnancy | 31 | 55.4 | 16 | 28.6 | 9 | 16.1 |
| | Non Predisposing factor | 5 | 45.5 | 4 | 36.4 | 2 | 18.2 |
| Symptoms | intense vulval pruritus | 16 | 72.7 | 4 | 18.2 | 2 | 9.1 |
| | burning | 4 | 25.0 | 7 | 43.8 | 5 | 31.3 |
| | erythema | 16 | 76.2 | 3 | 14.3 | 2 | 9.5 |
| | dyspareunia associated with a creamy-white, curd-like discharge | 29 | 64.4 | 13 | 28.9 | 3 | 6.7 |

Table of relation between age, residence ,predisposing factor and symptom and each sup groups of c.albicans:

| Title | Sub title | A no & % | B no & % | C no & % | Total no |
|---|---|---|---|---|---|
| Age Interval | | | | | |
| | (17-23) | 13(20%) | 10(37%) | 5(41.67%) | 28 |
| | (24-30) | 21(32.3%) | 8(29.6%) | 5(41.67%) | 34 |
| | (31-37) | 22(33.8%) | 7(25.9%) | 1(8.33%) | 30 |
| | (38-44) | 9(13.8%) | 2(7.4%) | 1(8.333%) | 12 |
| | Total no & % | 65(100%) | 27(100%) | 12(100%) | 104 |
| Residence | | | | | |
| | Jenin | 24(36.9%) | 14(51.8%) | 1(8.33%) | 39 |
| | Tubas | 21(32.3%) | 4(14.8%) | 5(41.67%) | 30 |
| | Tamon | 9(13.8%) | 4(14.8%) | 5(41.67%) | 18 |
| | Aqaba | 8(12.3%) | 4(14.8%) | 1(8.33%) | 13 |
| | Tayaser | 3(4.61%) | 1(3.7%) | 0(0%) | 4 |
| | Total no & % | 65(100%) | 27(100%) | 12(100%) | 104 |
| Predisposing factors | | | | | |
| | Tight clothes | 13(20%) | 2(7.4%) | 0(0%) | 15 |
| | Prolonged administration of antibiotics | 16(24.6%) | 5(18.5%) | 1(8.4%) | 22 |
| | Presence of vaginal candidiasis at pregnancy | 31(48%) | 16(59.3%) | 9(75%) | 56 |
| | No predisposing factor | 5(7.7%) | 4(14.8%) | 2(16.7%) | 11 |
| | Total no & % | 65(100%) | 27(100%) | 12(100%) | 104 |
| Symptoms | | | | | |
| | Intense vulval pruritus | 16(24.6%) | 4(14.8%) | 2(6.7%) | 22 |
| | Erythema | 4(6.2%) | 7(25.9%) | 5(41.7%) | 16 |
| | Burning | 16(24.6%) | 3(11.2%) | 2(16.7%) | 21 |
| | Dyspareunia assotiated with acreamy | 29(44.6%) | 13(48.1%) | 3(25%) | 45 |
| | Total no & % | 65(100%) | 27(100%) | 12(100%) | 104 |

جامعة النجاح الوطنية
كلية الدراسات العليا

# مقارنة الطرز الجينية لسلالات من فطر *Candida albicans* من مرضى مصابون ب Candidiasis المهبلي

إعداد
معتصم هاني ابو بكر

إشراف

الأستاذ الدكتور محمد سليم اشتيه

الدكتور صبري ناصر

قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس فلسطين.

2012

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## الملخص

**الخلفية:** فطر *Candida albicans* هو من اكثر الميكروبات التي تسبب الالتهابات الفطرية التي تصيب القناة المهبلية لدى النساء الحوامل.

تهدف هذه الدراسة الى التحقق ما اذا كانت هناك علاقة بين الانماط الجينية لسلالات الفطر ومتغيرات مختلفة مثل أعراض الالتهاب الفطري في القناة المهبلية لدى الحوامل، والفئة العمرية للمريض وأماكن سكناهم، والعوامل المهيئة للمرض.

**طرق البحث وموارده:** بدأت هذه الدراسة في شهر أيار 2010 وانتهت في شهر تشرين الثاني 2010 حيث تم عزل 104 سلالة من فطر *Candida albicans* من منطقة جنين من 104 نساء حوامل مصابات بفطريات القناة المهبلية. تم تصنيف سلالات الفطر بالإعتماد على أعراض الالتهاب الفطري في القناة المهبلية لدى الحوامل الفئة العمرية للمريض العوامل المهيئة للمرض وأماكن سكناهم. تم تحديد النمط الجيني باستخدام تقنية PCR من خلال مضاعفة الجين 25SrDNA ومضاعفة RPS.

**النتائج :** ظهر 14 نمطا جينيا مختلفا في هذه الدراسة، ووجدنا انه يوجد علاقة واضحة بين توزيع الانماط الجينية لفطر *Candida albicans* وأعراض الالتهاب الفطري في القناة المهبلية والفئة العمرية للمرض. بينما لم نستطيع ايجاد أي علاقة واضحة بين توزيع الانماط الجينية لفطر *Candida albicans* والعوامل المهيئة للمرض وأماكن سكناهم. وجدنا أن النمط الجيني A (62.5%) الأكثر نسبة بين الأنماط الجينية على أساس مضاعفة جين 25SrDNA بينما النمط

الجيني IV (42.3%) الأكثر نسبة بين الأنماط الجينية على أساس مضاعفة RPS بينما النمط الجيني A-IV (26%) الأكثر نسبة بين الأنماط الجينية على أساس مضاعفة جين 25SrDNA and RPS.

**الاستنتاجات**: هناك علاقة واضحة خرجت بين توزيع الأنماط الجينية لسلالات الفطر التي تسبب التهابات القناة المهبلية وأعراض الالتهاب الفطري في القناة المهبلية لدى الحوامل والفئة العمرية للمريض في هذه الدراسة. على الجانب الاخر، لا يوجد علاقة واضحة بين توزيع الانماط الجينية لسلالات الفطر والعوامل المهيئة للمرض وأماكن سكن المرضى. ان الاختلافات في توزيع الانماط الجينية للفطر بين نتائجنا ونتائج الدراسات الاخرى تعزو لاختلافات في مجتمع الدراسة والخصائص البيانية والمواقع الجغرافية.