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Genotype Comparison of Strains of *Candida albicans* from Patients with Cutaneous Candidiasis in Nablus Area

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Dedication

To my dear father, sisters, brothers, wife, and daughters for their patience and encouragement, with love and respect

A CKNOLEDGEMENT

I would like to express my sincere special thanks and gratitude to my supervisor Professor Dr. Mohammed 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 Comparison of Strains of *Candida albicans* from Patients with Cutaneous Candidiasis in Nablus Area

مقارنة الطرز الجينية لسلالات فطر *C. albicans* من مرضى مصابون ب Candidiasis الجلدي

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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List of Abbreviations

SDA	**Sabouraud Dextrose Ager**
YPD	**Yeast Peptone Dextrose**
GTT	**Germ Tube Test**
Kac	**Potassium Acetate**
EDTA	**Ethylenediamine Tetraacetic Acid**
SDS	**Sodium Dodecyl Sulfate**
EtOH	**Ethanol**
TE	**Tris –EDTA**
TBE	**Tris/Borate/EDTA**
ICU	**Intensive Care Unit**
PCR	**Polymerase Chain Reaction**
RPS	**Repetitive Sequences**
ALT	**Alternative Lengthening of Telomerase**
Dntps	**Deoxynucleotide Triphosphates**
CC	**Cutaneous Candidiasis**
VVC	**Vulvovaginal Candidiasis**
CAC	**Chromagar *Candida***
PFGE	**Pulsed-Field Gel Electrophoresis**
RAPD	**Random Amplification of Polymorphic DNA**
SDW	**Sterile Distilled Water**

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Abstract

Background *Candida albicans* is one of the most important etiologic agents causing cutaneous candidiasis. It is uncertain whether genotypes of *Candida albicans* (*C. albicans*) are associated with colonizing body locations. The aim of this study was to compare genotypes of *C. albicans* strains causing cutaneous candidiasis in Palestinian infants in Nablus area, and to investigate whether there are significant associations between strain genotypes and body sites of infection and to determine the potential pathogenesis of cutaneous candidiasis at multiple locations.

Methods This study was conducted during the period July 2010 to November 2011. Subjects of the study were infants with cutaneous candidiasis from Nablus area. A total of 100 isolates of *C. albicans* were isolated from 100 infant patients with cutaneous candidiasis. Patients were grouped according to the body sites infection, age, gender, place of residence, 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. A clear correlation was found between genotypes and gender, and age. In addition, isolates of *C.*

albicans from multiple cutaneous locations of the same patient had identical genotypes. But no clear correlation was found between genotypes and the sites of cutaneous infection or predisposing factors, or residence. Genotype B was found to have the highest frequency (47%) on the basis of amplification of 25S rDNA, while genotype V was the highest frequency (34%) on the basis of amplification of RPS, and genotype B-V was the highest rates with (15%) on the basis of amplification of both 25S rDNA and RPS.

Conclusion A clear correlation was found between genotypes and gender, and age . On the other hand, we could not find a correlation between location of the CC lesions, residence, or predisposing factors and *C. albicans* genotype.

CHAPTER ONE

INTRODUCTION

1.1 Epidemiology

1.1.1 Introduction

The colonization of *Candida spp.* could be endogenous or exogenous (Pittet *et al.,* 1991; Voss *et al.,* 1994). The infection could be arised from invasion by the patients 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).

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, subacute or chronic to episodic, involvement may be localized in mouth, throat, skin, scalp, vagina, nails, bronchi, lungs or in the gastrointestinal tract or became systemic as in septicemia, endocarditis and meningitis (Rippon, 1988).

Cutaneous candidiasis usually occurs in warm, moist and creased area, such as auxiliary folds, inguinal or intergluteal areas. Cutaneous candidiasis is fairly common opportunistic disease and is usually caused due to maceration and trauma in skin (Rippon,1988; Borzotta & Beardsley, 1999).

As a common and widespread opportunistic yeast pathogen, *Candida albicans* has caused an increasing number of human cutaneous

candidiasis infections in recent years (Sobel *et al.*, 1998; McCullough *et al.*, 1999).

The rash in premature infants is extensive and extremely erythematous with severe desquamation. Invasive lung disease can also occur. Whereas congenital candidiasis in full term infants is a relatively benign condition that can be treated with topical antifungal agents. In premature infants it should be treated with systemic antifungal agents as this syndrome is associated with high rates of mortality and morbidity (Hebert & Esterly, 1986; Baley & Silverman, 1988).

1.1.2 The need for rapid and robust identification of *Candida albicans* to the species level

The genus Candida includes around 154 species that show different levels of resistance to antifungal agents. Consequently, it is important to identify the causative organism to the species level correctly. Identification of *C. dubliniensis* in particular, remains problematic because of the high degree of phenotypic similarity between this species and *C. albicans* (Neppelenbroek *et al*., 2005). Morphological features and reproductive structures useful for identifying isolated yeasts may take days to weeks to develop in culture and evaluation of these characteristics requires expertise in mycology (Mirhendi *et al*., 2006).

Candida albicans is one of the most frequently isolated yeasts in clinical laboratories. Different studies have shown that this organism

accounts for up to 80% of the yeasts recovered from clinical specimens (Rex *et al*., 2000). It has been associated with infections, as well as colonization, in both immunocompromised and immunocompetent patients (Wade, 1993).

It is well accepted that candidiasis is caused by several species of the genus *Candida* and *C. albicans* is the major etiologic yeast species of both superficial and deep candidiasis while the incidence of infections caused by *Candida* species other than *C. albicans* has gradually increased (Hazen, 1995; Kamiya *et al*., 2005). Recently, studies concerning the genotypes of *C. albicans* have been increasing based on the assumption of nosocomial candidiasis or sexual transmission (Schmed *et al*., 1993; Eloy *et al*., 2006).

In order to determine the possibility of such infection or transmission it is necessary that *C. albicans* be exactly discriminated at the strain level, because the results by genotyping systems with low discriminatory potential lead to misleading ideas concerning the surveillance of candidiasis (Dalle *et al*., 2008).

The susceptibility varies among the various genotypes of *C. albicans*. Hence in the management of candidiasis it is important to take in consideration the genotype of *C. albicans* strain (Zhu *et al*., 2011).

C. albicans genotype A strains showed increased levels of resistance to the antifungal agent flucytosine (Stevens *et al.,* 1990; Mercure *et al.*, 1993), and it has been postulated that there is a direct causal relationship

between the presence of the group 1 intron in the 25S rDNA (the presence of which determines that the strain should be classified as genotype B) and a decrease in the level of resistance to flucytosine (Mercure *et al*., 1993). This indicates that this group 1 intron is only partially present throughout the rDNA repeats in the genomes of *C. albicans* genotype C strains. It may be postulated that we are observing these strains during a period when this intron is being lost and they are moving from a genotype B strain to a genotype A strain and concurrently developing an increased level of resistance to flucytosine (McCullough *et al*., 1999).

1.1.3 Age

In healthy individuals, *candida*l infections are usually due to impaired epithelial barrier functions and occur in all age groups, but are most common in the newborn and elderly, because of less of activity of the immune system (Murray *et al.,* 2000).

1.1.4 Etiological agents and source of infection with *Candida*

Candida is a part of human flora. It becomes pathogenic when certain conditions e.g., (immature immune system, low pH, moisture, and recent administration of antibiotics) are present and becomes opportunistic infection (Kwon-Chung & Bennett, 1992). The major etiological agent is *C. albicans*, whereas different *Candida* species can cause a variety of infections (Bodey, 1984), 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 specimens like *C. catenulata, C. intermedia, C. lambica*, and *C. zeylanoides*. These species are therefore not considered as agents of opportunistic infections (Crozier *et al.*, 1977; Odds, 1988).

1.1.5 Incidence and prevalence

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

The immature, compromised epidermal barrier of the preterm infant may predispose to cutaneous infection and enable the organisms to penetrate into deeper layers of the skin to enter the bloodstream and cause invasive disease (Baley & Silverman, 1998).

The incidence of neonatal candidiasis is inversely proportional to birth weight; 2 to 7% of infants 1,500 g, 10% of infants 1,000 g, and 20% of infants 800 g develop either catheter-related or disseminated candidiasis (Baley *et al.*, 1988). According to earlier reports, *C. albicans* was the cause of 80-95% of cases of symptomatic fungal vulvovaginitis, whereas other *Candida* species such as *C. glabrata, C. parapsilosis* and *C.*

tropicalis , were responsible for the remaining cases (Vincent *et al.*, 1995; Nolla- Salas *et al.*, 1997).

1.1.6 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). 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 & Buchard, 1996).

Candida infections usually remain superficial and respond readily to treatment. Systemic candidiasisis is usually seen in patients with cell-mediated immune deficiency, and those receiving aggressive cancer treatment, immunosuppressants, or transplantation therapies. *Candida* is considered as an opportunistic pathogen (Lamagni *et al.*, 2001).

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 catheterization and taking advantage of impaired immunity in a debilitated patient to establish the disease (Davis *et al.*, 2000).

1.1.7 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 *C. 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). This switching can occur at the site of infection and may be of advantage to the survival of *Candida* to escape its environment constraint imposed by pH and temperature; it may evade host surveillance by changing antigenicity; it may conceivably alter resistance to antifungal agents. The mechanism by which *Candida* damages the genital epithelium is uncertain. The association of filamentous forms of *C. albicans* in the deeper layer of the mucous membrane with active disease, and their greater adhesiveness to epithelial cells than to blastophores, suggests that filamentous forms are important in the pathogenesis of candidiasis (Oriel, 1977).

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, subacute, chronic to episodic. The location of infection is usually localized to the scalp, vagina, mouth, throat, skin, finger, nails, bronchi, lungs, or the gastrointestinal tract, or becomes more complicated in systemic septicemia, meningitis, and endocarditis (Murray *et al.*, 2000).

1.2.1 Oropharengeal candidiasis

Severe immunological impairments which caused by certain diseases like leukemia, malignancy, diabetes mellitus, lymphoma, neutropenia, inhaled steroids and HIV are the main cause of acute oral candidiasis. This type of infection may occur in up to 5% newborn (Murray *et al.*, 2000).

1.2.2 Cutaneous candidiasis

This type of candidiasis is also called intertriginous candidiasis commonly seen in the intergluteal folds, groin, axillae, interdigital spaces, intra-and sub-intra mammary folds, and umbilicus. Infants under unhygienic conditions are subjected to diaper candidiasis which results from ammonitic irritation due to irregular change of unclean diapers and thus erythematous lesions will be developed (Murray *et al.*, 2000).

1.2.4 Chronic mucocutaneous candidiasis

Occurs in patients with various metabolic disturbances to cell-mediated immunity (Murray *et al.*, 2000). Other clinical manifestations are: urinary tract candidiasis, oseophageal candidiasis, neonatal and congenital candidiasis, hepatosplenic candidiasis, gastointestinal candidiasis, pulmonary candidiasis, osteoarticular candidiasis, candidemia (septicemia), ocular candidiasis, and many other forms (Murray *et al.*, 2000).

1.3 Identification of *Candida albicans*

Identification to the species level of yeasts isolated from clinical specimens is often problematic for diagnostic laboratories (Koehler *et al*., 1999).

1.3.1 Conventional approaches

1.3.1.2 CHROMagar *Candida* medium

Use of chromogenic agar (CHROMagar *Candida* medium) allows for prompt speciation of common *Candida* species C. *albicans*, C. *glabrata*, C. *parapsilosis* and C. *krusei* based on isolate colony colors (Pfaller *et al*., 1996; Ha *et al*., 2010; Adam *et al*., 2010).

CHROMagar *Candida* differentiates *C. albicans* from all non-*albicans* except *C. dubliniensis* (Adam *et al*., 2010).

Identification of yeast pathogens by traditional methods requires several days and specific mycological media. Chromogenic media contain

chromogenic substrates which react with enzymes secreted by the target microorganisms to yield colonies of varying colors. CHROMagar *Candida* (CaC) is one such medium that, can identify three *candida* yeasts, *C. albicans* (green colonies), *C. tropicalis* (steel blue colonies), and *C. krusei* (fuzzy, rose colored colonies) after 48 hours of incubation at 30–37°C. Independent groups have reported success with CaC in differentiating *C. dubliniensis* from *C. albicans* (Duane *et al*., 2006).

1.3.1.2 Germ tube test

Germ tube test was used in identifying C. *albicans* (kwon-chung & Bennett, 1992). *C .albicans* can be reliably identified in 2-4 hours using a germ tube test (Ha *et al*; 2010). Rapid identification of *C. albicans* is a key step in the diagnostic and treatment algorithm for bloodstream *Candida* infection to guide targeted and cost-effective antifungal strategy (Pappas *et al*., 2004). 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 solid agar (Shepard *et al*., 2008).

Germ tube formation is induced at 37°C. Above 33°C mycelia are formed while below that temperature, pseudo-mycelia are formed. Divalent cations are necessary for germ tube formation. It has been shown that magnesium ions are required for germination (Walker *et al*., 1984).

1.3.2 PCR-based techniques as a tool for clinical diagnosis of candidiasis

1.3.2.1 Polymerase chain reaction, PCR

PCR using primers specific for fungal DNA directly from blood, has been reported to be more sensitive than blood cultures, thereby potentially facilitating early diagnosis of fungemia (Leon *et al*., 2006).

However, contamination difficulties, sample volume and sample imprecision, optimum sampling frequency and difficulty distinguishing colonization and infection for validation purposes, mostly limits utility of PCR to the research setting (Willinger, 2006).

PCR-based techniques have contributed to the identification of fungal species from clinical specimens (Posteraro *et al*., 2000; Luo & Mitchell, 2002; kanbe *et al*., 2003; Kanbe *et al*., 2005). The PCR technique is commonly used for identifying *Candida* species (Shin *et al*., 1999; Kanbe *et al*., 2003) and has allowed the distinction of *C. albicans* from its morphologically close species C. *dubliniensis* (Kanbe *et al*., 2002).

Furthermore, several investigators have reported that *C. albicans* can be grouped into several genotypes by Southern hybridization, pulsed field gel electrophoresis (PFGE) and random amplification of Polymorphic DNA (RAPD) techniques (Doi *et al*., 1992; Schmid *et al*., 1993; Mehta *et al*., 1999; Pujol *et al*., 2002; Lian *et al*., 2004). However, regarding the points of cost and time for identification of *C. albicans* as Kanbe *et al*. (2005) reported, the PCR targeting the ALT repeat is more convenient than PFGE, sequencing and RFLP techniques.

1.3.2.1.1 PCR targeting 25S rDNA

PCR targeting 25S rDNA, which has frequently been used for genotypic 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). Furthermore, it is acknowledged that genotype D *C. albicans* corresponds to C. *dubliniensis* (McCullough *et al*., 1999; Tamura *et al*., 2001). However, problems such as long time, cost limitations, resolution and or special equipments still remain to be optimized for expanding C. *albicans* typing at the strain level (McCullough *et al*., 1999; Hattori *et al*., 2006; Iwata *et al*., 2006). However, most studies regarding the genotyping of C. *albicans* have focused on C. *albicans* isolates from infected lesions, feces, and vaginal mucosa. lt is generally acknowledged that genital, groin and perianal candidiasis are endogenously caused by *Candida* species, especially C. *albicans*, distributed as ubiquitous commensal yeasts on the mucous epithelium of digestive organs including feces. There is a lack of genetic evidence to verify the relationships of groin candidiasis with commensal *Candida* species and was expected that a genotyping method with high resolution will be a powerful tool for identifying the dissemination area, infection route or infection source of C. *albicans* for management of candidiasis (Hattori *et al*., 2006).

1.3.2.1.2 PCR targeting repetitive sequences (RPSs)

lt has been accepted that *C. albicans* chromosomes contain characteristic repetitive sequences (RPSs), each of which contains a tandem short repeating unit of 172 bp, designated alternative lengthening of telomerase (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). 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). The combination of PCR system targeting 25S rDNA and RPS producing a high performance as a tool for *C. albicans* genotyping (Iwata *et al*., 2006; Hattori *et al.,* 2006).

C. albicans strains have been subdivided into different biological groups based upon genetic subtypes (Tamura *et al*., 2001). 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; Xu *et al*., 1999; Lian *et al*., 2004; Hattori *et al*., 2006).

However, data on strains obtained from cutaneous candidiasis are rare. Furthermore, there has been little or no systematic studies comparing the genotypes of *C. albicans* and non-*albicans* stains from cutaneous

candidiasis. Until now, polymerase chain reaction (PCR) amplification (Lian *et al*., 2004) and restriction enzyme digestion analysis (Clemons *et al*., 1997) are two of the most frequently used techniques in indentifying the genotyping of *C. albicans* and non-*albicans* (Schmid *et al*., 1993; Mehta *et al*., 1999, Pujol *et al*., 2002).

PCR and restriction fragment length polymorphism (RFLP) are selected methods to identify the genotypes of *C. albicans* and non-*albicans* strains (Sullivan *et al*., 1996; Lian *et al*., 2004).

The molecular techniques used for identification of *Candida* species are very important because it takes a minimum of 24-48 h to culture and identify a species of *Candida* from a clinical specimen by conventional mycological procedures (Coleman *et al*., 1993) and even then there may be some difficulty in identifying some isolates definitively so there is a compelling need for rapid sensitive and specific tests to aid in the diagnosis of *Candida* infections (Magee *et al*., 1987).

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 (51.4%) comprised the majority of the clinical isolates followed by genotype B (27.3%) while genotype C (21.2%) was found as the third group in Japan.

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 Genotype A was the most predominant (60%) followed by genotypes B, C and D respectively in Kenya.

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%).

Mercure *et al*. (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.

1.4 Aims of this study

In this study, PCR targeting 25S rDNA and RPS were selected as a typing method to compare the genotypes of *C. albicans* strains from infant patients with cutaneous candidiasis in order to (a) compare genotypes of *C. albicans* strains causing CC in Palestinian infants, (b) determine the

potential differences in genotypes among *C. albicans* in relation to body surface locations, age, gender, predisposing factors and residence, and (c) determine the potential pathogenesis of CC at multiple locations.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Patients and clinical specimens

A total of one hundred infants patients, comprising 52 males and 48 females with cutaneous candidiasis, aged from 1 day to 18 months from 4 pediatric clinics during the period of June 2010 - December 2010 in Nablus city (Appendix A); were recruited into the study.

Patients were divided into seven subgroups according to the location of their lesions (groin, armpit, perianal, hip, neck and back); two infant patients had multiple locations with cutaneous lesions (Appendix D).

A total of 351 clinical specimens were collected from the infant patients with cutaneous candidiasis attending four pediatrician clinics in Nablus city. All suspected cases were interviewed and data was recorded using specially designed questionnaires included demographic data on age, gender, predisposing factors, place of residence (Appendix B).

Scales and macerated skin of infected lesions were collected as clinical specimens by passing sterile swabs over the infected region.

Swabs were immediately placed in a sterile culture tubes filled with Yeast Peptone Dextrose (YPD) liquid medium amended with chloramphenicol (50 µg/ml) (Appendix C). The culture tubes were then transported in ice pockets to the laboratory, followed by incubation of the tube with shaking at 37 ˚C (Shaking incubator, Human lab. Co, Korea) for 24-48 hours.

2.2 Isolation and identification of *Candida* species from primary isolates cultures

Identification of *C. albicans* isolates was based on their physiological and morphological characteristics.

Yeast suspension from YPD broth medium was streaked out on Sabouraud dextrose agar plates (SDA, Oxoid, Ltd, Basingstoke, UK)

supplemented with chloramphenicol (0.05 g/l) and incubated at 37 °C for 24-48 hrs. Plates with white colonies were examined by germ tube test for *Candida albicans*. Other plates with different colors were excluded and considered as negative for *C. albicans* (Figure 2.1).

Figure 2.1 *Candida albicans* grown on SDA Agar

The germ tube test (GTT) was carried out by picking a pure colony from SDA agar with a sterile swab (Kwon-Chung & Bennett, 1992).

The colony was suspended in 0.3-0.5 ml human serum at room temperature and the loop was rubbed against the wall of the tube. Serum cultures was incubated at 37 °C for 2.5-3 hours. 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 was observed in positive isolates (Figure 2.2).

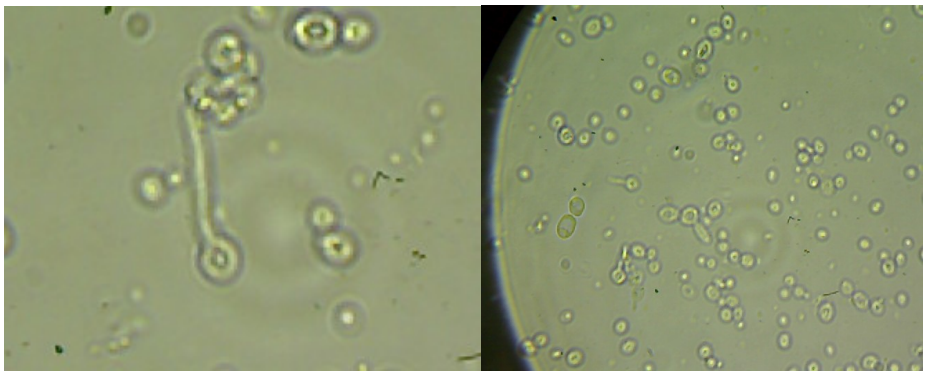

Figure 2.2 Micrograph showing formation of germ tubes by *C. albicans* grown in serum for 3 hours at 37 °C.

A single colony from SDA plates was streaked out on CHROMagar *Candida* (Oxoid, Ltd, Basingstoke, UK) and incubated for 24-48 hours at 30 °C (Pfaller *et al*., 1996). The appearance of light green colonies indicates the presence of *C. albicans* or *C. dublineinsis* (Figure 2.3).

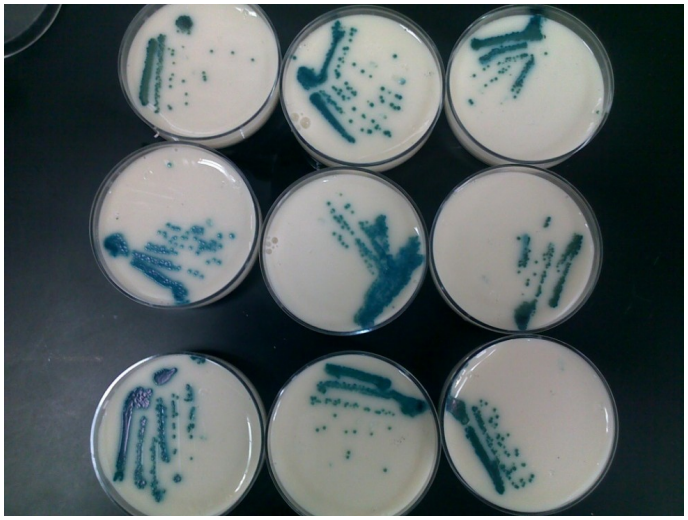

Figure 2.3 : *Candida albicans* grown on CHROMagar *Candida*

Single light green colonies from each plate were inoculated into a mixture of 800 µl of YPD broth and 200 µl of 86 % glycerol under highly sterile conditions and maintained at -80 °C**.**

2.3 Extraction and Purification of genomic DNAs

One single colony of each yeast isolate was inoculated in a tube containing 5 ml YPD broth and incubated overnight at 30 °C. The tubes were Centrifuged at 3000 rpm (Variable speed refrigerating multiple rotor, scientific Ltd, UK) for 5 minutes the supernatant was discharged . One ml of sorbitol (1M) was added to the pellet and transferred to 1.5ml tube followed by addition of 5u/µl 20 µl of lyticase (Sigma-Aldrich, Chemie GmbH, USA) to the tube 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 with 0.5 ml EDTA-SDS (50mM EDTA and 0.2% SDS) and incubated at 70 °C (Thermo mixer compact, Eppendorf, Germany) for 15 minutes, followed by addition of 50 µl of 5 M KAc. 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 (1:1) twice (vortex shortly) then centrifuged for 5 min., the supernatant was extracted twice with chlorophorm.

The supernatant was transferred to a new 2 ml tube and 1 volume 100% ethanol (stored at -20 °C) was added and the tube was inverted several times (DNA was seen). The tube was centrifuged 10 sec and the supernatant was discarded. The pellet was washed with 500 µl 70% EtOH

(stored at -20 °C), vortexed then incubated at room temp for 5 min. The tube was centrifuged for 10 seconds and the supernatant was discarded and the pellet was dried (Figure 2.4). Finally the pellet was resuspended in 50 µl TE buffer (Tris-EDTA) for 1 hour or overnight and DNA samples were stored at -20°C (Sambrook, 2001).

Figure 2.4 Tubes containing DNA samples before being resuspended in TE buffer

One hundred ml Tris- borate- EDTA (TBE) buffer was added to 0.8 gm agarose to prepare 0.8% agarose gel, boiled in microwave and the solution was poured in the rack of the electrophoresis chamber (Midi-Horizontal Electrophoresis Unit, Jencons, UK) for 30 min. Voltage 120 V (Electrophoresis power supply, Jencons, UK). The comb was put and left until the gel solidified. The rack of the electrophoresis chamber was transferred away from the electrophoresis system, TBE buffer was added as a running buffer. DNA samples were loaded as follow 2µl DNA, 3µl loading dye 10x commercially available and 5 µl sterile distilled water load the samples in the well by using micropipette. The first well was loaded with 5 µl 1 Kb DNA (Figure 2.5). Agarose gels were stained with 0.5

mg/ml ethidium bromide in distilled water at 21—25 °C for 20 min, and then washed 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(Nikon, Thailand).

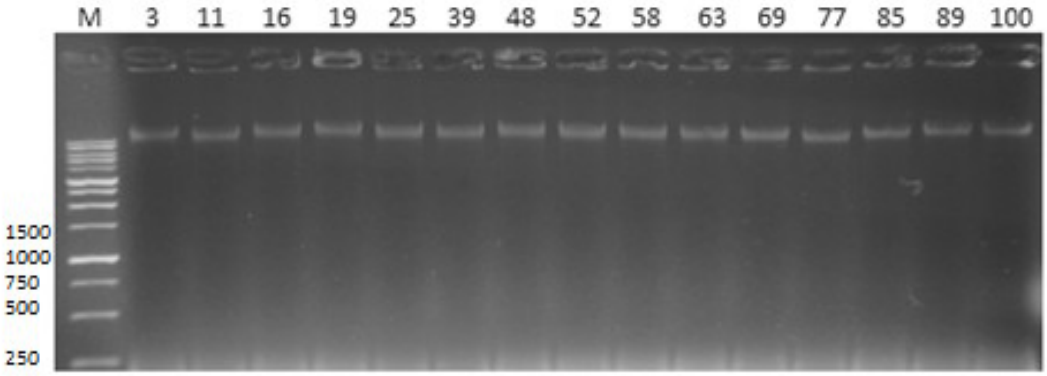

Figure 2.5 DNA samples from different isolates. M indicates DNA ladder. Numbers on the upper part of figure indicate numbers of *C. albicans* isolates.

2.4 PCR primers

For genotype determination of *C. albicans* on the basis of amplification of 25S rDNA, primers CA-INT-L and CA-INT-R were used. This primer set is referred as P-I in this study (Tamura *et al.*, 2001). For typing of *C. albicans* on the basis of ALT repeats, two further primers were newly designed on the basis of the nucleotide sequences of *C. albicans* RPS (Chibana *et al*., 1994), and were designated as ASDcF and pCSCR. This primer set is referred to as P-II in this study (Table 2.1).

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

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) as above.

C. albicans was grouped into six genotypes on the basis of the number of the most intense bands of PCR products of RPS (Table 2.2).

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 (25 µl) 1 µl genomic DNA, 2.5 µl 10 ×buffer, 2.5 µl MgSO4, 0.5 µl dNTPs (10mM), 1 µl forward primer (CA-INT-L for 25S rDNA or ASDcF for RPS) (10 µM) (Hylabs, Ltd., Israel) 1 µl reverse primer (CA-INT-R for 25S rDNA or pCSCR for RPS) (10 µM) (Hylabs, Israel), 0.2 µl Taq DNA polymerase (2.5 U/ml; Hylab, Ltd., Israel), And 16.3 µl sterile distilled water (Hattori *et al*., 2006; Iwata *et al*., 2006).

All the reaction mixtures and components were kept on ice.

PCR cycle parameters were as follows: Preheating at 96 °C for 120s; then 35 cycles of 96 °C for 30s, 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 % agarose gel for identification and genotyping of *C. albicans* on the basis of 25S rDNA. (Voltage 120 V for 30 minutes). While for RPS 1.2% agarose gel was used.

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

PCR products were loaded as follows: 3µl DNA, 2µl loading dye 10x commercially available and 5 µl sterile distilled water. The samples were loaded in the well by micropipette. 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 (Nikon, Thailand).

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 associations with other variables such as site of infection, gender, age, residence and predisposing factors. A P value < 0.05 was considered statistically significant.

CHAPTER THREE

RESULTS

3.1 Study population

One hundred infant patients with cutaneous candidiasis (CC) were recruited into the study. The patients, aged 1-18 months, comprising 52 males and 48 females; were divided into seven subgroups according to the location of their lesions (Table 3.1). Two of the infant patients had multiple locations with cutaneous lesions.

3.2 Strain number and *Candida* species

A total of one hundred isolates of *Candida* species were obtained from infant patients with cutaneous candidiasis (CC). Based on phenotypic identification, all isolates from infant patients were *C. albicans*.

3.3 Genotypic identification for all *C. albicans* genomic DNA isolates by PCRs targeting 25S rDNA and the RPS from different infant patients with (CC)

The genomic DNAs of the *C. albicans* isolates obtained from the above clinical specimens were amplified by PCR using P-I and P-II to determine the genotypes based on variations in the 25S rDNA and RPS, respectively.

The PCR profiles amplified with P-I defined DNA products of 450 bp for genotype A (Figure 3.1, lanes 6, 10, 11, 86, and 88), 840 bp for genotype B (Figure 3.1, lanes 4, 16, 36, 74, and 99) and both 450 bp and 840 bp for genotype C *C. albicans*, (Figure 3.1, lanes 56, 77, and 93). None of the PCR products in our study corresponded to 1040 bp long *C. dubliniensis*. Of the 100 *C. albicans* isolates 47 (47%), 37 (37%), and 16 (16%) were recognized as genotypes A, B, and C, respectively.

Frequency of genotypes A, B, and C among CC locations shown in (Table 3.1).

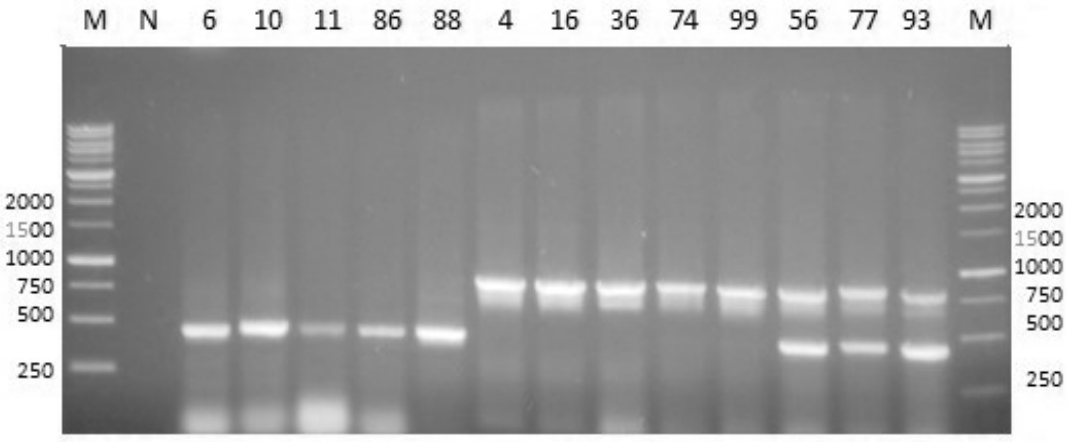

Figure 3.1 Amplification patterns and genotyping of *C. albicans* by PCR targeting 25S rDNA. Genomic DNAs were amplified by P-I for 25S rDNA- based genotyping. P-I amplifies DNA products of 450 bp for genotype A lanes (6, 10, 11, 86, 88), 840 bp for genotype B lanes (4, 16, 36, 74, 99), both 450 bp and 840 bp for genotype C lanes (56, 77, 93). 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 (Table 3.1) and subsequently were 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, lanes 6, 30, 32, 83: genotype II (698 bp); lane 29: genotype III (870 bp); lane 60: genotype IV (698/870 bp); lanes: 57, 59, 81, and 88: genotype V (698/870/1042); lanes: 37, 72, and 86: genotype VI).

Groups of clinical specimens and 25S rDNA type												Totals (%)			
Perianal (%)				Armpit (%)				Back (%)							
A	B	C	Total	A	B	C	Total	A	B	C	Total	A %	B %	C %	Total %
												1 (2.7)	1 (2.1)		2 (2)
	1		1 (7.7)	1			1 (7.7)	4			4 (33.3)	10 (27)	3 (6.4)		13 (13)
									5		5 (41.7)	2 (5.4)	12 (25.5)		14 (14)
2	2		4 (30.7)	1			1 (7.7)					9 (24.3)	7 (14.9)		16 (16)
1	3	3	7 (53.8)	2	4	2	8 (61.5)		1	1	2 (16.7)	9 (24.3)	15 (29.8)	10 (62.5)	34 (34)
1			1 (7.8)	1	1	1	3 (23.1)			1	1 (8.3)	6 (16.3)	9 (21.3)	6 (37.5)	21 (21)
4	6	3	13 (100)	5	5	3	13 (100)	4	6	2	12 (100)	37 (100)	47 (100)	16 (100)	100 (100)

Table. 3 . 1 Genotype variations of *C. albicans* isolates recovered from infant patients with cutaneous candidiasis on the basis of 25S rDNA and RPS.

RPS	Neck (%)				Groin (%)				Mouth (%)				Hips (%)			
	A	B	C	Total	A	B	C	Total	A	B	C	Total	A	B	C	Total
I	1			1 (7.7)						1		1 (5.3)				
II	1			1 (7.7)	3			3 (17.6)	1			1 (5.3)		2		2 (15.4)
III		2		2 (14.1)	2	2		4 (23.6)		1		1 (5.3)		2		2 (15.4)
IV	1	2		3 (23.3)	1	3		4 (23.6)	3			3 (15.7)	1			1 (7.8)
V	3	1	1	5 (38.7)		2	1	3 (17.6)		4	1	5 (26.3)	3		1	4 (30.7)
VI	1			1 (7.7)		3		3 (17.6)	3	3	2	8 (24.1)		2	2	4 (30.7)
Total	7	5	1	13 (100)	6	10	1	17 (100)	7	9	3	19 (100)	4	6	3	13 (100)

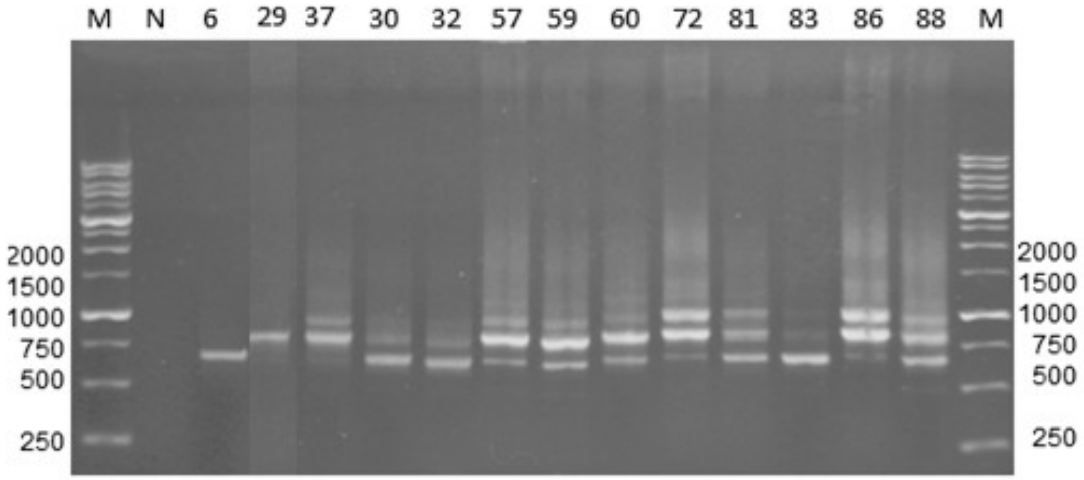

Figure 3.2 Amplification patterns and genotyping of *C. albicans* by P-II targeting (RPS). Numbers on the upper part of the figure refer to clinical isolates that gave 450 bp with P-I. lanes (6, 30, 32, and 83) were classified as A-II, (29) as A-III, (60) as A-IV, (57, 59, 81, and 88) as A-V, and lanes (37,72, and 86) as A-VI. 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 ratio of these six RPS genotypes and the genotype diversities in the cutaneous candidiasis locations are presented in Table (3.1).

Genotype V was found to be the most frequent one in all locations especially in armpit (61.5%), perianal (53.8%), neck (38.7%), hips (30.7%) and mouth (26.3%) among all CC isolates except in back (41.7%) and groin (23.6%) were genotype III was the most frequent. Genotype V comprised the highest frequency of all RPS types (34%) followed by VI, IV, III, II and I with(21%), (16%), (14%), (13%) and (2%), respectively (Table3.1).

Figure 3.3 shows the distribution of RPS types (P-II) among (P-I) genotypes A, B, C). Genotype II (27%)was the most frequent in genotype A, genotype V was the most frequent in genotype B (31.9%), and C (62.5%), distribution of RPS types in the 25S rDNA genotypes(A, B, C) see also (Table 3.1).

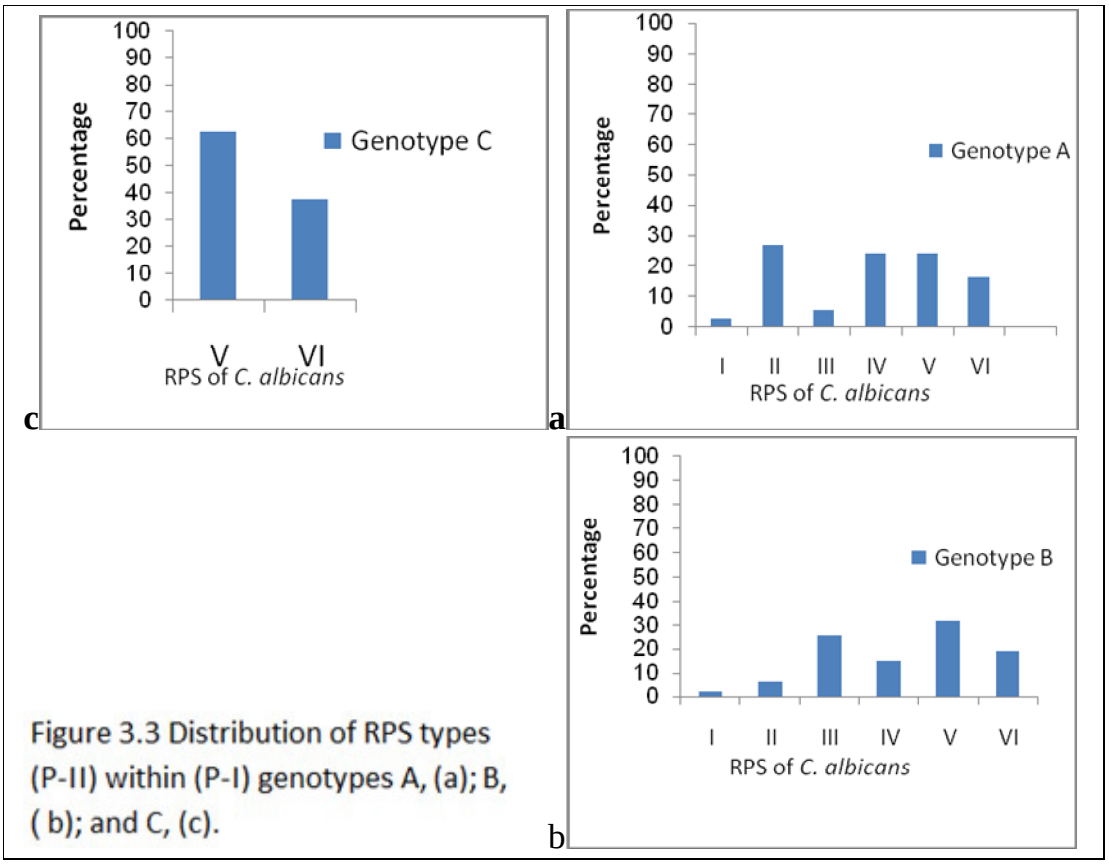

Figure 3.3 Distribution of RPS types (P-II) within (P-I) genotypes A, (a); B, (b); and C, (c).

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 genotypes were identified in our study (Table 3.2).

Table 3.2 The frequency and distribution of combined (25S rDNA and RPS) genotypes among CC locations:

Genotype	Number of isolates							Total %
	Neck	Groin	Mouth	Hips	Perianal	Armpit	Back	
A-I	1(14.3)							1 (1)
A-II	1(14.3)	3(50)	1(14.2)			1(20)	4(100)	10 (10)
A-III		2(33.3)						2 (2)
A-IV	1(14.3)	1(16.7)	3(42.9)	1(25)	2(50)	1(20)		9 (9)
A-V	3(42.8)			3(75)	1(25)	2(40)		9 (9)
A-VI	1(14.3)		3(42.9)		1(25)	1(20)		6 (6)
Subtotal A	7(100)	6(100)	7(100)	4(100)	4(100)	5(100)	4(100)	37(37)
B-I			1(11.1)					1 (1)
B-II				2(33.3)	1(16.7)			3 (3)
B-III	2(40)	2(20)	1(11.1)	2(33.3)			5(83.3)	12 (12)
B-IV	2(40)	3(30)			2(33.3)			7 (7)
B-V	1(20)	2(20)	4(44.5)		3(50)	4(80)	1(16.7)	15 (15)
B-VI		3(30)	3(33.3)	2(33.4)		1(20)		9 (9)
Subtotal B	5(100)	10(100)	9(100)	6(100)	6(100)	5(100)	6(100)	47(47)
C-V	1(100)	1(100)	1(33.3)	1(33.3)	3(100)	2(66.7)	1(50)	10 (10)
C-VI			2(66.7)	2(66.7)		1(33.3)	1(50)	6 (6)
Subtotal C	1(100)	1(100)	3(100)	3(100)	3(100)	3(100)	2(100)	16(16)
Total	13	17	19	13	13	13	12	100(100)

B-V (15%) was found to be the most frequent genotype among all CC isolates on the basis of amplification of P-I and P-II followed by B-III (12%), C-V (10%), A-II (10%) A-IV (9%), A-V (9%), and, B-III(9%) (Table 3.2).

Genotype A-V was the most frequent on the hips (75%) followed by neck (42.8%), and armpit (40%). While, genotype A-II had its highest frequency on the back (100%) and groin(50%), genotype A-VI on the perianal(50%) and mouth (42.9%), and the highest frequency of genotype A-VI was found on the mouth (42.9%) and hips (33.4%), B-III on the back (83.3%), neck (40%), and hips (33.3%). Genotype B-IV on the neck(40%),

B-V in the armpit (80%), perianal (50%), and mouth(44.5%), B-VI on the hips (33.4%) and mouth (33.3%), C-V on the neck, groin, and perianal (100%) for all, C-VI on the mouth (66.7%), hips (66.7%), and back (50%) (Table 3.2).

3.4 Genotypes of *C. albicans* isolated from multiple cutaneous locations in the same patient

In order to examine the homogeneity of the genotypes of *C. albicans* isolates from different infected lesions of the same patient, the PCR products amplified by P-I and P-II of four recovered isolates from two patients were compared (Table 3.3).

One female had two infected sites from mouth and armpit (34, 82) both of CC sites gave genotype A-V (Figure 3.4, lanes 34 and 82) and one male had two infected sites from neck and hips (9, 50) (Appendix D). Both of CC sites gave genotype A-IV (Figure 3.4, lanes 9 and 50).

Table 3.3 Genotypes of *C. albicans* from the same infant patients with cutaneous candidiasis at multiple locations:

Genotypes	No. of patients	Gender	Age	CC location
A-V	1	Female	6 months	Mouth/Armpit
A-IV	1	Male	18 months	Neck / Hips

The PCR profiles revealed that there were two genotypes among the four isolates of *C. albicans* in these two patients. There was some

genotyping variation among different patients. However, all isolates from each patient were identical regardless of the cutaneous locations.

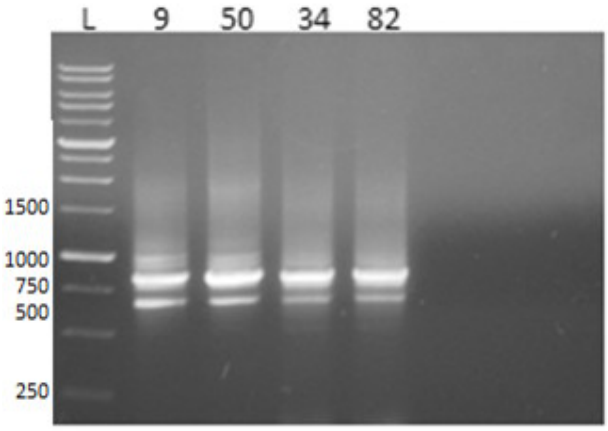

Figure 3.4 Amplification products with P-II from the same patients.

Isolates 9 and 50 from one patient, while 34 and 82 from another patient. L refers to 1kb ladder. Numbers on the upper part of the panel refers for isolates number.

3.5 Patterns of *C. albicans* genotypes distribution among CC infant patients in relation to different cutaneous locations and demographic characteristics of study population

Table 3.4, summarizes *C. albicans* 25S rDNA genotypes distribution among CC infant patients in relation to different cutaneous locations and demographic characteristics of study population. The frequency and distribution of genotypes among CC sites of the sample group indicates that there was not a CC site that contained only one genotype. Analysis of genotypes distribution of *C. albicans* in every subgroup of cutaneous locations did not show any obvious association between the isolates with a

certain genotype colonizing a specific cutaneous location (χ^2 = 5.144, df =12 *P*=0.953), with genotype A showing the highest frequency in neck (53.8%) and genotype B in groin (58.8%) and back (50%) (Table 3.4 and Figure 3.5).

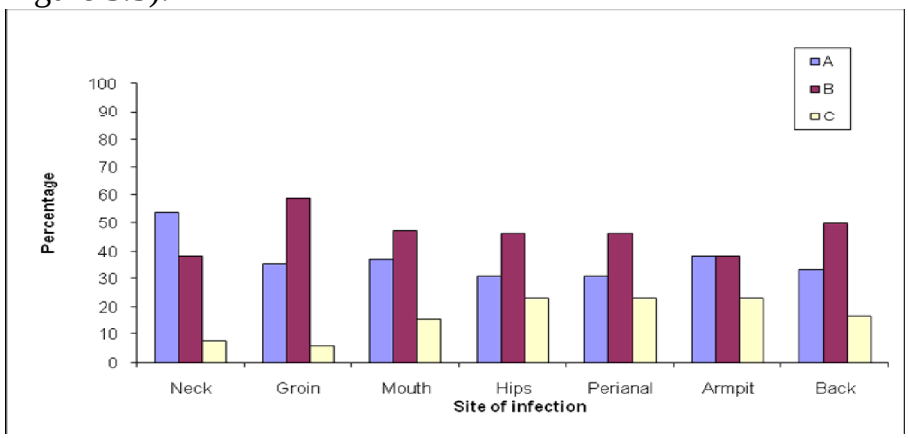

Figure 3.5 Distribution of CC isolates P-I genotypes in relation to site of infection.

The distribution of *C. albicans* genotypes (A, B, C) differed significantly between male and female patients(χ^2 = 16.876, df=2, *P* =.00001), with genotype A showing the highest frequency in females (52.1%) and genotype B in males (48.1%) (Table 3.4 and Figure 3.6).

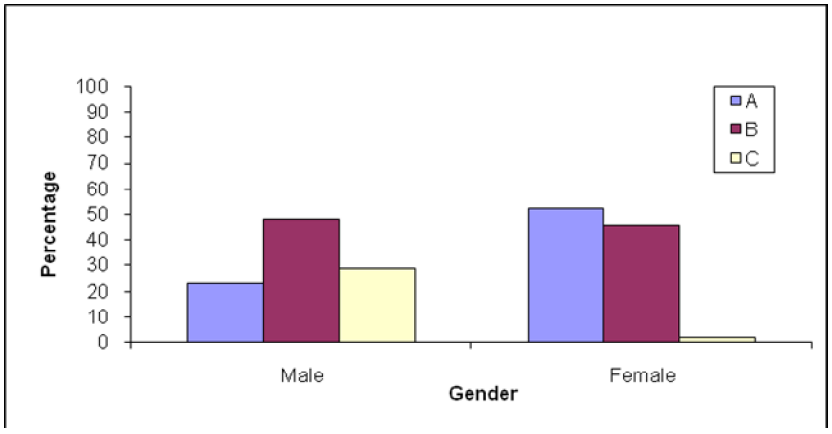

Figure 3.6. Distribution of CC isolates P-I genotypes in relation to gender.

Table 3.4 *C. albicans* 25S rDNA genotypes distribution among CC infant patients in relation to different cutaneous locations and demographic characteristics of study population

Variable	Genotype A Num (%)	B Num (%)	C Num (%)	Total	*P Value**
Site of infection					
Neck	7 (53.8)	5 (38.5)	1 (7.70)	(100)13	0.953
Groin	6 (35.3)	10 (58.8)	1 (5.90)	17 (100)	
Mouth	7 (36.8)	9 (47.4)	3 (15.8)	19 (100)	
Hips	4 (30.8)	6 (46.2)	3 (23.1)	13 (100)	
Perianal	4 (30.8)	6 (46.2)	3 (23.1)	13 (100)	
Armpit	5 (38.5)	5 (38.5)	3 (23.1)	13 (100)	
Back	4 (33.3)	6 (50.0)	2 (16.7)	12 (100)	
Gender					
Males	12 (23.1)	25 (48.1)	15 (28.8)	52(100)	0.00001
Females	25 (52.1)	22 (45.8)	1 (2.10)	48(100)	
Age (months)					
(0-4)	11 (28.9)	19 (50.0)	8 (21.1)	38(100)	0.033
(5-9)	13 (54.2)	9 (37.5)	2 (8.3)	24(100)	
(10-14)	4 (21.1)	14 (73.7)	1 (5.3)	19(100)	
(15-19)	9 (47.4)	5 (26.3)	5 (26.3)	19(100)	
Predisposing factors					
Low weight birth	3 (33.3)	5 (55.6)	1 (11.1)	9 (100)	0.946
Recent administration of antibiotics	17 (40.5)	19 (45.2)	6 (14.30)	42 (100)	
Intravenous catheters	1 (50.0)	1 (50.0)	0 (00.0)	2 (100)	
Diaper	5 (35.7)	5 (35.7)	4 (28.6)	14 (100)	
Tight clothes	3 (50.0)	3 (50.0)	0 (00.0)	6 (100)	
Iatrogenic immunosuppression	1 (50.0)	1 (50.0)	0 (00.0)	2 (100)	
No predisposing factors	7 (28.0)	13 (52.0)	5 (16.7)	25 (100)	
Residence					
City	12 (33.3)	17 (47.2)	7 (19.4)	36(100)	0.781
Village	15 (34.9)	22 (51.2)	6 (14.0)	43(100)	
Camp	10 (47.6)	8 (38.1)	3 (14.3)	21(100)	
Total	37	47	16	100	

* Statically significant at ($\alpha = 0.05$).

The distribution of genotypes also differed significantly among infant patients at different age groups (χ^2 = 13.698, df = 6, *P* = 0.033), with genotype A showing the highest frequency in age group (5-9) month (54.2%), B in age group (10-14) month (73.7%) and C in age group (15-19) month (26.3%) (Table 3.4 and Figure 3.7).

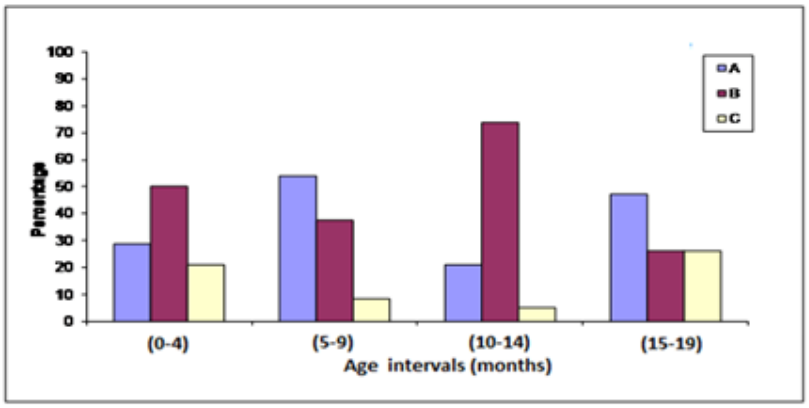

Figure 3.7 Distribution of CC isolates P-I genotypes in relation to age groups.

However, no statistically significant association was identified between genotypes and other demographic characteristics including predisposing factors (χ^2 =5.324, df=12,*P*= 0.946) and residence (χ^2 = 1.754 df =4 *P* =0.781)(Table 3.4). However, the highest frequency of genotype A in relation to predisposing factors was found in patients with low weight birth (53.8%), B in patients with recent administration of antibiotics (58.8%) (Table 3.4 and Figure 3.8).

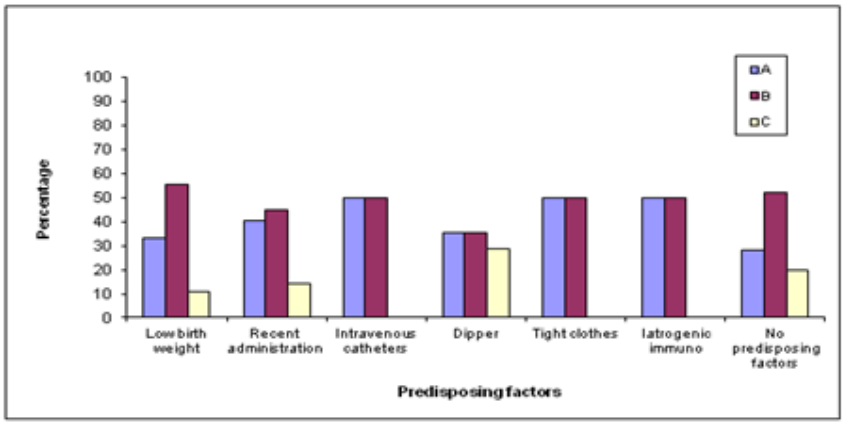

Figure 3. 8 Distribution of CC isolates P-I genotypes in relation to predisposing factors.

While the highest frequency of genotype A was found in patients residing in refugee camp (47.6%), B in patients living in rural areas and C in urban areas (19.4%) (Table 3.4 and Figure 3.9).

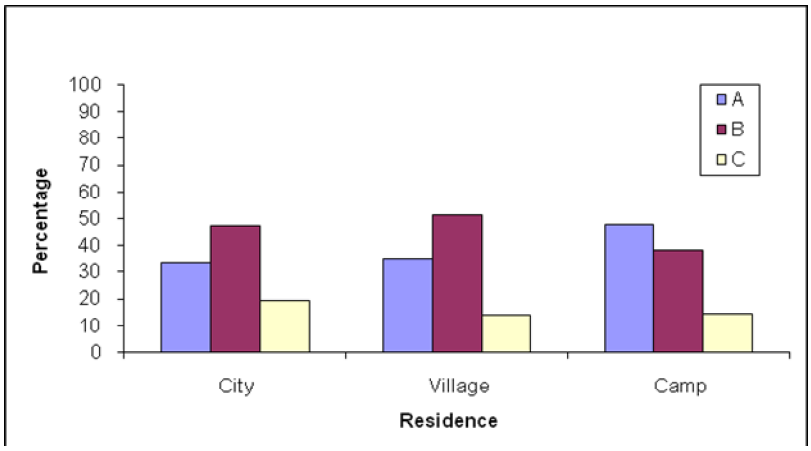

Figure 3.9 Distribution of CC isolates P-I genotypes in relation to residence.

Among variables the highest frequency of genotype A was found in females, recent administration of antibiotics, and patients living in rural areas, 67.6%, 45.9%, and 40.5%, respectively; genotype B in males 53.2%, and patients living in rural areas 48.6%, while genotype C was predominant

in males, , age group 0-4, and patients living in urban areas 93.8%, 50%, and 43.9%, respectively (Table 3.5).

Table 3.5 The percentage and distribution of 25S rDNA genotypes (A,B, C) in the variables (site of infection, gender, age, predisposing factors, and residence)

		***C. albicans* genotypes**		
		A	**B**	**C**
Variable type		**%No.**	**%No.**	**%No.**
Age (months)	(0-4)	11(29.7)	19(40.4)	8(50)
	(5-9)	13(35.1)	9(19.1)	2(12.3)
	(10-14)	4(10.8)	14(29.8)	1(6.2)
	(15-19)	9(24.4)	5(10.7)	5(31.5)
	Subtotal	**37(100)**	**47(100)**	**16(100)**
Gender	Male	(32.4)12	(53.2)25	(93.8)15
	Female	(67.6) 25	(46.8)22	(6.2)1
	Subtotal	**37(100)**	**47(100)**	**16(100)**
Residence	City	(32.4)12	(36.1)17	7(43.9)
	Village	(40.5)15	(48.6)22	(37.6)6
	Camp	(27.1)10	(17.1)8	(18.5)3
	Subtotal	**37(100)**	**47(100)**	**16(100)**
Site of infection	Neck	(18.9)7	(10.6)5	(6.2)1
	Groin	(16.3)6	(21.3)10	6.2)1
	Mouth	(18.9)7	(19.1)9	(18.8)3
	Hips	(10.8)4	(12.8)6	(18.8)3
	Perianal	(10.8)4	(12.8)6	(18.8)3
	Armpit	(13.5)5	(10.6)5	(18.8)3
	Back	(10.8)4	(12.8)6	(12.4)2
	Subtotal	**37(100)**	**47(100)**	**16(100)**
Predisposing factors	Low birth weight	(8.1)3	(10.6)5	(6.2)1
	Recent administration of antibiotics	(45.9)17	(40.5)19	(37.6)6
	Intravenous catheters	(2.7)1	(2.1)1	
	Diaper	(13.6)5	(10.6)5	(25)4
	Tight clothes	(8.1)3	(6.4)3	
	Iatrogenic immunosuppression	(2.7)1	(2.1)1	
	No predisposing factors	(18.9)7	(27.7)13	(31.2)5
	Subtotal	**37(100)**	**47(100)**	**16(100)**

CHAPTER FOUR

DISCUSSION

In this study, cutaneous 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), no *C. dubliniensis* was detected in our clinical specimens. To our knowledge no *C. dubliniensis* has been reported from Palestinian patients yet, although it is possible that *C. dubliniensis* does exist in a low frequency in the Palestinian population.

An epidemiological study on CC on school children was conducted in Nablus city by Aslan (2004), in that study *C. dubliniensis* was not detected, but she found that the most prevalent species observed in her study among school children were found to be *C. guillermondii* (males, 62.6%; females, 26.2%), *Tricosporon spp*. (males; 5.5%, females; 21.4%) and *C. albicans* (males, 5.5%; females, 19%). While in a study in Israel for genotyping clinical isolates from stool, urine, vaginal, throat, and blood in Israel, 3.8% of them were *C. dubliniensis* (McCullough *et al*., 2004).

PCR methods can detect extremely small quantities of DNA and cause earlier detection of pathogenic fungi and consequently allow earlier beginning of antifungal therapy that may improve chances of survival. These methods can directly detect the presence of fungi with high degree of specificity and sensitivity (Mirhendi, & Makimura, 2003).

For accurate treatment of candidiasis using antifungal drugs, it is necessary to discriminate correctly at the species level because of the different levels of susceptibility to antifungal drugs among the species. Furthermore, to elucidate the infection route of candidiasis, it is necessary to discriminate the pathogen at the strain level (Hattori *et al*., 2009).

For determination of *C. albicans* on the basis of 25S rDNA, primers CA-INT-L and CA-INT-R that span the site of the transposable intron in the 25S rDNA (Tamura *et al*., 2001) were used in our study. This method has been confirmed as a specific and reproducible method of genotype analysis of *C. albicans* (Hattori *et al*., 2006).

In our work *C. albicans* was genotyped from clinical sources in Nablus city from infants with cutaneous candidiasis and identified three genotypes namely, A, B, and C on the basis of targeting 25S rDNA. This is the first study documenting the genotype distribution of *C. albicans* from infants with CC in the Palestinian Authority territories.

Genotypic analysis not only categorizes *C. albicans* into five (A, B, C, D, E) genotypes but can also discriminate against *C. dubliniensis*, according to Sullivan and Coleman (1998).

In our study the most prevalent *C. albicans* genotype among CC isolates on the basis of amplification of 25SrDNA was genotype B (47%), followed by genotype A (37%),and C (16%). Similar studies were done in Turkey by Gurbaz and kaleli (2010) on a total of 194 *C. albicans* isolates which were isolated from various body location such as sputum, urine, blood and other locations, were genotyped on the basis of amplification of transposable group-1 intron in the 255 rDNA. The frequencies of genotypes A,B,C and D were found as 51%, 29.4%, 19.1% and 0.5%, respectively. Another study in Turkey by Karahan *et al*. (2004) showed that genotype A (50.2%) was the most predominant genotype among invasive isolates and

non-invasive isolates from sputum, throat, urine, and feces, followed by genotype C (31.9%), and genotype B (17.9%).

A study in Israel by McCullough *et al*. (2004) in which *C. albicans* was isolated from stool, urine, vaginal, throat, and blood showed that genotype A has the highest frequency 40%, followed by 33% and 27% for genotypes C and B, respectively.

The ratios of genotypes A, B and C, of *C*. *albicans* varied among the reports, where the ratio of genotype B or C to genotype A *C.albicans* varied in each group of clinical specimens. These findings may be affected by the kinds of clinical specimens colonized by *C*. *albicans* (Iwata *et al.*, 2006). No *C. dubliniensis* was found in our study but a study in Kuwait by Ahmad *et al*. (2005) on 390 germ tube positive isolates of *Candida* species only 3% of them were identified as *C. dubliniensis* by using the sequences of the universal forward and reverse fungal primers for amplifying of 3' end of both 5.8S and 28S rDNA. In Saudi Arabia Fotedar and Al-Hedaithy (2003) studied 823 germ tube and chlamydospore positive *C. albicans* isolates at the University Hospital in Riyadh, 27(3.3%) isolates were identified as *C. dubliniensis*. In Jordan an epidemiological study by Issa *et al*. (2011) on the distribution of *Candida* species isolates colonizing oral and rectal sites of 61 infant patients, revealed that *C. albicans* comprised the majority (67.1%) of *Candida* species while no *C. dubliniensis* were observed.

It is important to identify *C. albicans* at the strain level because there is a correlation between the *Candida* groups and antifungal susceptibility (McCullough *et al.*, 1999; Tamura *et al*., 2001). *C. albicans* genotypes (genotypes A, B, and C) and the susceptibility, results indicated that strains of *C. albicans* genotype A were significantly less susceptible to flucytosine than either *C. albicans* genotype B or C *C. albicans* strains (McCullough *et al.*, 1999; Tamura *et al*., 2001). Another study in Nigeria on VVC showed that genotype A resistance to fluconazole was quite high (Emmanuel *et al.,* 2012).

According to the definition of McCullough *et al*. (1999) genotype C isolates carry the two bands belonging to genotype A and B (450 and 840 bp). The existence of these two bands can be explained with the occurrence of the transposable group 1 intron in some of the repetitions of 25S rDNAs in genotype C genome while in some of the repetitions it does not carry this intron. For that reason genotype C isolates can be seen as an intermediate form occurring during the transition from genotype A to genotype B or genotype B to genotype A or as a result of the sexual reproduction between genotype A and genotype B (McCullough *et al.,* 1999).

Targeting the repetitive sequence of *C. albicans,* the PCR has been used by several researchers for targeting the reported repetitive sequences (RPS) in order to differentiate *C. albicans* from non-*albicans* (Knabe *et al*., 2005; Iwata *et al*., 2006; Hattori *et al.,* 2006 and 2009). In this study we

used this method with all isolates in order to check the accuracy of *C. albicans* identification.

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 number of ALT repeats in the RPS vary in each chromosome, thereby leading to variation in the molecular sizes of RPSs (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. Iwata *et al*. (2006) reported that molecular techniques using PCR provide the evidences that the PCRs targeting the 25S rDNA and the ALT repeats in the RPS sequences were rapid and simple techniques for genotyping of *C. albicans*, and were useful not only for discrimination of *C. albicans* from its related species *C. dubliniensis* and *C. stellatoidea*, but also for management and

control of *Candida* infections at the molecular level in the field of the dermatological science.

So in this study, we used PCR targeting the RPS that can rapidly, simply and specifically identify *C. albicans* at the species level, and we found six genotypes named genotypes I, II, III, IV, V and VI based on PCR amplification of RPS profiles generated by primer pair P-II these genotypes were based on the number of the intense bands.

We found that RPS type V constituted the highest genotype of all RPS types (34%) followed by VI, IV, III, II and I with 21%, 16%, 14%, 13% and 2%, respectively.

These findings differ from those of Hattori *et al*. (2006) and (2009) who reported that genotype III (70.7%) *C. albicans* constituted the majority of the isolates in all of the 25S rDNA-based genotypes, followed by genotype VI (17.1%) *C. albicans* were found from different body locations of patients with superficial candidiasis in Japan.

These findings also differs from those of Iwata *et al*. (2006) who reported that genotype III (70.4%) constituted the majority of the isolates in all of the 25S rDNA-based genotypes, followed by genotype VI (21.3%) *C. albicans* were found from clinical specimens from scale, nail, vaginal secretion, sputum and others in Japan.

In this study genotype B-V (15%) was found to have the highest frequency among all CC strains genotypes. This finding differed from that

found in a study by Xiao-dong *et al*. (2008) in which A-I (53.6%) had the highest frequency among CC strains, and those of Hattori *et al*. (2006) on superficial candidiasis and Iwata *et al*. (2006) from scale, nail, vaginal secretion, sputum, and other clinical specimens, in which genotype A-III (34%) had the highest frequency among all strains. These variations could be correlated with type of isolate, strain and patient's age.

However, regarding the points of cost and time for identification as Kanbe *et al*. (2005) reported, the PCR targeting the ALT repeat is more convenient than PFGE, sequencing and RFLP techniques.

Hattori *et al*. (2009) reported that in PCRs using P-II *C. albicans*, different electrophoretic patterns were observed between strains indicating that both the repeat numbers and arrangement order of the ALTs in each RPS are variable among strains. While Knabe *et al*. (2005) reported that *C. dubliniensis* showed relatively constant profiles among the strains of each species, and the amplification profiles of each species were unique, the major band numbers of *C. albicans*, based on intensities were bands 3-5 while for *C. dubliniensis* 5-7 bands . Kanbe *et al*. (2005) reported also that the major patterns of RPS in *C. albicans* contains 3 or 4 ALT repeats, and that differences between the amplification patterns of *C. dubliniensis, C. albicans* and *C. stellatoidea* are due to the annealing site for the forward primer rather than the repeat number, indicating that the nucleotide sequences of the RPSs differ between *C. dubliniensis, C.albicans* and *C. stellatoidea.*

The relationships between genotype and CC locations, predisposing factors, age, gender, and residence.

After analyzing the genotypes in the cutaneous candidiasis isolates, there was no significant association between genotypes of isolates and there invasive body surface locations, or predisposing factors. This finding is in agreement with that of Xiao-dong *et al*. (2008) who also found no clear association between genotypes and the site of cutaneous infections, probably due to the fact that the influences of different skin surfaces in the cutaneous locations were minor and could not influence the genotype of strains.

On the other hand, we demonstrated that there was a significant association between genotypes of strains isolated from CC in this study, and gender or age of patients. These findings support the hypothesis that genotypic differences among *C. albicans* strains might be correlated with their invasive environments (Kwon-Chung & Bennett, 1992; Hattori *et al*., 2006; Xiao-dong *et al*., 2008), these variations of genotypes among *C. albicans* among these studies also may be due to differences in geographic locales (Clemons *et al*., 1997) and related to differences in study populations of *C. albicans* strains such as patient's age.

***C. albicans* isolated from different locations from the same patients with CC**. We found that different infected regions from the same

individual were found to have the same genotype (Table 3.3 and Figure 3.4).

The genotypes of strains isolated from different cutaneous locations were found to be identical.

This finding is in agreement with that of Xiao-dong *et al.*(2008), Abbes *et al*. (2008), and Hattori *et al*. (2006) who found that the genotypes of isolates recovered from different CC locations were similar. It was thus assumed that the pathogen of multiple *C. albicans* cutaneous infections in infants were not caused by different exogenous pathogenic strains (Xiao-dong *et al.*, 2008).

In conclusion, a strong correlation was found between the genotypes of *C. albicans* and patient's gender and age. Furthermore, in the same patient there was usually one genotype of *C. albicans* colonizing various CC locations. On the other hand, we could not find a correlation between location of the CC lesions, residence, or predisposing factors and *C. albicans* genotype.

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Appendixes

Appendix A

Name of pediatricians from where isolates were taken

Table 2.1 Distribution of infants with cutaneous candidiasis in Nablus city.

Pediatrician	**Male**	**Female**	**Total**
Dr. Abdellah othman	**24**	**20**	**44**
Dr. Abdellah hamdan	**4**	**3**	**7**
Dr. Omar abu zytoon	**2**	**1**	**3**
Dr. Ayoob hamdan	**22**	**24**	**46**
Total	**52**	**48**	**100**

Appendix B

Genotype comparisons of strains of *Candida albicans* from patients (infants) with cutaneous candidiasis from Nablus area

Number: Date of collection:

Sampling site	Back	Groin	Armpit	perianal	hip	Neck

Health institution and person in-charge :

Patient name : age : gender :

Medical history :

Previous (before 2 months) : type	duration
Present disease	Initiation date
Drugs used	Treatment period
Symptoms:	

Predisposing Factors

Low-birth (Yes , No)	preceding surgery (Yes , No)

weight	
tight clothes (Yes , No)	diaper (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)	Iatrogenic immunosuppression (Yes , No)

Appendix C

solutions Preparations

SDA broth:

0.5 gm yeast extract 1 gm peptone 2 gm dextrose were dissolved in 100ml distilled water then autoclaved.

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

70% Ethanol:

30 ml distilled water were added to 70 ml of pure ethanol(100%)

YPD agar:

65 gm of commercially available powder were dissolved in 1 L distilled water and autoclaved then chloramphenicol (50µg /ml) was Dissolved in 2 ml of absolute alcohol.

CHROMagar candida :

16.5 gm of powder were dissolved in 500 ml distilled water and boiled with shaking for 4 minutes (do not autoclave).

86% glycerol:

14 ml of distilled water were added to 86 ml of pure glycerol and autoclaved

1 M Sorbitol:

18.217gm were dissolved in 100ml sterile distilled water.

5u/ul lyticase:

2001.24 µl sterile distilled water were added to the components7.65 mg (1308 unit/ 1mg contains in 261.6 ul /mg) and stored at -20 c .

50mM EDTA - 0.2% SDS:

- 50mM EDTA

1.46 gm were dissolved in 50 ml sdw at PH 8 by using NaOH 1 M

- 0.2% SDS until dissolved completely.

then 0.2 gm SDS were added then volume was completed to 100 ml

5 M (potassium acetate) KA c :

4.9075gm of potassium acetate were dissolved in10ml of s terile distilled water

TE buffer (Tris-EDTA):

1. 2.92 gm were dissolved in 50 ml sdw at PH 8 by using NaOH (1 M)

2.then 1. 2114 gm Tris –HC l were added then volume was completed to 100 ml

10 µ M CA-INT-L a:

590 µl sterile distilled water were added to 59 n mol of the primer the molarity should be 100 µ M then 10µ L of 100 µ M primer were added to 90 µ L sterile distilled water to prepare 10 µM of the primer

10 µM CA-INT-R a:

636 µl sterile distilled water were added to 63.6 n mol of the primer the molarity should be 100 µ M then 10µ L of 100 µ M primer were added to 90 µ L sterile distilled water to prepare 10 µM of the primer

10 µM PcSCRC-R:

824 µl sterile distilled waterwere added to 82.4 n mol of the primer the molarity should be 100 µ M then 10µ L of 100 µ M primer were added to 90 µ L sterile distilled water to prepare 10 µM of the primer

10 µM ACDCFC-F:

686 µl sterile distilled water were added to 68.6 n mol of the primer the molarity should be 100 µ M then 10µ L of 100 µ M primer were added to 90 µ L sterile distilled water to prepare 10 µM of the primer

TBE buffer(1 L) 5X:

2.922g EDTA were dissolved in 20ml of sdw at PH 8, 54 gm of TRIS – base, and 27.5 gm boric acid then volume were completed too 1 liter.

1 liter of 1x TBE buffer:

200 ml of 5x TBE buffer were added to 800 ml distilled water.

Appendix D

List of patients, infected lesions, residence, age and *Candida albicans* subgroups according to amplification of 25S rDNA:

Specimen No.	Residence	Gender	Age	Lesion	*C. albicans* Subgroups (P-I)
1	City	F	14 days	Neck	B
2	Village	F	6 months	Neck	A
3	City	M	11 months	Neck	A
4	City	F	12 months	Neck	B
5	Camp	F	13 months	Neck	B
6	Village	M	5 months	Neck	A
7	Village	M	16 months	Neck	C
8	Village	F	14 months	Neck	B
9	Village	M	18 months	Neck	A
10	Camp	F	10 months	Neck	A
11	Village	M	14 days	Neck	A
12	City	F	5 months	Neck	A

13	Camp	F	10 months	Neck	B
14	Village	F	4 months	Groin	B
15	Camp	F	8 months	Groin	B
16	City	F	11 months	Groin	B
17	Camp	M	18 months	Groin	C
18	City	M	18 months	Groin	B
19	Village	F	17 months	Groin	B
20	Camp	M	11 months	Groin	B
21	Village	M	12 months	Groin	B
22	City	F	4 months	Groin	A
23	Village	F	7 months	Groin	A
24	Camp	M	8 months	Groin	A
25	Village	M	8 months	Groin	B
26	Village	M	17 months	Groin	B
27	Camp	M	16 months	Groin	A
28	City	F	15 days	Groin	B
29	City	F	2 months	Groin	A
30	Camp	F	2 months	Groin	A
31	Camp	M	8 months	Mouth	B
32	Camp	F	2 months	Mouth	A
33	City	M	14 days	Mouth	B
34	City	F	6 months	Mouth	A
35	City	M	5 months	Mouth	C

36	City	F	20 days	Mouth	B
37	City	M	8 months	Mouth	A
38	Camp	F	9 months	Mouth	A
39	City	F	14 days	Mouth	B
40	City	M	40 days	Mouth	B
41	Camp	F	5 months	Mouth	B
42	Camp	M	1 month	Mouth	B
43	City	M	2 months	Mouth	C
44	Village	M	4 months	Mouth	B
45	Village	M	1 month	Mouth	C
46	Village	F	4 months	Mouth	A
47	Camp	F	9 months	Mouth	A
48	Village	F	15 months	Mouth	A
49	Village	M	10 months	Mouth	B
50	Village	M	18 months	Hips	A
51	Village	M	4 months	Hips	B
52	City	M	3 months	Hips	C
53	City	F	2 months	Hips	B
54	Camp	F	7 days	Hips	C
55	Village	M	4 months	Hips	B
56	Camp	M	22 days	Hips	C
57	Village	F	4 months	Hips	A
58	Village	F	35 days	Hips	B

59	City	M	6 months	Hips	A
60	Camp	F	16 months	Hips	A
61	City	M	4 months	Hips	B
62	Village	M	8 months	Hips	B
63	Village	M	2 months	Perianal	B
64	Village	M	11 months	Perianal	C
65	City	M	15 months	Perianal	B
66	Village	M	2 months	Perianal	C
67	Village	M	3 months	Perianal	C
68	City	F	12 months	Perianal	B
69	Village	F	17 months	Perianal	A
70	Village	M	3 months	Perianal	B
71	City	F	11 months	Perianal	A
72	City	F	20 days	Perianal	A
73	Village	F	16 months	Perianal	A
74	Village	M	7 months	Perianal	B
75	Village	F	6 months	Perianal	B
76	Village	F	11 months	Armpit	B
77	City	M	15 months	Armpit	C
78	Camp	M	15 months	Armpit	B
79	City	M	4 months	Armpit	C
80	City	M	1 month	Armpit	B
81	Camp	F	1 month	Armpit	A

82	City	F	6 months	Armpit	A
83	Village	F	3 months	Armpit	A
84	City	F	13 months	Armpit	B
85	City	F	7 months	Armpit	B
86	City	F	6 months	Armpit	A
87	Village	M	15 months	Armpit	C
88	Village	M	8 months	Armpit	A
89	Village	M	11 months	Back	B
90	Village	F	7 months	Back	B
91	Village	M	11 months	Back	B
92	Village	M	14 days	Back	B
93	City	M	15 months	Back	C
94	City	M	15 months	Back	A
95	Camp	F	16 months	Back	A
96	village	M	10 months	Back	A
97	City	M	5 months	Back	C
98	Village	F	3 months	Back	A
99	City	F	10 months	Back	B
100	Village	M	4 months	Back	B

Appendix E

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

5'(CAACCAAGCGCGGGTAAACGGCGGGAGTAACTATGACTCTC
AACCTATAAGGGAGGCAAAAGTAGGGACGCCATGGTTTCCAG
AAATGGGCCGCGGTGTTTTTGACCTGCTAGTCGATCTGGCCAG
ACGTATCTGTGGGTGGCCAGCGGCGACATAACCTGGTACGGG
GAAGGCCTCGAAGCAGTGTTCACCTTGGGAGTGCGCAAGCAC
AAAGAGGTGAGTGGTGTATGGGGTTAATCCCGTGGCGAGCCG
TCAGGGCGCGAGTTCTGGCAGTGGCCGTCGTAGAGCACGGAA
AGGTATGGGCTGGCTCTCTGAGTCGGCTTAAGGTACGTGCCGT
CCCACACGATGAAAAGTGTGCGTGCAGAATAGTTCCCACAGA
ACGAAGCTGCGCCGGAGAAAGCGATTTCTTGGAGCAATGC
TTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGC)3'

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 F

One of the nucleotide sequences of ALT repeats (172 bp) of *C. albicans*

5'(GAATTTGCGGTGATGTCCGTTGAAGACTGCGCGATGAAAAAT
AACGCTACAAAAATCAAACTAGTGCCGATTTATACCTTTTTCTTA
TGAGTGCTAACCATGCAAGAACTGTTAGAAACGAAATACAACTG
CTATCTGTGGAACAAAAAAGGCCGTTTTGGCCATAGTTAAG)3'
(Hattori *et al*., 2009)

Appendix G

Predisposing factors for infants with cutaneous candidiasis:

Patients No	Low birth weight			Recent administration of antibiotics			Intravenous catheters			Diaper			Tight clothes			Iatrogenic Immuno-suppression			No predisposing Factors		
	C. albicans subgroups			*C. albicans* subgroups			*C. albicans* subgroups			*C. albicans* subgroups			*C. albicans* subgroups			*C. albicans* subgroups			*C. albicans* subgroups		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
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	107																				

جامعة النجاح الوطنية
كلية الدراسات العليا

مقارنة الطرز الجينية لسلالات فطر *Candida albicans* من مرضى مصابون بـــ Candidiasis الجلدي

إعداد
نهاد حسين أحمد العثمان

إشراف
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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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الأستاذ الدكتور محمد سليم اشتيه

مشرف مشارك

الدكتور صبري ناصر

الملخص

الخلفية:

يعد فطر *C. albicans* من أهم الميكروبات التي تصيب الجلد خاصة لدى الأطفال. وبات من غير المؤكد فيما إذا كان نوع النمط الجيني لسلالات الفطر له علاقة بمواقع الإصابة. من هنا تهدف هذه الدراسة إلى التحقق فيما إذا كانت هناك علاقة معنوية بين الأنماط الجينية لسلالات الفطر ومواقع الالتهاب الفطري في الجلد.

الأساليب:

امتدت الدراسة من شهر تموز 2010 حتى شهر تشرين الثاني 2011 حيث تم عزل 100 سلالة من فطر *C. albicans* من 100 طفل من منطقة نابلس مصابين بمرض فطري جلدي. ثم تم تصنيف سلالات الفطر بالاعتماد على مواقع الإصابة، والجنس، ومكان السكن، والعوامل المؤهبة، والعمر وأخيرا تم تحديد النمط الجيني باستخدام تقنية PCR من خلال مضاعفة الجين 25S rDNA و مضاعفة RPS.

النتائج:

ظهر 14 نمطا جينيا مختلفا، فقد وجدنا أن النمط الجيني B (47%) الأكثر نسبة بين الأنماط الجينية الناتجة عن مضاعفة الجين 25S rDNA بينما النمط الجيني V (34%) الأعلى نسبة بين الأنماط الجينية الناتجة عن مضاعفة RPS والنمط الجيني B-V (15%) الأكثر تكرارا عند مضاعفة الجين 25S rDNA و RPS. فقد وجد الباحث علاقة بين توزيع

الأنماط الجينية والعمر إضافة إلى أن هناك علاقة بينها وبين الجنس، بينما لم يجد علاقة واضحة بين توزيع الأنماط الجينية ومواقع الإصابة أو مكان السكن أو العوامل المؤهبة.

الاستنتاجات:

توصلنا في هذا البحث إلى وجود علاقة واضحة بين الأنماط الجينية وبين عمر وجنس الأطفال. اضافة إلى ذلك، في نفس المريض المصاب بأكثر من موقع كان هناك نوع واحد من الأنماط الجينية،بينما لم نستطع التوصل إلى علاقة واضحة بين الأنماط الجينية ل *C. albicans* ومواقع الإصابة، السكن، والعوامل المؤهبة للمرض.