Oral Candida Carriage and Fungi Species Prevalence

By

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This thesis was submitted in partial fulfillment for requirement of master degree of science in

Oral Medicine

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June (2022)

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آية قرآنية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ فَتَعالَى اللَّهُ المَلِكُ الحَقُّ وَلا تَعجَل بِالقُرآنِ مِن قَبلِ أَن يُقضى إِلَيكَ وَحيُهُ وَقُل رَبِّ زِدني عِلمًا ﴾[طه: ١١٤]

Dedication

To

My parents;
With love!

I dedicate this thesis to my father and mother for their continuous and unlimited support and without it, I would not have reached this level of education.

I wish I could always be what they like

Acknowledgement

I wish to express my deep sense of gratitude to my supervisors Mohamed S H Ingafou for his outstanding guidance and support, which helped me in completing my thesis work. My deep thanks go to my supervisor Dr. Mohamed Ganim who spent his valuable time teaching me patiently every aspect of this work until it becomes a reality. I would like to thank MR. Moussa EL wafi as an assistant technician for, Erba medical company for the use of the microbiology device Erba scan (installation, operation and training), Thanks to Manara Benghazi for importing medical supplies, for helping me in order to facilitate the necessary laboratory material and provide the opportunity to use ErbaScan microbiology devices. It is a matter of great privilege for me to present this project to my thesis examiners, for their cooperation and being a part of this work.

Words are inadequate in offering my thanks to my parents and family for their continuous support and cooperation throughout my life, without their support and encouragement this work would not have been completed.

I would like to express my heartfelt thanks to my colleagues and teachers for unconditional support and encouragement to pursue my interests, for listening to my complaints, frustrations and for believing in me, Also my friends and colleagues for their help and wishes for the successful completion of this project.

Logien Saleh Mustafa

Declaration

I confirm that this thesis entitled ***"Oral Candida Carriage and Fungi Species Prevalence"*** is a record of research carried out by myself. Except where otherwise stated, the research design and analysis were my own work, subject to the help and advice received from those who were acknowledged. I have consulted all the references cited. This research has not previously been submitted for a high degree.

Logien Saleh Mustafa

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List of abbreviation

AIDS	Acquired Immunodeficiency Syndrome
APP	Acute Periapical Periodontitis
CP	Chronic Periodontitis
CPP	Chronic Periapical Periodontitis
DMFS	Decayed, Missing and Filled Surfaces
GT	Germ Tube
HIV	Human Immunodeficiency Virus
LG	Localized gingivitis
NAC	Non-Albicans Candida
OC	Oral Candidiasis
OLP	Oral Lichen Planus
SDA	Sabouraud's Dextrose Agar

Oral Candida Carriage and Fungi Species Prevalence

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Abstract

This study aims to identify the prevalence of oral fungal species in routine dental patients via the laboratory culturing of the isolates and identification by the Erba expert identification program plus the evaluation of the antifungal susceptibility pattern of the isolated fungi by agar diffusion method with Neo-Sensitabs.

Subjects and Methods: A total 310 dental patients examined at Al salmani central dental clinic in Benghazi throughout 9 months in the year 2021 in full observation of the measures imposed to combat COVID-19 pandemic. The patients were adults aged 18 years or above, with different dental complaints (dental caries, gingivitis, periodontal disease, dental prosthesis problems), few of them had associating medical illness such as (diabetes, hypertension, asthma, paranasal sinusitis, hyperthyroidism, hypothyroidism or anemia) which might have modified their oral fungal carriage. The later can also be modified by personal habits such as smoking or alcoholism. All relevant information was recorded prior taking an oral smear and immediately transferring it to the laboratory for cultivation on a Sabouraud's Dextrose Agar (SDA) by standard mycological methods. The isolates were first tested by gram stain then by germ tube test. The colony color and culture characteristics were recorded. CANDIDAtest 21 was used along with antifungal susceptibility test by Agar Diffusion method with Neo-Sensitabs method. The susceptibility to seven antifungal agents (fluconazole, amphotericin B, Nystatin, fluocytosine, Clotrimazole, Itraconazole, and voriconazole) was tested.

Results: In 310 cases, the prevalence of oral carriage of yeasts was 32% (100 patients; 54 females and 46 males). No correlation was found between clinical variables and candida carriage except for minor increased tendency of fungal carriage in the patients using dental prostheses. Twelve strains of candidal and non-candidal species could be isolated. *Candida albicans* was the most predominant species and found in 68% of the isolates, while *Candida dubliniensis* was the second isolated species in 15 cases, *Trichosporon spp* in 5 cases, *Candida catenulata* in 2 cases,

Candida krusei in 2 cases, *Candida glabrata* in 2 cases, and (1 case each) for *Candida magnolia*, *Candida pelliculosa*, *Cryptococcus humicola complex*, *Cryptococcus Laurentii*, *Geotrichum capitatum*, and *Rhodotorula rubra*. Almost all isolates expressed high resistance to (amphotericin, fluocytosine, and Clotrimazole) 98%, 97% and 83% respectively, while the resistance was intermediate to (fluconazole and Nystatin), 67% and 60% respectively and high sensitivity was recorded to (voriconazole and Itraconazole) 91% and 71% respectively. Interestingly, fluconazole has performed well in this study, as the isolates were either sensitive (67%) or intermediately sensitive (33%) to it, and no single resistant was isolated.

Conclusions: *Candida albicans* is the most predominately isolated species followed by *Candida dubliniensis*, while different figures were recorded for the other less commonly encountered species. Resistance to antifungal agents is high to some agents particularly amphotericin, fluocytosine, and Clotrimazole and less profound to other agents such as voriconazole and Itraconazole and no fluconazole resistant strains could be found.

INTRODUCTION

Chapter: 1 Introduction

Microbes as an integral part of living environment have an intimate relationship with human being. At birth, the oral cavity is generally lacking significant microbial colonization. However, microbes are continually introduced into the mouth from contaminated animate and inanimate objects. While the majority of these microbes are transients, successful oral colonizers will be obtained from exogenous saliva. *Few months later*, most mouths possess a microbiota consisting of recognizable oral organisms such as bacteria, fungi and actinomyces. *Years after that*, the eruption of deciduous teeth (at around 6 months of age) provide hard non-shedding surfaces that allows further colonization of organisms that are exquisitely adapted to this environment. Significant proportion of these organisms are in dental plaque on tooth surfaces. The oral microbiota continues to develop, changing with age in composition and overall activity.

Hormonal changes during puberty can contribute to increased colonization by groups of gram-negative anaerobes and spirochetes, with some hormones possibly acting as nutritional sources. *In adults,* gradual age-related changes, physical exercise levels, and psychological stress can all influence the numbers or proportions of oral microbiota, often through effects on immune function or salivary flow rate, lifestyle events such as smoking, frequency of carbohydrate consumption, or pregnancy can affect the microbial composition in the oral cavity. *In later life*, the decline in salivary flow rate and in general health status leads to changes in microbial colonization, such as increased carriage of the yeast Candida albicans, with subsequent higher risk of oral candidiasis (Lamont et al, 2014).

Fungi are normal, harmless commensals found in the mouth of approximately 40% of healthy individuals and can and do, however, cause oral mucosal diseases, particularly in immunocompromised individuals. The main fungal species are *Candida albicans, Candida Dubliniensis, Candida glabrata, Candida tropicalis, Candida krusei, Candida parapsilosis,* and *Candida guillermondi*. Although several other fungi can cause oral lesions, *Candida albicans* is the causative agent of the most common oral fungal infection (i.e. candidiasis), which has a variety of clinical presentations (Hu et al, 2019).

Fungal cells have a diameter of approximately 3 to 6 μm, and in general they are larger than bacteria and smaller than mammalian cells. Those fungi that exist predominantly in the unicellular state are usually ovoid and termed yeasts; while those grow as hyphae are commonly

called molds. Hyphae consist of chains of individual cylindrical cells, each containing a nucleus and divided from adjacent cells by walls called septa. The initial emergence of a hypha from a *Candida albicans* yeast cell is referred to as a germ tube (Hu et al, 2019).

Candida albicans is often referred to as a dimorphic fungus, as it exists mostly in either the yeast or hyphal morphological form. Macroscopically, yeast colonies on agar plates tend to be smooth with well-defined edges, whereas mold colonies are furry with individual hyphal threads visible at the edge of the colony, yeast can be cultured from the saliva of approximately 40% of healthy individuals (Hu et al, 2019).

Microbiological resistance is characterized as a fungal pathogen's non-susceptibility to an antifungal agent as determined in vitro. Susceptibility testing and comparison with other isolates of the same species can be further divided into inherent and acquired categories. While intrinsic resistance develops naturally in some fungal strains without prior drug contact, acquired resistance develops after exposure to medicines. Following drug treatment, in previously vulnerable fungal strains, and can often develop as a result of changed gene expression (Kanafani and Perfect, 2008) . Clinical resistance, on the other hand, refers to the persistence of a fungal infection after adequate treatment. Although microbiological resistance can contribute to clinical resistance, additional variables such as diminished immune function, underlying illness, lower medication bioavailability, and increased drug metabolism may also play a role (Rex et al, 1997).

Microscopic examination of clinical samples can be informative. Samples can be obtained by hard swabbing from both sites of the buccal cavity with sterile swab sticks or scraping mucosal surfaces with a wooden spatula, or tongue depressor, and transferring the material to a clean glass slide for wet-mount microscopy. Alternatively, a biopsy whereby the sample is stained before microscopic examination may be indicated for some lesions. However in some studies, phosphate-buffered saline in oral rinses is used. After that, fungi can be cultured from clinical specimens on Sabouraud’s agar. An antibiotic such as chloramphenicol or gentamicin or cefotaxime is to be included in the agar to inhibit the growth of bacteria from the samples. The agar plates are generally incubated aerobically at 30°C for 48 hours (Madhavan et al., 2011).

There are several methods for identifying fungi, however the identification of fungi by growth characteristics is time-consuming, thus techniques have been developed to utilize the differences in the nucleic acid sequences of different fungal species in fungal identification for

species identification, but they are humbled by the high false positive results as a result of specimen contamination. Hybridization of nucleic acids with labeled nucleic acid probes specific for a particular fungus have been in use for a while to detect that fungus in clinical samples. Many candidal characterization studies had found that *Candida albicans* is the most prevalent yeast species isolated (Akram et al., 2018).

Oral candidiasis is a common oral fungal infection, which clinically presented as pseudomembranous, erythematous, and plaque like/nodular forms. Angular cheilitis and median rhomboid glossitis are mostly caused by fungal infection. The mere isolation of Candida from intraoral surfaces is not interpreted as a predictive signal for disease as 40% of healthy individuals from diverse populations can carry such yeasts without any clinical symptom (Rosa, 2015).

Furthermore, oral candidiasis can be a manifestation to a number of underlying systemic conditions such as diabetes mellitus, hypertension, dehydration, malnutrition, and certain medication intake particularly those used to treat anxiety or depression as they can result in severe reductions in salivary production, incurring in converting the saprophytic yeasts into opportunistic pathogens (Akram et al., 2018).

Many studies showed increased candida carriage among diabetics, cigarette- and water-pipe-smokers, electronic cigarette users, than in never smokers (Mokeem, et al., 2019), (Alaizari, 2020). Studies have also shown that asymptomatic oral colonization of *Candida spp.* may lead to oral lesions or become a source of disseminated infections (Lourenço et al, 2017).

Other studies suggested a potential role of oral candidiasis in the development of dental caries and increases its severity (Lourenço et al, 2017) . Oral candidiasis has been suggested to play a role in the pathogenesis of many other oral conditions such as oral lichen planus (OLP) (Zeng et al, 2009) .

This study tries to evaluate the possible role of dental or systemic conditions of oral candida carriage through clinical examination and laboratory studies of the positive growth of the isolates and their susceptibility to antifungals.

LITERATURE REVIEW

Chapter: 2 Literature review

2.1. Introduction

Candida spp. are widely spread habitat among people in different parts of the world. Differently from other microbes their isolation from the mouth doesn't mean an indication of disease status (Rosa, 2015). The commensal status of this fungal genus was evaluated for many years and according to different authors, about 54% to 71.4 % of healthy individuals from diverse populations may carry such yeasts without any symptom (Hauman et al, 1993), (Darwazeh, 1995), (Kindelan et al.,1998), (Blignaut et al., 2002).

2.1.1. Prevalence of carriage of oral fungi

In a study of candida carriage the investigators (Akpan and Morgan, 2002) have compiled data concerning to carrier status of individuals from different risk groups and stated that "in general population, the carriage rates have been reported to range from 20 to 75 % without any symptoms". According to them, the incidence of Candida isolates from oral cavity (not related to OC episodes) has been reported to be 45 % in neonates, 45–65 % in healthy children, 30–45 % in healthy adults, 50–65 % in people who wear removable dentures, 65–88 % in those residing in acute and long-term care facilities. Furthermore, candida carriage reported in 90 % of patients with acute leukemia undergoing chemotherapy, and 95 % of patients with HIV (Rosa, 2015).

In another study of 482 Japanese dental staff and students, the oral candidal carriage was 18.3%, where *Candida albicans* accounted for 80.7% of the isolates (Majima et al., 2014). Oral candidal carriage in routine Kuwaiti patients seeking dental treatment was investigated in Kuwait University Dental Clinic, where 350 patients were investigated, 160 (46%) had Candida growth in culture. The isolation rate of Candida was significantly higher in individuals who were smokers or with underlying medical condition (62%) compared to healthy individuals (38%) ($P<0.01$). The species isolated were predominated by *Candida albicans* (63.7%), then *Candida dubliniensis* (14.3%), *Candida krusei* (8.1%), *Candida tropicalis* (7.5%) and *Candida glabrata* (6.2%). *Candida spp* were also more prevalent in the immunocompromised patients in the same study. *Candida albicans* was the most prevalent species, followed by *Candida dubliniensis*, the high prevalence of *Candida dubliniensis* over other non-albicans species in the oral cavity in that study was considered as an

epidemiological shift in the oral flora of Candida isolates in the Kuwaiti population (Ellepola et al., 2011).

In a study from Saudi Arabia of 104 voluntary adults at the college of medicine-Jouf University, *Candida spp* were isolated from oral cavity of 45 (43.4%) subjects. Of these 55.6% were identified as *Candida albicans*, *Candida glabrata* (11.1%), *Candida krusei* (11.1%), *Candida dubliniensis* (8.9%), *Candida parapsilosis* (6.7%), *Candida tropicalis* (4.4%), and *Candida famata* (2.2%). Subjects with very poor plaque control status, severe gingivitis and diabetes had significantly (P = 0.001) high concentration of *Candida spp* (Alrayyes et al., 2019).

In Portugal oral Candida carriage of patients attending a dental clinic in Braga, the samples analyzed 54.6% (n=53) were Candida positive, and *Candida albicans* was the most frequently isolated species, accounting for 79% of all the species identified. Non- *Candida albicans* (NCA) species recovered included *Candida parapsilosis*, *Candida glabrata*, *Candida tropicalis*, and *Candida guilliermondii*. There was a lack of association between the presence of *C. albicans* or NCA species, and age, gender, or prostheses wearing in this population (Martins, M., et al, 2010).

In another study, Oral candidal carriage in asymptomatic patients 203 patients attending the Royal Dental Hospital of Melbourne, Oral yeast carriage was found in 98/203 patients (48.3%), and of these, 83 (84.7%) patients carried *C. albicans*. There was no statistical difference in carriage when comparing gender, age, or presence of a removable prosthesis (Mun, M., et al, 2016).

Oral candidal carriage and its association with dental carious lesions in asymptomatic adults from the UAE, Candida colonies were identified in 49 (30.6%) patients with CFUs ranging from 103 to 105 colonies per mL. The quantity of Candida CFUs increased with age ($r = 0.200$; $p < 0.05$). Among all dental and periodontal health indices, only DMFS was significantly associated with higher values of Candida carriage ($p = 0.034$), and this association was independent from sex, age, smoking, diabetes mellitus and plaque index (Al-Amad, S. H., et al, 2021).

2.1.2. Oral Candida species

There are several species of candida can cause disease in oral cavity and the other parts of human body. These are considered as normal habitat in healthy individuals, but they become pathogenic in certain circumstance of decreased immunity. The most common spp are *Candida albicans, Candida catenulate, Candida dubliniensis, Candida glabrata, Candida krusei, Candida magnoliae, Candida pelliculosa, Crypt. Humicola complex, Cryptococcus Laurentii, Geotrichum capitatum, Rhodotorula rubra, Trichosporon sp.*

2.1.2.1. Candida albicans

It is an opportunistic fungal pathogen that exists as a harmless commensal in the gastrointestinal and genitourinary tracts in about 70% of humans and about 75% of women suffer from Candida infection at least once in their lifetime (Kabir et al., 2012) , *Candia albicans*, as the most common cause of candidiasis, is studied more extensively than any other Candida species (Singh et al., 2020) .

2.1.2.2. Candida dubliniensis

It is an opportunistic fungal pathogen originally isolated from AIDS patients. It is also occasionally isolated from immunocompetent individuals. *Candida dubliniensis,* which has a high degree of phenotypic similarity to *Candida albicans*, has the ability to induce oral candidiasis (Colman et al., 1997) *Candida dubliniensis* isolates have much higher levels of proteinase activity and adhesion to buccal epithelial cells than conventional *Candida albicans* isolates, suggesting that they are more virulent.(McCullough et al., 1995). *Candida africana,* whose taxonomy was evaluated is another unusual isolation of *Candida albicans* (Romeo and Criseo, 2011).

2.1.2.3. Candida glabrata

It was considered as relatively nonpathogenic commensal fungal organism of human mucosal tissues; however, with the increased use of immunosuppressive agents, mucosal and systemic infections caused by *Candida glabrata* have increased significantly, especially in the human immunodeficiency virus-infected population. A major obstacle in *Candida glabrata* infections is their innate resistance to azole antimycotics therapy, which is very effective in treating infections caused by other Candida species. Depending on the site of infection, *Candida glabrata* is often the second or third most common cause of candidiasis after *Candida albicans* (Fidel et al., 1999*)*

2.1.2.4. Candida Krusei

This species is the fifth most common cause of candidemia, but probably is most noteworthy for its innate resistance to the antifungal agent fluconazole in addition to somewhat reduced susceptibility to other drugs (Pelletier et al., 2005) Most commonly isolated from neutropenic patients, *Candida krusei* has sometimes been inadvertently selected as a pathogen in some patients receiving prophylactic fluconazole therapy. This yeast, which is commonly recovered from various environmental sources, is a significant etiological agent of vaginitis although it is not typically recovered from mucosal surfaces of healthy persons (Cooper et al., 2011) .

2.1.2.5. Candida catenulate

This candida species was formerly also named *Candida ravautii* and *Candida brumptii*, is considered a natural contaminant of dairy products and has not yet been associated with invasive infection in humans (Radosavljevic et al., 1999). *Candida catenulata* is a fungus commonly found in Australian cheeses. *Candida catenulata* has been identified as the causative pathogen for one report of onychomycosis and one report of candidaemia (Ha et al., 2018).

2.1.2.6. Candida magnoliae

Candida magnoliae, is commonly used in the food industry because of its high capacity to produce erythritol and mannitol, which are used as functional substitutes for sugar in various foods. This microorganism has the ability to quickly consume fructose and grow in various pH levels (Hernandez et al., 2018)

2.1.2.7. Candida Pelliculosa

Candida pelliculosa is a rare fungal pathogen that is mainly found in soil, lakes, fermented fruits, and industrial pollutants. Recently, *Candida pelliculosa* has been identified as an opportunistic pathogen causing sexually transmitted infections, dacryocystitis via penetrating keratoplasty, and fungal infections after cardiac surgery and fungal hemorrhagic pancreatitis, Preterm infants with extremely low birth weights account for a majority of *Candida pelliculosa* infections. Fluconazole has been recommended as a prophylactic and therapeutic agent for fungal infections caused by *Candida pelliculosa* (Cai et al., 2021).

2.1.2.8. Cryptococcus Laurentii

Cryptococcus Laurentii was previously considered saprophyte and thought to be non-pathogenic to humans. However, in favorable circumstances like diminished immunity, it seems to be an important pathogen, and *Cryptococci* are generally found in soil contaminated by pigeon feces, as well as on the surfaces of certain vegetables and in milk from infected dairy cowherds. *Cryptococci* are transmitted to humans primarily through inhaled fomites, although direct entry through the digestive tract or skin can also occur. fungemia due to *Cryptococcus Laurentii* has been reported (Cheng et al, 2001).

2.1.2.9. Geotrichum Capitatum

Geotrichum capitatum mainly grows in food, soil, mucous membranes and on skin surfaces. It can usually be separated from the normal flora of human skin, respiratory tract and gastrointestinal tract. In general, *Geotrichum capitatum* has low virulence, and it is an opportunistic pathogen. In patients with normal immunity, it is generally not pathogenic, in vitro studies confirmed that voriconazole and amphotericin B were effective medications (Gao et al., 2015).

2.1.3. Non-candida oral yeasts

2.1.3.1. Trichosporon spp

It is a yeast-like fungus macroscopically. Approximately 50 species of the genus Trichosporon have been characterized, 16 of which are associated with diseases in humans. Guého et al, When *Trichosporon spp* develops as an invasive infection, the disease is known as trichosporonosis. This infection has a mortality rate between 50% and 80%, and it is the second or third cause of fungemia in immunocompromised patients just after *Candida spp*. Trichosporonosis is considered an endogenous disease because the microorganism is commonly found as a part of the flora in the gastrointestinal tract, lungs, and skin (Montoya et al, 2014).

2.1.3.2. Rhodotorula spp

They are yeasts, which are normal inhabitants of skin and environments such as shower curtains and toothbrushes. *Rhodotorula rubra* has been reported in rare instances to cause fungemia in patients with acute leukemia and after bone marrow transplantation. Most of the cases reported were related to catheters, and the source of infection was likely the skin, as opposed to the gastrointestinal tract of many candidemia (Navarro et al, 2001).

2.1.4. Oral diseases caused by candida (Candidiasis)

Oral candidiasis is considered the most common oral disease caused by fungi. As the causative agent is an opportunistic microbarom it usually seen in circumstances of decreased immunity or presence of other conditions favorable to candida growth such as hormonal imbalance or immunosuppression. Other factors include excessive smoking, decreased vertical dimension of the face and the poor denture hygiene. Oral candidiasis clinically presentes as pseudomembranous, erythematous, and plaque like/nodular forms. Angular cheilitis, and median rhomboid glossitis are mostly caused by fungal infection as well. The mere isolation of Candida from intraoral surfaces is not interpreted as a predictive signal for disease as 40% of healthy individuals from diverse populations can carry such yeasts without any clinical symptom (Rosa, 2015).

2.2. Oral candidal carriage

The carrier status of individuals from different risk groups in general population have been reported to range from 20 to 75 % of healthy individuals without symptoms (Akpan and Morgan, 2002). The incidence of Candida isolates from the oral cavity, not related to oral candidiasis (OC) episodes has been reported to be 45 % in neonates, 45–65 % of healthy children, 30–45 % of healthy adults, 50–65 % of people who wear removable dentures, 65–88% in those residing in acute and long-term care facilities, 90 % of patients with acute leukemia undergoing chemotherapy, and 95 % of patients with HIV (Rosa 2015) . After 80 years of age, there is a considerable increase in Candida non-albicans species and a reduced susceptibility to fluconazole (Benito-Cruz et al., 2016).

In a study by Mun and co-associates to determine the presence and amount of oral yeast in the mouths of healthy patients without mucosal lesions, the oral yeast carriage was (48.3%), which was not statistically different from those individuals with a comparable gender, age with a removable prosthesis.

2.2.1.1. Diabetes mellitus

Diabetes mellitus as a commonly encountered condition in routine dental patient is thought to influence candidal carriage. In one study, investigators tried to investigate the candida colonization in patients with diabetes mellitus and its relationship with factors such as *Candida species*, serum glucose level, and the susceptibility rate of isolated yeasts to antifungals in 113 patients with type 2 diabetes, 24 patients with type 1 diabetes, and 105 healthy control and concluded that significant association exists between the poor glycemic

control and the higher prevalence rates of candidal carriage and density in diabetic patients. In addition, a high prevalence of *Candida dubliniensis* in diabetic patients was found, which might be misdiagnosed with its morphologically related species, *Candida albicans* (Zomorodian et al, 2016) .

Several other studies reported that candida is significantly higher among diabetics than the non-diabetics, however, *Candida albicans* was the most prevalent species isolated from the diabetics and the non-diabetics denture-wearers (Javed et al, 2017). In a recent study to establish a relationship between salivary glucose levels and Candida carriage rate in type 2 diabetes mellitus patients in 60 patients couldn't find any correlation between salivary PH levels and Candida carriage rate, despite that the increased salivary glucose level was associated with increased prevalence of oral Candida in diabetic subjects and the growth of Candida in saliva was accompanied by a rapid decline in PH, which in turn favored their growth (Balan et al., 2016).

Another study to establish a relationship between salivary glucose levels and Candida carriage rate in type 2 diabetes mellitus patients in 60 patients, couldn't find any correlation between salivary PH levels and Candida carriage rate, despite that the increased salivary glucose level is associating with increased prevalence of oral Candida in diabetic subjects and the growth of Candida in saliva was accompanied by a rapid decline in PH, which in turn favored their growth (Balan et al, 2015).

2.2.1.2. Smoking

Smoking is a hard habit to break because tobacco contains the very addictive chemical nicotine, Smoking is predisposing factor for many diseases such as heart disease pulmonary diseases and oral carious problems, However, both smoking and the presence of active carious lesions were found to be positively correlated with the carriage of oral Candida. Individuals who are current smokers are nearly seven times more likely to have oral Candida, and participants with high candidal colonization are more likely to be current smokers. Participants with active carious lesions were also more likely to carry oral Candida (Mun et al, 2016).

The association between smoking and smokeless tobacco with oral Candida carriage has been studied by meta-analysis including 14 studies and concluded that there was a significant relationship between smoking/smokeless tobacco users and oral Candida carriage. However, observational studies cannot clarify whether the observed epidemiologic

association is a causal effect or the result of some unmeasured confounding variables (Alaizari and Al-Anazi, 2020).

More detailed study included the daily frequency of smoking and its duration and reasons plus daily oral hygiene maintenance habits have conclude the same findings (Akram et al., 2018). Oral *Candida albicans* carriage (but not the other Candida species) in one study was significantly higher among cigarette and waterpipe-smokers and E-Cigarette users than never-smokers. Periodontal disease is associated with increased Candida species carriage in HIV-infected patients and may be a predisposing factor to clinical manifestations of candidiasis (Lourenço et al, 2017). However, both smoking and the presence of active carious lesions were found to be positively correlated with the carriage of oral Candida. Individuals who are current smokers are nearly seven times more likely to have oral Candida, and participants with high candidal colonization are more likely to be current smokers. Participants with active carious lesions were also more likely to carry oral candida (Mun et al, 2016).

Oral *Candida albicans* carriage (but not the other Candida species) in one study was significantly higher among cigarette and waterpipe-smokers and E-Cig users than never-smokers (Mokeem et al, 2019).

2.2.1.3. Periodontal disease

Periodontal disease is associated with increased Candida species carriage in HIV-infected patients and may be a predisposing factor to clinical manifestations of candidiasis (Lourenço et al, 2017).

2.2.1.4. Dental caries

Higher Candida carriage rate, is associated with the highest level of caries severity among school children and high salivary candida carriage rate is associated with presence of specific species of this fungus (such as *Candida albicans* and C. *dubliniensis*) which appear to be related to the severity of caries experienced by preschool children (Lorano Moraga et al, 2017), similar findings were obtained in a more recent study from a Saudi sample.

2.2.1.5. HIV

Another study was carried out to identify the oral carriage of *Candida spp* in 246 patients infected by human immunodeficiency virus (HIV) and the possible correlation with clinical characteristics has found that Candida yeasts were present in 41.87% of the samples, and Candida albicans was the most prevalent (32.52%) species. Other identified Candida

species in that study were *Candida tropicalis* (4.88%), *Candid parapsilosis* (2.85%), *Candida dubliniensis* (0.81%), and *Candida famata* (0.81%). Interestingly, there had been lower rate of oral Candida carriage in patients infected by HIV who were on highly active antiretroviral therapy. A greater prevalence of *Candida albicans* than non-albicans Candida species was found at the species level. Prior candidiasis predicted the oral carriage of *Candida albicans*; however, it did not influence the carriage of non-albicans species (Ribeiro et al, 2015).

2.2.1.6. Alcoholism

Alcohol consumption significantly decreases oral carriage of *mutans streptococci*, whereas there was no effect on *Candida albicans* colonization levels. Tobacco users were found to harbor elevated levels of *Candida albicans*, however, there was no observe consumption of the stimulants analyzed. Microbial colonization of the oral cavity changes in a species-specific manner in response to dietary and social habits such as drinking alcohol and smoking. The effect on bacterial colonization by *mutans streptococci as well as t*he carriage of other species which were investigated in the past, such as *Candida krusei*, *Candida tropicalis* and *lactobacilli*, did not show a response to the species (Sheth et al., 2016).

2.2.1.7. Oral dysesthesia

Many factors are thought to associate with the increase of oral candida carriage. Investigators advocated an increased carriage of candida in the patients with clinical signs of xerostomia, no such association could be established between oral dysesthesia and the increased load of oral Candida in a study in 79 patients diagnosed with oral dysesthesia. Their oral carriage of Candida detected in 50 patients 63.3% which was not higher than other oral conditions (Farah et al, 2018).

2.2.1.8. Xerostomia

Although, patients with clinical signs of xerostomia usually present with an increased carriage of Candid, there is no association between oral dysesthesia and the presence or load of oral Candida (Javed et al., 2017).

2.2.1.9. Poverty

In a study of 82 USA socioeconomically disadvantaged women (48 pregnant and 34 non pregnant) it has been found that their oral Candida carriage is positively associated with hypertension and the number decayed teeth (Xiao et al, 2019).

2.2.1.10. Poor oral health

In study of the relationship between candida carriage and oral health, a cross-sectional study involving 160 patients investigated the associations between Candida species collected by oral rinse technique, and Decayed, Missing, and Filled Surfaces (DMFS), and periodontal health indices. Candida colonies were identified in 49 (30.6%) patients with CFUs ranging from 10^3 to 10^5 colonies per ml. The quantity of Candida CFUs increased with age ($p < 0.05$). Among all dental and periodontal health indices, only DMFS was significantly associated with higher values of Candida carriage ($p = 0.034$), and this association was independent from sex, age, smoking, diabetes mellitus and plaque index (Al-Amad et al. 2021).

2.2.1.11. Oral contraceptives

Oral contraceptives containing estradiol can lead to Candida colonization in the oral cavity. This has been found in a relatively recent case-control study of 40 non-pregnant women divided into two groups: 20 who used oral contraceptive pills and 20 who did not. This study found an increased frequency of positive cultures of *Candida albicans* (P value = 0.03) for the case group. In addition, the number of *Candida albicans* and *Candida krusei* was significantly higher for the case group compared to the control group (P value = 0.04, P value = 0.03) (Aminzadeh et al., 2016) .

2.3. Pathogenicity of candida

Candida spp. have virulence factors that help them colonize and multiply in the oral mucosa and, presumably, periodontal pockets these fungi can co-aggregate with bacteria in dental biofilm and adhere to epithelial cells. These interactions, which are linked to their ability to infiltrate gingival conjunctive tissue, could play a role in microbial colonization and disease development (Haynes, 2001) , (Järvensivu et al., 2004) .

Candida spp. also produce enzymes that breakdown extracellular matrix proteins and as immunoglobulin's, collagenases and proteinases (Haynes, 2001) . Netea, *et al*., confirmed that genetically homogenous strains of *Candida albicans* were present in these patients' oral cavity, and that these strains were capable of producing large quantities of exoenzymes (Netea et al., 2008) . Species of Candida, especially *Candia albicans*, have been recovered

from periodontal pockets in 7.1% to 19.6% of patients with chronic periodontitis (Slots et al., 1988), (Reynaud et al., 2001).

Candida albicans and *Candida dubliniensis* were capable of colonizing periodontal pockets in patients with chronic periodontitis, while only *Candida albicans* was identified in the sub gingival microflora of healthy individuals and patients with aggressive periodontitis. Cancer, diabetes mellitus, and immunosuppressive conditions such as acquired immunodeficiency syndrome (AIDS) increase host susceptibility to these infections (Urzúa et al., 2008). Feller et al. observed higher prevalence of Candida spp. in the oral cavity, and specifically in the sub gingival biofilm, of HIV-seropositive patients (Feller et al., 2008).

2.4. Susceptibility to the Antifungals

Many studies examined different aspects of antifungal susceptibility. Such studies mostly involved *Candida albicans* as well as NAC species particularly *Candida dubliniensis* followed by *Candida glabrata* and *Candida parapsilosis*. Candida species are susceptible to the commonly used antifungal agents in general, but with considerable variation among species. Occasionally, some NAC exhibited lower antifungal susceptibility (Meurman et al., 2011). It has been demonstrated that although most isolates of *Candida dubliniensis* are sensitive to the commonly used antifungal agent fluconazole (Gilfillan et al., 1998) .

Non-albicans Candida (NAC) species such as *Candida glabrata, Candida tropicalis, Candida guillier-mondii, Candida dubliniensis, Candida parapsilosis*, and *Candida krusei* are emerging as colonizers and pathogens that can cause superficial and systemic infections, despite *Candida albicans*' dominance (Colman et al, 1997), (Gutiérrez et al., 2002), (Bassetti et al, 2006) in comparison, some of these species are more resistant to antifungal drugs. *Candida glabrata and Candida krusei* (Yang, 2003) have an intrinsic resistance to fluconazole, a routinely used antifungal drug (Pfaller et al., 1999) *Candida palmioleophilia*, a newly discovered species in Denmark, has a distinctive antifungal susceptibility profile, being highly susceptible to echinocandins, less susceptible to Itraconazole, posaconazole, and voriconazole, and fluconazole resistant (Jensen et al., 2011) Furthermore, most isolates of *Candida dubliniensis* are sensitive to fluconazole (Moran et al., 1997), (Kirkpatrick et al, 1998) encodes multidrug transporters that mediate fluconazole resistance quickly during clinical treatment (Moran et al., 1997) , (Moran et al, 1998). As a result, early and accurate identification of Candida species can aid in infection management and reduce mortality rates.

2.5. Antifungal agents

2.5.1. Amphotericin B

Amphotericin B is fungistatic or fungicidal depending on the concentration obtained in body fluids and the susceptibility of the fungus. The drug acts by binding to sterols (ergosterol) in the cell membrane of susceptible fungi. This creates a transmembrane channel and the resultant change in membrane permeability allowing leakage of intracellular components. Asthmatics using steroid inhalers, gargling with water or even weak concentrations of amphotericin B does not prevent colonization of the throat with *Candida albicans*. This group at high risk of developing oral candidiasis should gargle with amphotericin B at concentrations higher than 100 times dilution that can prevent clinically detectable oral candidiasis (Fukushima et al., 2001).

2.5.2. Nystatin

Nystatin is a polyene antifungal drug that has broad-spectrum fungicidal and fungistatic activity against a number of yeasts and fungi, most notably Candida species. It is one of the most effective antifungal agents synthesized by bacteria, in this case a strain of *Streptomyces noursei*, and is closely related to amphotericin B, differing only slightly in structure. Nystatin has a greater antifungal activity than amphotericin B - parenterally administered nystatin, however, is associated with significant toxicity and is not available in a formulation appropriate for systemic use. As it undergoes very little absorption following oral or topical administration, nystatin's efficacy is limited to the treatment/prevention of cutaneous, mucocutaneous, and gastrointestinal fungal infections.

2.5.3. Clotrimazole

Clotrimazole is a broad-spectrum antifungal agent that inhibits the growth of pathogenic yeasts by changing the permeability of cell membranes. The action of Clotrimazole is fungistatic at concentrations of drug up to 20 mcg/mL and may be fungicidal in vitro against *Candida albicans* and other species of the genus Candida at higher concentrations Label. Unfortunately, resistance to Clotrimazole, which was rare in the past, is now common in various patient populations. Clotrimazole is generally considered to be a fungistatic, and not a fungicidal drug, although this contrast is not absolute, as Clotrimazole show fungicidal properties at higher concentrations (Wu et al., 1999)

2.5.4. Fluocytosine

Fluocytosine is an antimetabolite that acts as an antifungal agent with in vitro and in vivo activity against Candida and Cryptococcus. Fluocytosine enters the fungal cell via cytosine permease; thus, fluocytosine is metabolized to 5-fluorouracil within fungal organisms. The 5-fluorouracil is extensively incorporated into fungal RNA and inhibits synthesis of both DNA and RNA. The result is unbalanced growth and death of the fungal organism.

2.5.5. Itraconazole

One of the triazole antifungal agents that inhibits cytochrome P-450-dependent enzymes resulting in impairment of ergosterol synthesis. This enzyme converts lanosterol to ergosterol, and is required in fungal cell wall synthesis.

2.5.6. Voriconazole

Voriconazole is a triazole antifungal medication used to treat serious fungal infections; it is used to treat invasive fungal infections that are generally seen in patients who are immunocompromised. These include invasive candidiasis, invasive aspergillosis, and emerging fungal infections. The increased affinity of voriconazole for 14-alpha sterol demethylase makes it useful against some fluconazole-resistant organisms (Clancy and Nguyen, 2011).

2.5.7. Fluconazole

Fluconazole is a triazole antifungal agent available for oral or intravenous use in the treatment of a number of localized and disseminated mycoses fluconazole inhibits the synthesis of ergosterol to increase cellular permeability (Zervos M, Meunier, 1993).

AIMS AND OBJECTIVES OF THE STUDY

Chapter: 3 Aims and Objectives of the study

1- To determine the oral Candida carriage and species prevalence in healthy dental patients.
2- To isolate and identify candida spp in the samples collected.
3- To determine the antifungal susceptibility pattern for the isolated fungus.

MATERIAL AND METHODS

Chapter: 4 Material and Methods

4.1.1. Study design

This is a mycological investigation involves clinical examination of the consecutive routine dental patients for their systemic health and dental findings in order to determine the relationship between these clinical findings and candidal carriage and other fungi. The positive growths of the smears were studied for the prevalence of fungal species and their susceptibility to antifungal agents.

4.1.2. Setting

Samples were collected from Benghazi dental clinic as it has good flow of patients. The laboratory work was conducted in the laboratories of the faculty of public health of the University of Benghazi.

4.1.3. Study sample

Patients seen were 310 patients from routine consecutive patients with different oral diseases. Involves comprehensive clinical examination and laboratory investigations.

4.1.4. Sample size

Was designed to target 310 consecutive dental patients at the same dental center and to be examined by the same clinician. The ted number was successfully achieved in the period between first of March 2021 to the end of November 2021

4.1.5. Study registration

The protocol of this study was in accordance with the principals established by the declaration of Helsinki and was approved and registered in the postgraduate studies office and an ethical approval was obtained from the ethical committee of the dental faculty, which authorized for that according to the roles.

4.1.6. Inclusion criteria

Patients aged 18 years or above, and be born or permanently resident in Libya for at least 10 years, both genders are included.

4.1.7. Exclusion criteria

- Patient younger than 18 years.
- Patient, who do not permanently resident in the country, tourists & immigrants.
- Patient received chemotherapy, radiotherapy, or treatment of malignancy.
- Patients currently taking antifungal antibiotics for any reason.

4.1.8. Clinical Study conduct

Patients at first registered at reception desk, interviewed for their general health status and case history was taken on the scene. The inclusion in this study begins by explaining the purpose of the study, and obtaining implied verbal consent from the patient.

The patient then seated on a dental chair to conduct clinical examination, to confirm the clinical signs of any underlying systemic illness, further investigation (hematological, histopathological or radiological) had been ordered whenever deemed necessary, a smear sample was obtained from both buccal mucosae of every patient.

4.1.9. Information specifically obtained for this study

In the clinical setting, the personal data (age, gender occupation and smoking habits) will be collected as well as drug hypersensitivity. and information regarding the status of general health of the patient and the current systemic diseases such as bronchial asthma, cardiovascular diseases, endocrine disorders such as diabetes mellitus and thyroid diseases, gastrointestinal diseases, hepatitis, hematological disease, infectious disease, kidney disorders and further diseases which known to predispose to oral candidiasis.

4.1.10. Tools used in the clinical examination

Dental mirror, dental probe, tongue spatula, condescend chair light, gauze, cotton roll and tweezers, Information recorded in a specially designed clinical form. The working environment was adapted according to the measures imposed for COVID-19 pandemic.

4.1.11. Samples collection

To evaluate the oral carriage of yeasts, swabs was collected from both buccal mucosae, palate and oral commissures and immediately transferred to laboratory for cultivation.

4.2. Laboratory examination

4.2.1. Equipment and Material used

Incubator, autoclave, light microscope, sterile cotton swab SDA, microscope slide, oversleep, petri dishes sterile plastic inculcated loops, lactose phenol blue stain, Gram stain, CANDIDAtest21 cefotaxime 1g antibiotic, amphotericin B, nystatin, Clotrimazole, fluocytosine, ketoconazole, Itraconazole, voriconazole.

4.2.2. Media preparation

Sabroaud dextrose agar was used for the isolation, cultivation, and maintenance of non-pathogenic and pathogenic species of fungi and yeasts. SDA was formulated by Sabouraud in 1892 for culturing dermatophytes. The pH was adjusted approximately 5.6 in order to enhance the growth of fungi, especially dermatophytes, and to slightly inhibit bacterial growth in clinical specimens.

4.2.2.1. Composition of SDA

The standard composition of SDA (Ingredients in gram/liter) includes dextrose (Glucose, 40 gm), peptone 10 gm, and agar 15 gm, final pH 5.6 +/- 0.2 at 25 º C.

4.2.2.2. Principle constituents of SDA

Peptone (Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue) provide the nitrogen and vitamin source required for organism growth in SDA.

Dextrose was added as the energy and carbon source. Agar is the solidifying agent.

Cefotaxime was added as broad-spectrum antimicrobials to inhibit the growth of a wide range of gram-positive and gram-negative bacteria

4.2.2.3. Preparation of SDA

65g of the medium Suspended in one liter of distilled water. Then Heated with frequent agitation and boil for one minute to completely dissolve the medium. Autoclaved at 121°C for 15 minutes. 1g of cefotaxime dissolved in sterile water added to the medium. The medium Cooled to (45 to 50°C) in water bath before poured into petri dishes as in (Figure 1).

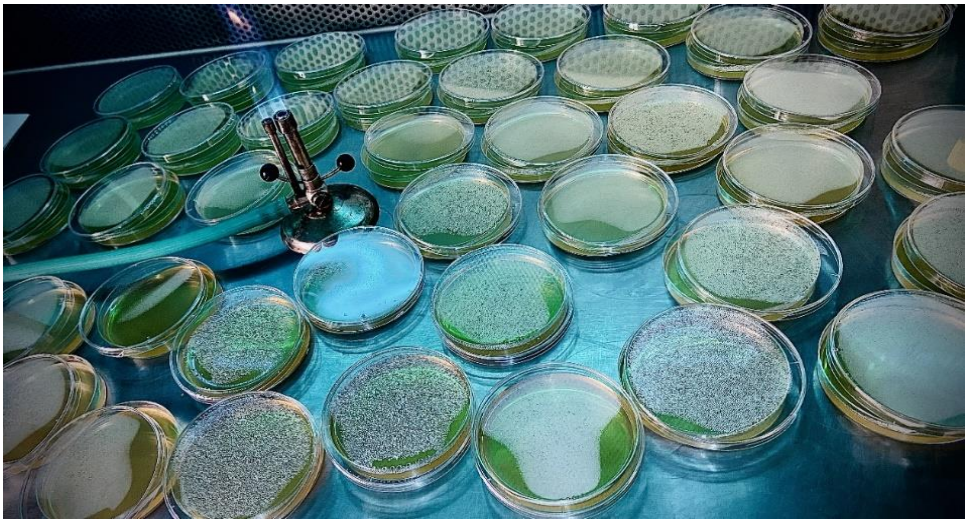

Figure 1: Media poured in petrri dishes

4.2.3. Specimen collection and culture -

Patient samples were collected using sterile cotton swab from the right and the left side of buccal pouch, post part of hard palate in case of denture wearer, commissure of mouth in case of angular cheilitis. Swabs for mycological examination were submitted to the microbiology laboratory and immediately plated on Sabouraud agar. The plates then incubated at 37°C for 48 hours.

4.2.4. Macroscopic Examination of Isolated Fungus

After successful cultivation, macroscopic and microscopic examination were done, macroscopically, the cultures were closely observed for the color of the colony, type of growth, texture, and size and shape of the colony. The color of colonies ranged from white to creamy in color as a useful guide to identify the type of species, while texture of most colonies either creamy, or occasionally hard. The size the colony ranges from small to large. There are some differences in the shape of colonies, some of them are circular and others are irregular. All these characteristics were used to differentiate between various types of the grown microbiota as following and could be differentiated into three main forms:

1- Mold: The basic morphological elements of filamentous fungi are long branching filaments or hyphae, which intertwine to produce a mass of filaments or mycelium. Colonies are strongly adherent to the medium and unlike most yeast colonies cannot be emulsified in water. The surface of these colonies may be velvety, powdery, or may show a cottony aerial mycelium. Reproduce by the formation of different types of spores, (Figure 2).

2-Yeast: These occur in the form of round or oval bodies, which reproduce by asexual process called “Budding” in which the cell develops a protuberance, which enlarges and eventually separates from the parent cell. Yeasts colonies resemble bacterial colonies in appearance and in consistency.

3- Yeast like Yeast like the one fungus grow partly as yeast and partly as elongated cells resembling hyphae. Which are called pseudomycelium. e.g. *Candida albicans* (Figure 3)**.**

Figure 2: Macroscopic appearance of growth of mold on SDA

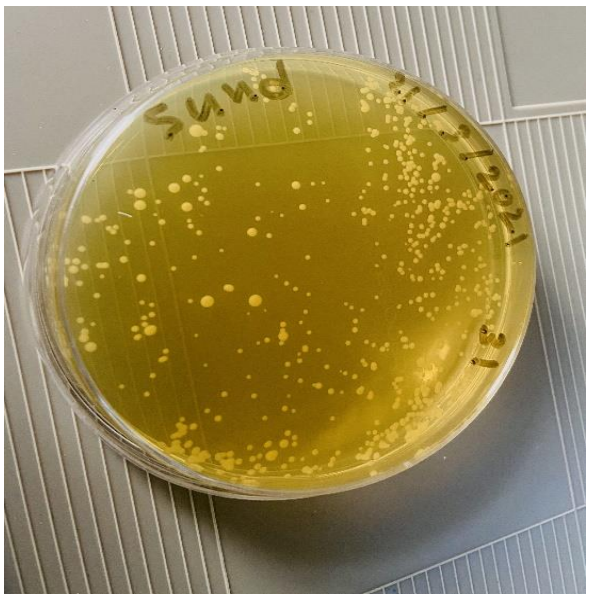

Figure 3: Growth of yeast on SDA

4.2.5. Microscopic examination

Thin smears of the organisms were prepared on microscope slides, two drops of lactose phenol cotton blue were dropped on the slide , then, covered with coverslip and viewed using light microscope at X40 (Figure 4).

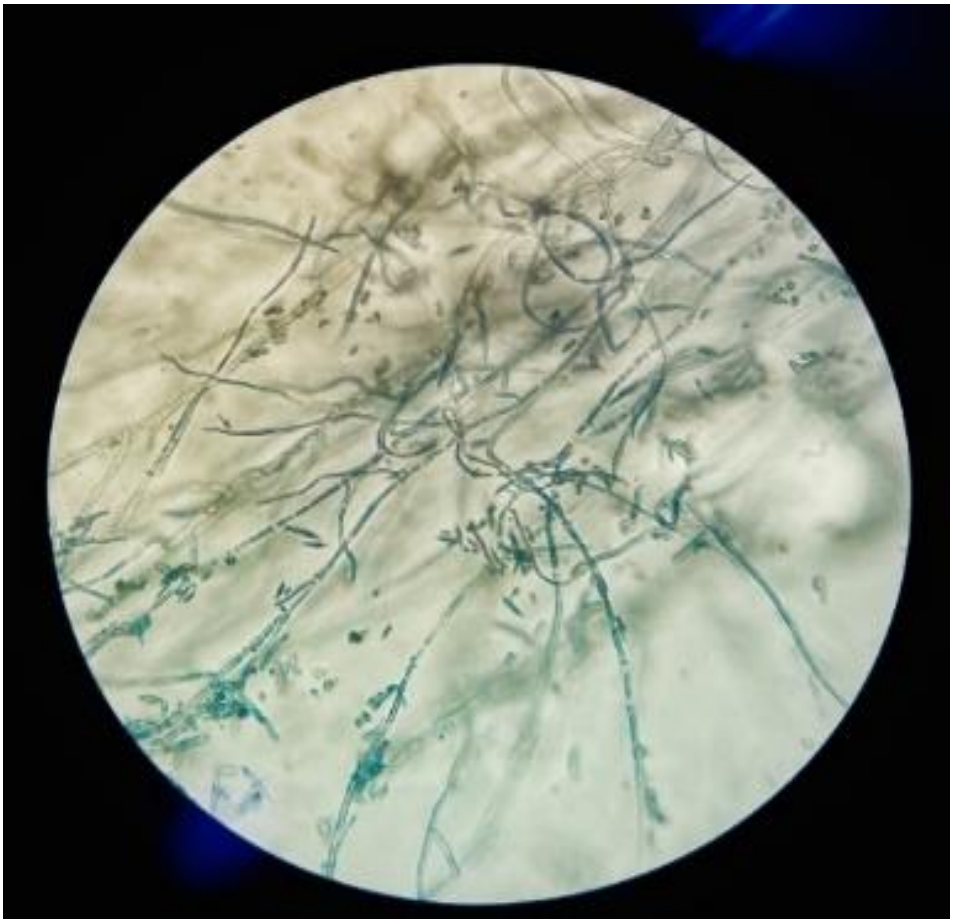

Figure 4: Microscopic appearance of fungal hyphae with lacto phenol stain at X40 power

4.2.6. Gram stain

Gram stain was used at 100 oil magnification. The microscopic features of Candida spp. also show species-related variations. All species produce blastoconidia singly or in small clusters. Blastoconidia may be round or elongate. Most species produce pseudohyphae, which may be long, branched or curved. True hyphae and chlamydospores are produced by strains of some *Candida spp* (Figure 5).

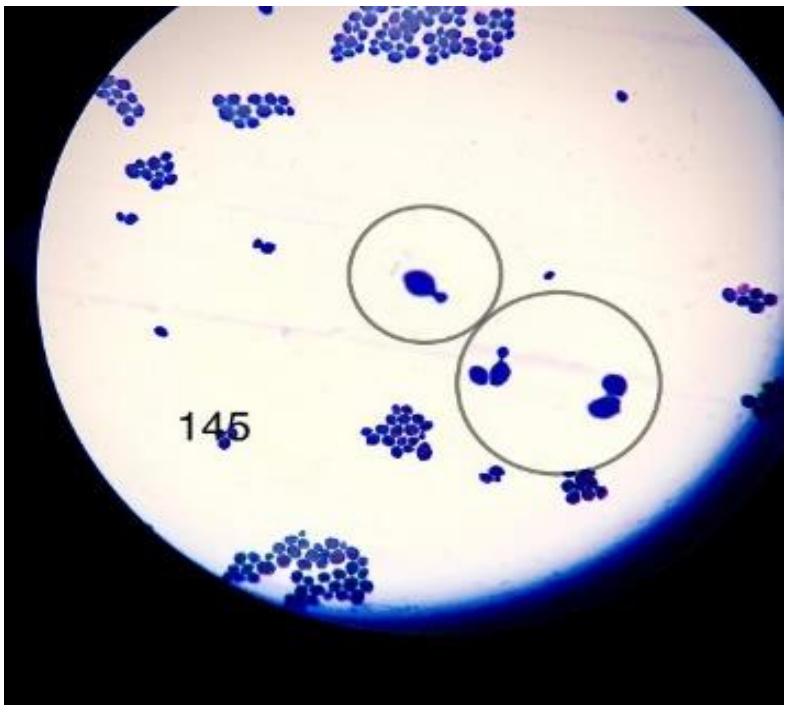

Figure 5: Microscopic view of candida with gram stain at 100-oil magnification

1.1.1. Germ Tube Test

The germ tube test is a screening test is considered a simple, economical, and efficient procedure for differentiating C. albicans from other Candida. Germ tube (GT) formation was first reported by Reynolds and Braude in 1956. When Candida is grown in human or sheep serum at 37°C for 3 hours, they form germ tubes, which can be detected with a wet KOH films as filamentous outgrowth extending from yeast cells. It is positive for *Candida albicans* and *Candida dubliniensis*. Approximately 95 – 97% of Candida albicans isolated develop germ tubes when incubated in a proteinaceous media. Germ-tube test provides a rapid identification test for C. albicans that can be carried out on primary or purified cultures.

4.2.6.1. Principle of Germ Tube Test

Formation of germ tube is associated with increased synthesis of protein and ribonucleic acid. Germ Tube solutions contains tryptic soy broth and fetal bovine serum, essential nutrients for protein synthesis. It is lyophilized for stability. Germ tube is one of the virulence factors of Candida albicans. This is a rapid test for the presumptive identification of *Candida albicans*.

4.2.6.2. Procedure of Germ Tube Test

0.5 mL of human serum was put in tiny tube, a colony of yeast touched with a Pasteur pipette and gently emulsified in the serum, (Figure 6). Then incubated at 37°C for 2 to 4 hours. After incubation a drop of serum placed on a slide to be examined (Figure 7). The slides were stained with gram stain and examined microscopically under high power objectives,

4.2.6.3. Interpretation of Germ Tube Test

Positive Test: A hyphal (filamentous) extension that grows laterally from a yeast cell and has no constriction at the point of origin. The size of the germ tube is half the diameter and three to four times the length of a yeast cell, with no nucleus (Figure 8 & figure 9). Candida albicans and Candida dubliniensis are two examples. Negative test. There is no hyphal (filamentous) extension originating from a yeast cell, or a small hyphal extension that is constricted at its origin. *Candida tropicalis, Candida glabrata*, and other yeasts are examples.

4.2.6.4. Limitations of Germ Tube Test

Candida tropicalis might develop early pseudo hyphae that are mistaken for germ tubes. This test is merely one aspect of a larger system for yeast identification, for a definitive identification, more testing is required.

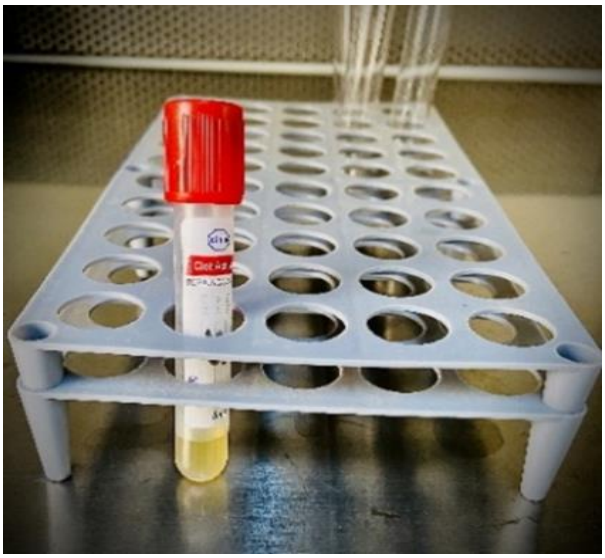

Figure6: Yeast emulsified in serum

Figure7: Serum distributed on slide

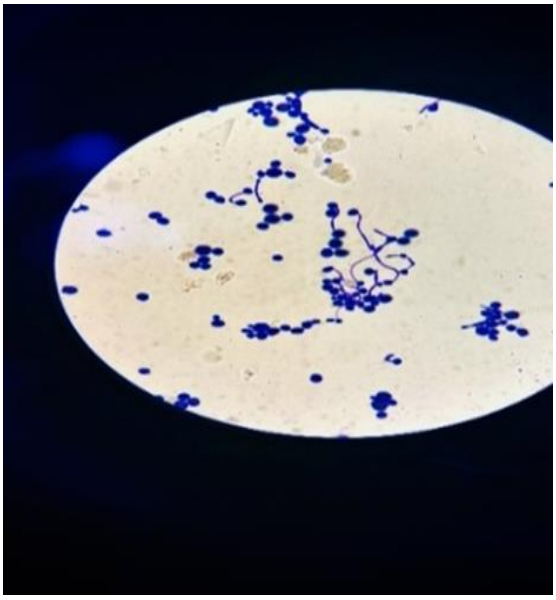

Figure 8: Germ tube formation 1

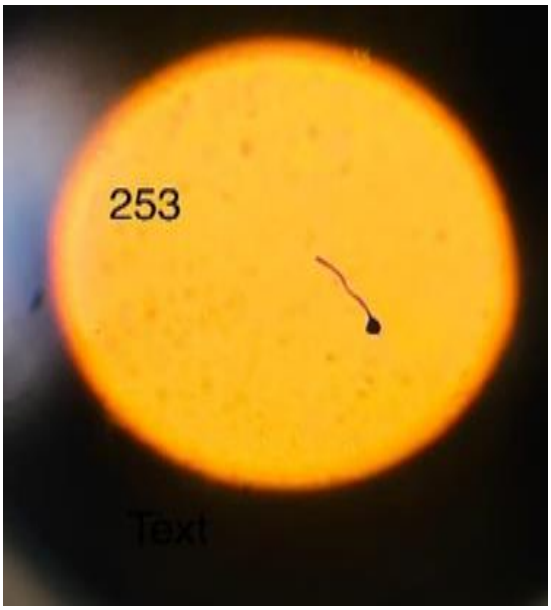

Figure9: Germ tube formation 2

4.2.7. CANDIDAtest 21

4.2.7.1. Presumptive Identification Test Principle, Procedure

ERBA SCAN 21 identification method was used. It was provided by Erba Diagnostics (former Diamedix Corporation and Immunovision) in the USA. The kit CANDIDAtest 21 (Cat. No.: 10010220) is designed for routine identification of the most clinically relevant yeasts. The kit enables the identification of thirty-four strains by means of twenty-one biochemical tests (chromogenic substrates, decarboxylase and assimilations). The tests were placed in wells of micro titration plates. Three rows, each consisting of eight wells, were intended for the identification of one strain. Negative tests for each group of reactions were placed on the plate to help with the correct readings of positive results.

4.2.7.2. CANDIDAtest 21 kit contents

1- 5 microwell plates with tests (each for the identification of 4 isolates)
2- Instructions for use
3- Color scale for CANDIDAtest 21
4- 20 record sheets
5- Lid
6- 20 foils for incubation
7- Storage bag for an open plate
8- 20 suspension media in glass test tubes

4.2.7.3. The Basic Method of Erba Scan 21 Test

based on assimilation reactions of different types of sugars and enzyme.(N-acetyl-b-D-galactosaminidase, alpha-galactosidase, prolinaminopeptidase, alpha-glucosidase, phenylalanine-aminopeptidase, p-nitrophenyl-alpha-glucuronidase, beta-glucosidase, urease, melibiose assimilation, acid from gentiobiose, rhamnose assimilation, xylose assimilation, acid from D-glucose, myo-inositole assimilation, cellobiose assimilation, acid from galactose, trehalose assimilation, saccharose assimilation, acid from maltose, lactose assimilation, raffinose assimilation.

4.2.8. Preparation of candida Erba Scan 21 plate

One side of the packaging foil were Cut off and the plate removed from the package, the required number of the strips were Cut off from the plate (3x8 wells per identification) and inserted them into a prepared empty frame (Figure 10). The unused strips were inserted into a

storage bag freely. Identification numbers of tested strains were wrote on the corresponding strips.

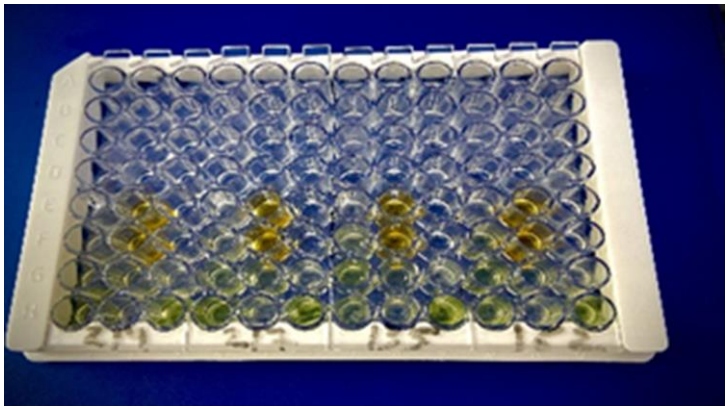

Figure10: Plate inserted into frame

4.2.8.1. Inoculation

The colonies emulsified in 5 mL of sterile saline until the turbidity is similar to McFarland No. 0.5 turbidity standard (Figure 11), densilameter device (Figure 12) were used to measure turbidity, 0.1 ml of the suspension Inoculated into all wells of the respective three row-strip.

4.2.8.2. Incubation

The test strips covered with the incubation foil. CANDIDAtest 21 plate Incubated at 30°C for 48 hours for species growing slowly (Figure 13).

4.2.8.3. Evaluation

The Erba Expert Identification Program were used to evaluate the reactions after 48 hours of the incubation .The incubation foil was removed, and all the results read and printed out by the machine (Figures 14 & Figure 15).

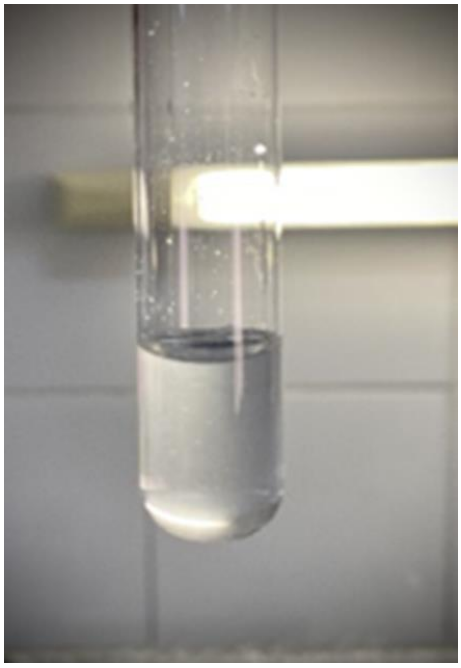

Figure11: McFarland suspension

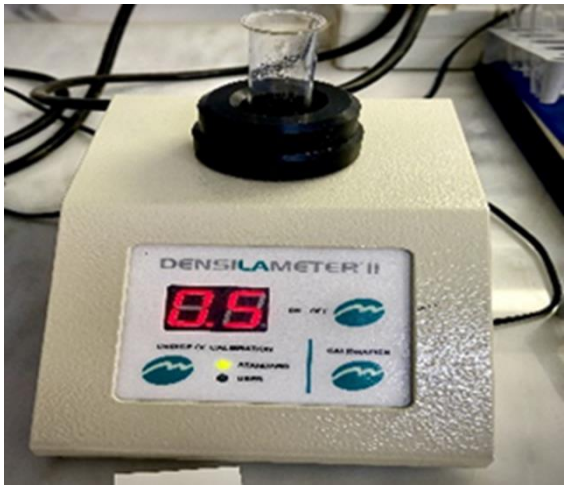

Figure12: Densilameter device

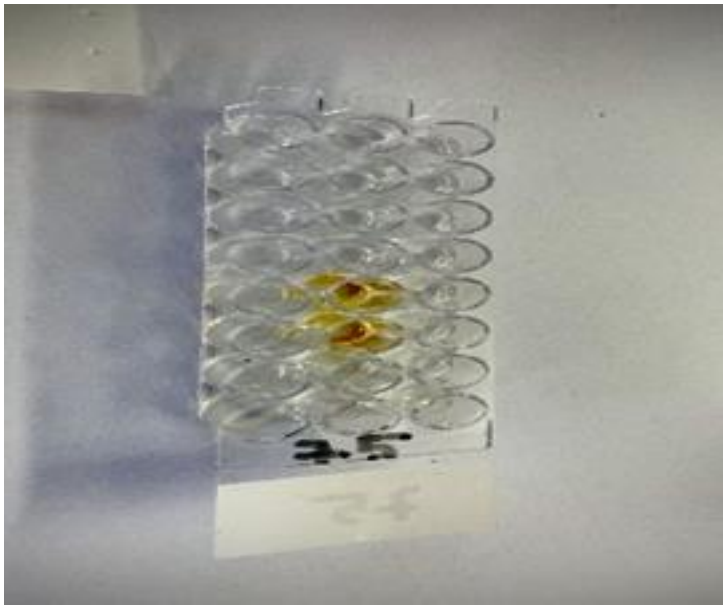

Figure13: Strips enucleated by the suspension

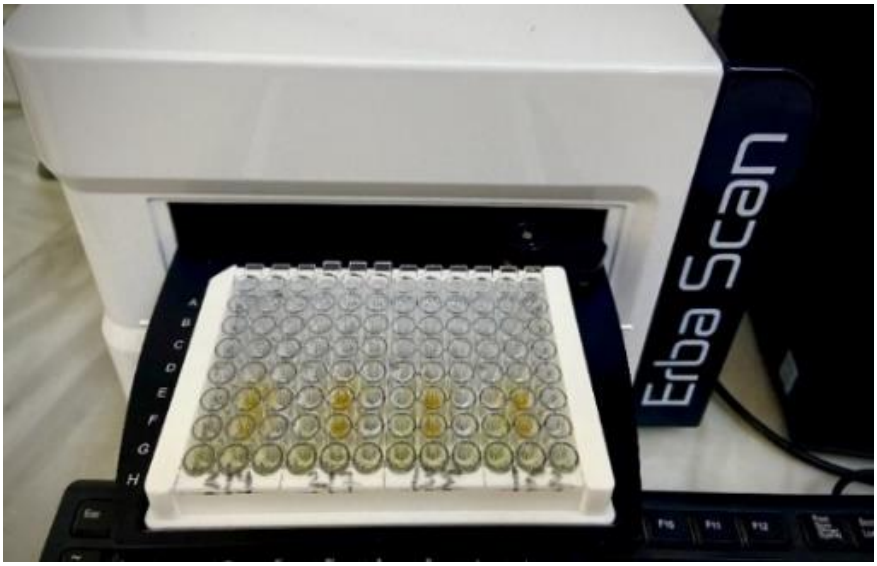

Figure14: ErbaScan device

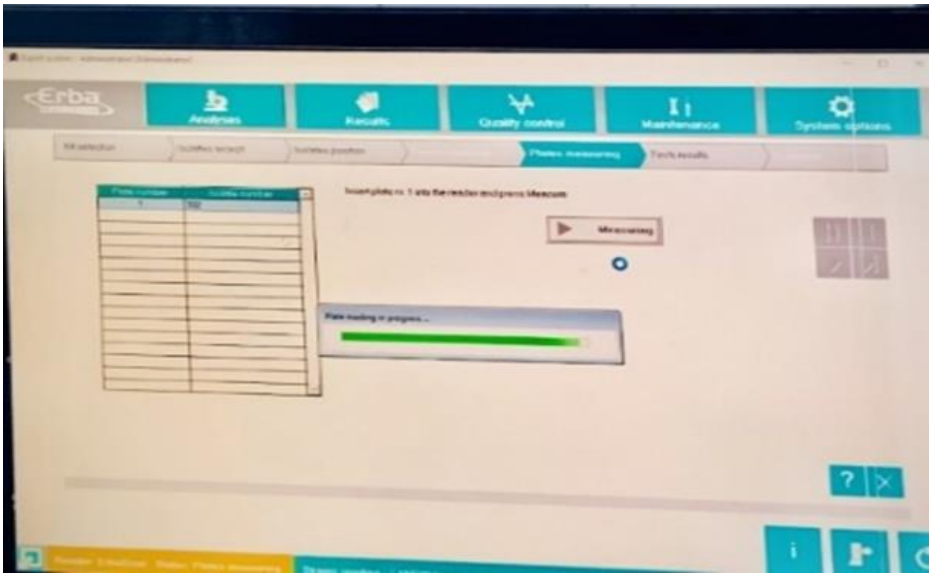

Figure15: Evaluation process of identification

4.3. Sensitivity to antimycotics

4.3.1. Laboratory procedures

The standardized filter paper disk agar diffusion method or Kirby-Bauer method is the most commonly used testing method. In this test, sterilized and cooled inoculating loop used to touch 1 to 3 fungal colonies. The colonies emulsified in 5 mL of sterile saline until the turbidity is similar to McFarland No. 0.5 turbidity standard drug.

A swab dipped into the fungal suspension, squeezed out extra fluid against the tube's side, (Figure 16), then inoculated on agar plate's surface as follows: First, the swab used to streak the entire surface of the plate; then the plate rotated 45 degrees and the entire surface streaked again; finally, the plate rotated another 90 degrees and streaked once more (Figure 17). The swab Discarded in disinfectant, the forceps heated in the Bunsen burner flame and waited to cool.

With the forceps, Antifungal disks from *Oxoid Company* were picked up and put it on the agar surface of one of the inoculated plates. By using the forceps' tips, the disk carefully pressed into full contact with the agar. The forceps reheated and cold again. Steps repeated 5 and 6 until there are around seven different disks on one plate, evenly spaced apart, the plates Inverted and incubated for 24 hours at 37°C.

4.3.2. Interpretation and measurements

After incubation, the plates were checked for zones of fungal growth inhibition (clear rings) around the antimicrobial disks. If no inhibition is present, growth extends to the rims of the disks on all sides, and the organism is classified as resistant (R) to the antimicrobial agent present in that disk. The organism is not automatically considered sensitive (**S**) to the antifungal being tested if a zone of inhibition surrounds the disk. The zone's diameter must first be measured (in millimeters) and compared to values specified in a standard chart for size (Table 1). In some cases, the organism is classed as "intermediate" (**I**) susceptibility to a specific medicine since it cannot be defined as sensitive or resistant.

On each plate culture, the presence or absence of growth around each antimicrobial disk was observed. The sizes of any zones of inhibition Measured with a ruler with millimeter markings and recorded them on the chart. If the organism grows right up to the edge of a disk recorded a zone diameter of 0 mm.

Table 1: Interpretation Table** (Systemic)

Ref. No	Neo-Sensitabs	Potency	Code	Zone diameter in mm			Break-points MIC mg/ml	
				S	I	R	S	R
82512	Fluconazole	25 mg	FLUCZ	> 19	18 – 15 (DD)	< 14	< 8	> 64
82312	Voriconazole	1 mg	VOR.1	> 17	16 - 14 (DD)	< 13	< 1	> 4
281012	Amphotericin B	10 mg	AMPH	> 15	14 – 10	< 10	< 1	> 2
81812	Itraconazole	10 mg	ITRAC	> 23	22 – 14 (DD)	< 13	< 0.12	> 1
81912	Ketoconazole	15 mg	KETOC	> 28	27 – 21	< 20	< 0.12	> 0.5
82412	Caspofungin*	5 mg	CASP5	> 16	15 – 13	< 1 2	< 0.25	> 1
82612	N Posaconazole	5 mg	POSAC	> 17	16 – 14(DD)	< 13	< 1	> 4

DD = dosis dependent
*** Potencies of antifungals, MIC breakpoints and zone breakpoints as recommended by CLSI for Fluconazole and Voriconazole (1). For Amphotericin B, Itraconazole, Ketoconazole and Posaconazole the MIC breakpoints recommended by CLSI are used.*

Figure16: A swab dipped into the fungal suspension

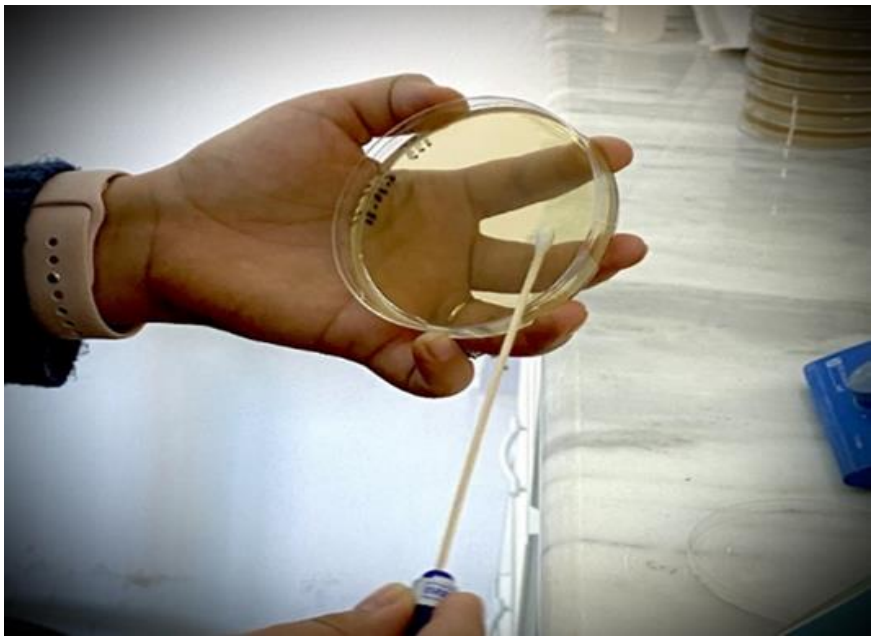

Figure17: Inculcation Sabroaud agar with fungal suspension

4.4. Data analysis

All the obtained information's were tabulated in a Microsoft excel spreadsheet then validated and exported into an SPSS package for statistical analysis. All statistical analyses were performed using SPSS v23 (SPSS Inc). The descriptive analysis was used to describe the demographic characteristics. Chi-square test was performed for comparison. Statistical significance was assumed if p-value≤0.05. Correlations were tested by Spearman Chi square at a level of significance (p value≤ 0.05).

RESULTS

Chapter: 5 Result

5.1. Clinical characteristics of the study group

5.1.1. Introduction

After study registration and approval from the ethical committee for 9 months of the year 2021 this cross-sectional study was carried out to evaluate the oral candidal carriage in 310 dental patients from both genders (female=184; males 126) at central dental clinic in Benghazi city. Patients were recruited from routine dental patients came for different dental and oral complaints. Patients were aged 18 years or over, their dental complaints were related to different dental conditions such as (dental caries, gingivitis, periodontal disease, and fixed prosthesis or denture problems). Few patients have an associated systemic disease such as (diabetes, hypertension, asthma, sinusitis, thyroid diseases and anemia). The patients are included if they met the inclusion criteria and the patient agrees to participate in this study.

Oral samples were collected from the both sides of buccal mucosae by sterile cotton swab, then immediately transported to the microbiology laboratory of the faculty of public health in Benghazi university for culturing the fungi and yeasts on (dextrose Sabroaud agar media and incubated at 36± 1 ^{0}C for 48 hours.

Out of 310 collected samples there were one hundred 100 (32%) positive growths for fungi and yeasts, their ages ranged from 29-71 years (45 males and 55 females). Twelve strains of different fungi were isolated; these strains were exposed to seven types of antifungals agents to evaluate their susceptibility to these agents.

5.1.2. Age groups

This study included patients of different age groups ranged from 18 years to 78 years. Most of patients selected in this study lie in the age group (20-29 years). However, it seems that there is no statistically significant correlation between the age group and the prevalence or the type of oral fungal carriage (Pearson Chi-Square=53.223, df=66, p-value=0.872) (Table 2) and (Figure 18).

Table 2: Age of the study groups

Age group	No	%
< 20	14	04.5%
20-29	83	26.8%
30-39	72	23.2%
40-49	68	21.9%
50-59	41	13.2%
> 60	32	10.3%
Total	310	100%

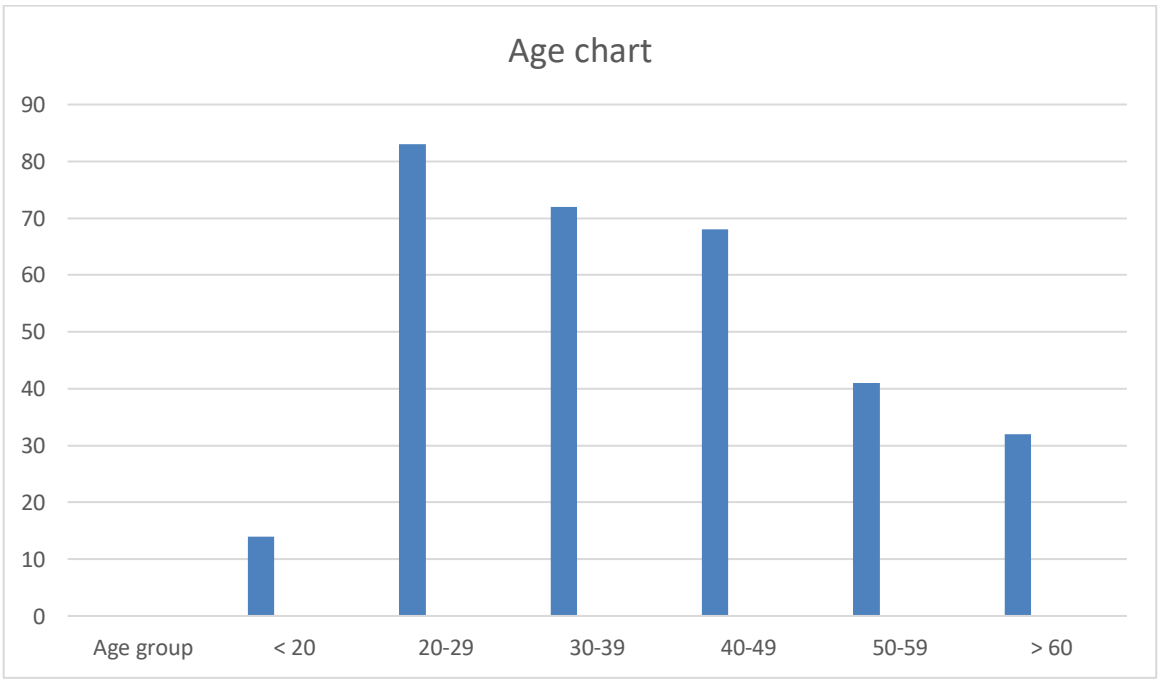

Figure18: Age of study groups

5.1.3. Gender of the study group

The total number of patients was 310, from which there were 126 male patients and 184 female patients (Figure 19).

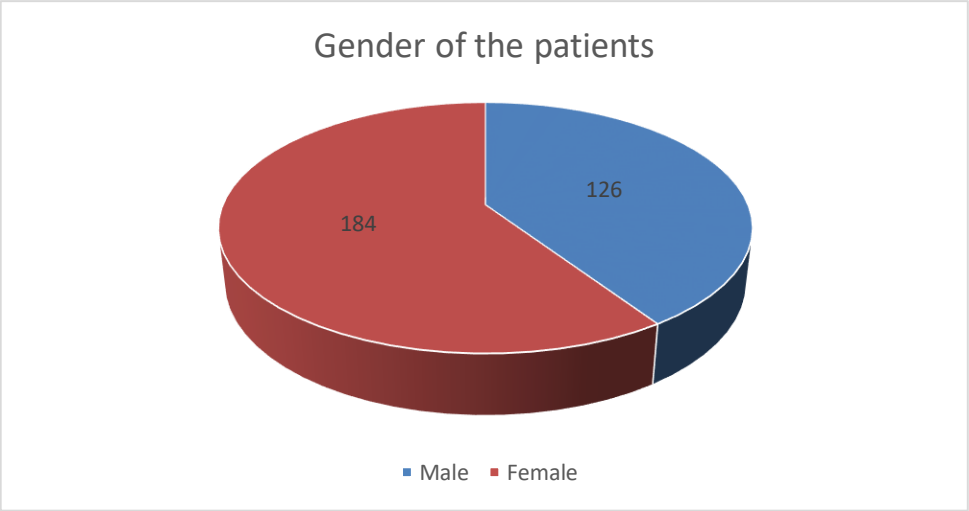

Figure19: Gender of the study group

5.1.4. Smoking and alcoholism

There were 56 male smokers in the study group (18.1%). About half of them 27 patients had positive growths of oral candida (Pearson Chi-Square=7.963, 1, p-value=0.005).

5.1.5. Medical conditions

Only 97 patients (31.6%) reported having medical condition or medical illness, may require medication intake. 70 females in this group has medical problems in comparison to only 27 males. No statistically significant association could be established between these variables and oral candida carriage (Table 3).

Table 3: Cases with medical illnesses in 310 patients

Condition	No of patients		Males	Female
Hypothyroidism	3	(1.0%)	0	3
Asthma	9	(2.9%)	2	7
Hypertension	20	(6.5%)	6	14
Sinusitis	32	(10.3%)	9	23
Diabetes	22	(7.1%)	9	13
Anaemia	10	(3.2%)	0	10
Cardiac Disease	1	(0.3%)	1	0
Total	97	(0.313)	27	70

5.1.6. Dental findings in the whole study group

The dental findings, which may affect the oral candida carriage, were carefully evaluated in study group regardless of their actual candida carriage or not. Interestingly, dental caries was detected in 271 (87.4%) of the patients while periodontal disease ranging from localized gingivitis to advanced generalized periodontitis in about 139 patients. These in turn led to accelerated cases of missing teeth. As the patients living with more than 4 missing teeth in this group was 101 (32.6 %). Fewer patients were lucky to have prosthesis or dental restorations. This indicates poor oral hygiene in the study group (Table 4).

Table 4: Dental findings of the group

Dental condition	No of patients	Male	Female
Dental caries lesion	271 (87.4%)	106	165
Having dental restoration	135 (43.5%)	54	81
Localized chronic gingivitis	22 (7.1%)	8	14
Generalized chronic gingivitis	16 (5.2%)	5	11
Chronic adult periodontitis	91 (29.4%)	43	48
Acute periapical periodontitis	55 (17.7%)	16	39
Chronic periapical periodontitis	150 (48.4%)	60	90
>4 missing teeth	101 (32.6%)	43	58
Wearing complete denture	04 (1.3%)	3	1
Diminished vertical dimension	02 (0.6%)	0	2
Wearing partial denture	06 (1.9%)	3	3
Wearing fixed prosthesis	25 (8.1%)	13	12

5.2. Laboratory findings of the positive growths (n=100)

5.2.1. Culture Characteristics

Culture characteristics of the growth fungi in different samples few colonies (40), heavy growth (17), medium growth (42), single colony (1) figure (22). These characteristics are used to macroscopic identification of the fungi (Table 5).

Table 5: Culture characteristics

	Characteristics				
Isolated M.O ErbaScan	few colonies	heavy growth	medium growth	Single colony	Total
Candida albicans	29	10	28	0	68
Candida catenulate	0	0	2	0	2
Candida dubliniensis	5	4	6	0	15
Candida glabrata	0	0	2	0	2
Candida krusei	2	0	0	0	2
Candida magnoliae	1	0	0	0	1
Candida pelliculosa	0	1	0	0	1
Crypt. Humicola coplex	0	0	1	0	1
Cryptococcus Laurentii	0	0	1	0	1
Geotrichum capitatum	1	0	0	0	1
Rhodotorula rubra	0	0	0	1	1
Trichosporon sp	2	1	2	0	5
Total	40	17	42	1	100

5.2.2. Color characteristics

Colony color characteristics 60% creamy medium, 21% creamy large, 9%creamy small, 7% mixed size, 3% white hard medium (Table 6) and (Figure 20).

Table 6: Color characteristics

Character	Number
Creamy large	21
Creamy medium	60
Creamy small	9
Mixed size	7
White hard medium	3
Total	100

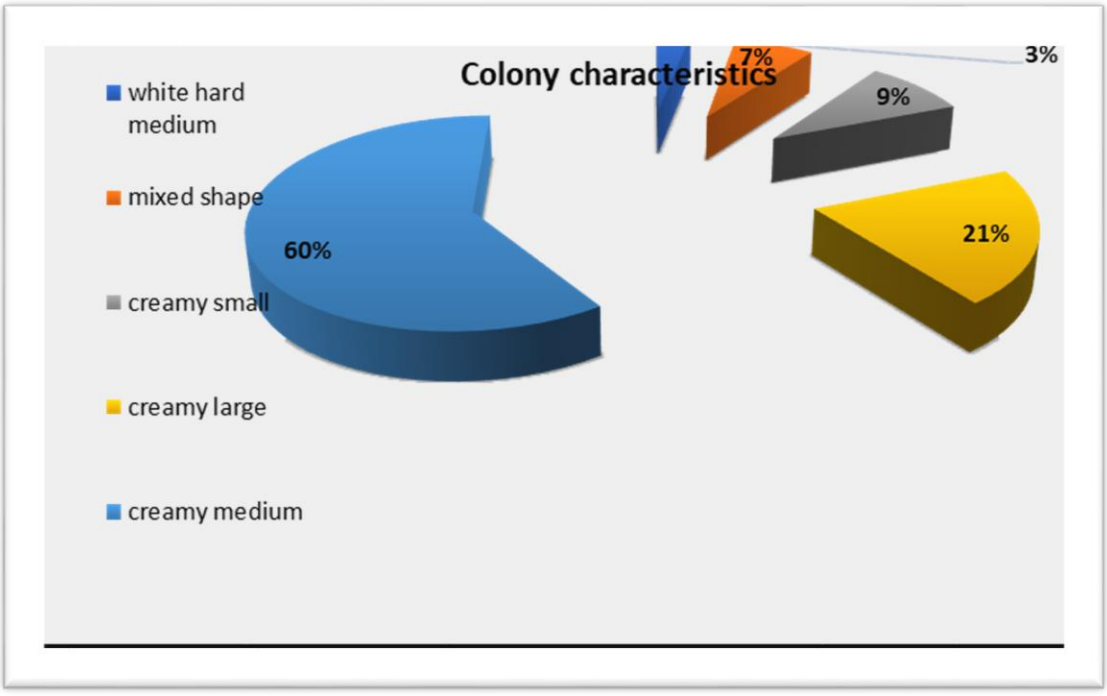

Figure20: Colony color characteristics

5.2.3. Type of candida species in the isolates

One hundred positive growth (32%) samples were isolated from the totally collected 310 samples. On laboratory examination, 12 candidal species could be isolated for different samples. *Candida albicans* were the most prevalent species and found in (68 cases), *Candida dubliniensis* (15 cases), *Trichosporon spp* (5 cases), *Candida catenulata* (2 cases), *Candida glabrata* (2 cases), *Candida krusei* (2 cases), *Candida magnolia* (1), *Candida pelliculosa* (1), *Cryptococcus humicola complex* (1), *Cryptococcus Laurentii* (1), *Geotrichum capitatum* (1), *Rhodotorula rubra* (1) (Table 7) & (Figure 21).

Table 7: Prevalence of fungal species in the isolates

Isolated M.O ErbaScan	**Number of cases**	*Isolated M.O ErbaScan*	**Number of cases**
Candida albicans	68	*Candida magnoliae*	1
Candida dubliniensis	15	*Candida pelliculosa*	1
Trichosporon spp	5	*Crypt. Humicola complex*	1
Candida catenulata	2	*Cryptococcus Laurentii*	1
Candida glabrata	2	*Geotrichum capitatum*	1
Candida krusei	2	*Rhodotorula rubra*	1

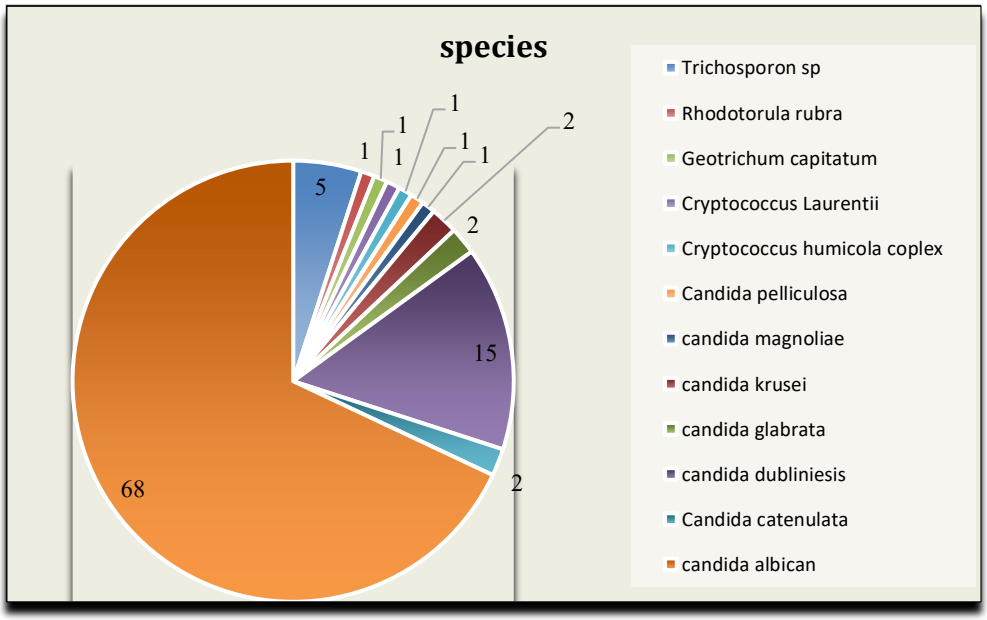

Figure21: Fungal Species isolates

1.1.1. Germ tube test

Germ tube is usually carried out to initially confirm the type of isolated species as it is well known that it only positive in certain strains. This test was positive in 74 cases. In the total 68 cases of *candida albicans* there were 64 cases had positive germ tube test. In addition, out of there were 10 positive cases out of 15 of *candida dubliniensis.* No positive germ tube test results were seen in any other species, as shown in (Table 8) and (Figure 22).

Table 8: Germ tube test

Isolated M.O ErbaScan	Positive growth	Positive GT test	Negative GT test
Candida albicans	*68*	*64*	*4*
Candida catenulate	*2*	*0*	*2*
Candida dubliniensis	*15*	*10*	*5*
Candida glabrata	*2*	*0*	*2*
Candida krusei	*2*	*0*	*2*
Candida magnoliae	*1*	*0*	*1*
Candida pelliculosa	*1*	*0*	*1*
Cryptococcus humicola complex	*1*	*0*	*1*
Cryptococcus Laurentii	*1*	*0*	*1*
Geotrichum capitatum	*1*	*0*	*1*
Rhodotorula rubra	*1*	*0*	*1*
Trichosporon spp	*5*	*0*	*5*
Total	***100***	***74***	***26***

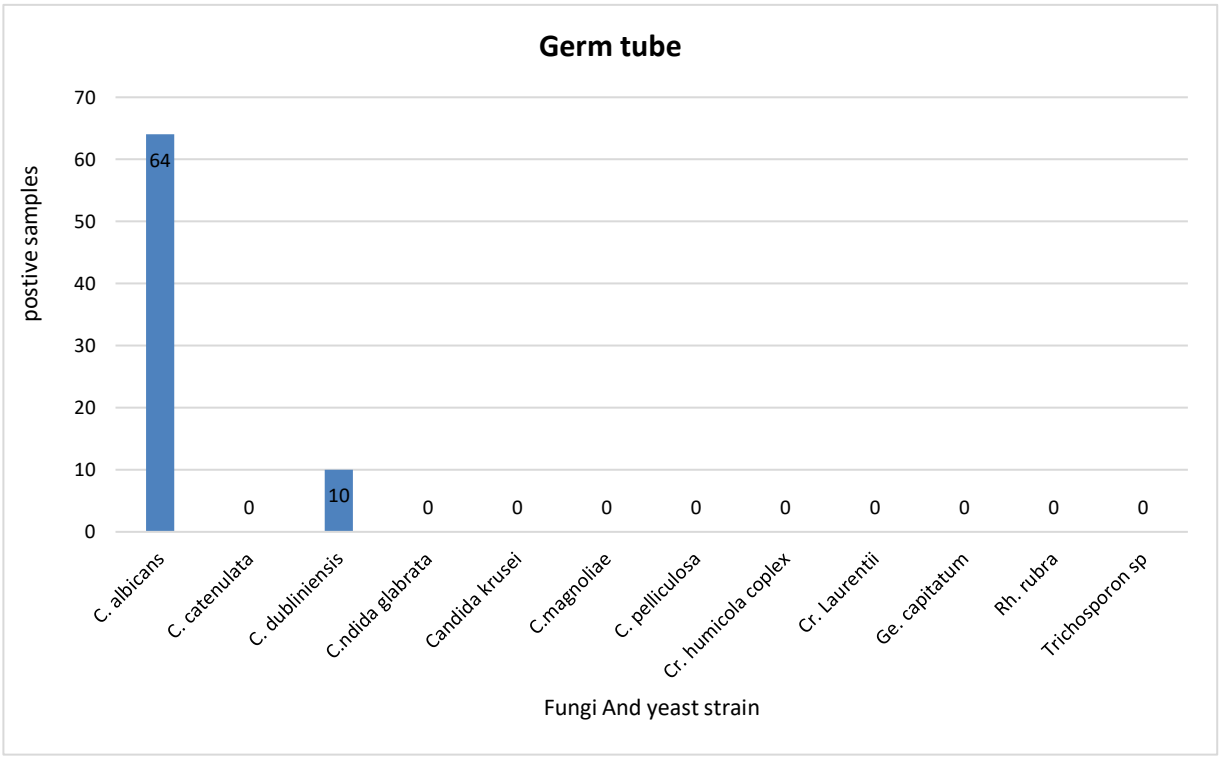

Figure 22: Germ tube test

1.1.1. Frequency of candida species according to age group

The age of patients with positive growths has ranged from (18-73 years) (<20) 9%, (20 to 29) 31%, (40 to 49) 20%, (30-39) 15%, (>60) 15% years old, as it has been shown in Figure 17 (Table 9) shows species isolates distribution among different age groups in this study, *Candida albicans* is the most prevalent species among (20-49 years), while *Candida dubliniensis* was mostly prevalent at the age (20-29 years), as such the 2 cases of *Candida krusei* were found in this age group only. *Trichosporon spp.* Cases seen at age (40-49 years). Generally, Candida oral carriage peaks at middle age groups. There is no correlation between the age group of the patient and the type of candida isolate (Pearson Chi-Square=54.352, df=55, p-value=0.499) (Table9) & (Figure 23).

Table 9: Age groups of the patients with positive growths

	Candida albicans	***Candida catenulata***	**C. dubliniensis**	**C. glabrata**	**C. krusei**	**C. magnoliae**	***Candida pelliculosa***	**Crypt. H. comp**	**Crypt. Laurentii**	**Geo. Capit.**	**Rhodotorula rubra**	**Trichosporon spp**	**Total**
< 20	6	0	2	0	0	1	0	0	0	0	0	0	9
20-29	16	1	7	1	2	0	1	0	1	0	1	1	31
30-39	14	0	1	0	0	0	0	0	0	0	0	0	15
40-49	15	1	1	0	0	0	0	0	0	0	0	3	20
50-59	7	0	1	0	0	0	0	0	0	1	0	1	10
> 60	10	0	3	1	0	0	0	1	0	0	0	0	15
Total	68	2	15	2	2	1	1	1	1	1	1	5	100

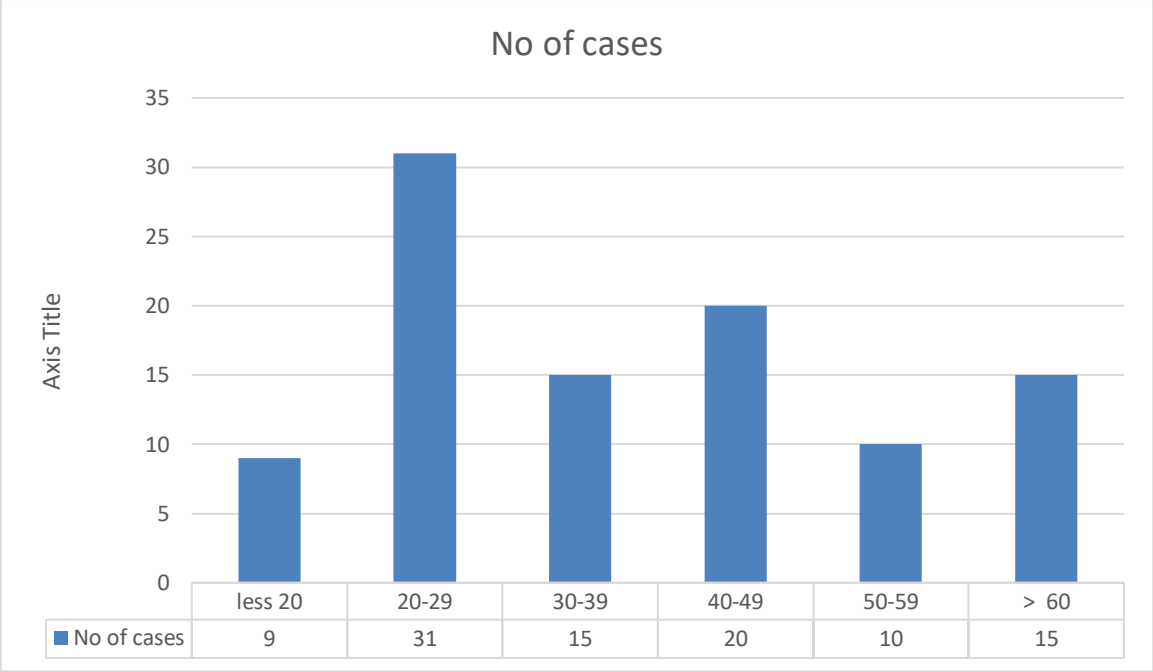

Figure23: Age group of the patients with positive growths (n=100)

1.1.1. The type of fungal isolates according to gender

Males constituted 41% of the total number of the samples with positive growths in comparison to 59 females (Table 10). There is no statistically significant correlation between gender and the type of fungal isolate in the 100 cases with positive growth (Pearson Chi-Square=11.169, df=11, p-value=0.429) (Table10).

Table 10: Frequency of candida species according to gender

Isolated M.O ErbaScan	**Male (n=46)**	**Female (n=54)**	**Total (100)**
Candida albicans	29	39	68
Candida catenulata	1	1	2
Candida dubliniensis	7	8	15
Candida glabrata	2	0	2
Candida krusei	2	0	2
Candida magnoliae	0	1	1
Candida pelliculosa	1	0	1
Crypt. Humicola complex	0	1	1
Cryptococcus Laurentii	1	0	1
Geotrichum capitatum	0	1	1
Rhodotorula rubra	1	0	1
Trichosporon spp	2	3	5
Total	46	54	100

5.2.4. Relationship between smoking, alcoholism & type of candida isolates

Out of 56 smokers in the study group 27 of them had positive growth to oral fungi. *Candida albicans* in 17of them, 4 *Candida dubliniensis*, 1 *Candida catenulata*, 1 *Trichosporon spp*. There is no correlation between smoking habit and the type of oral candida species carriage. Pearson Chi-Square=13.834, df=11, p-value=0.242). There was only one patient admitted alcohol drinking in this group (Table 11).

Table 11: Relationship between candida species and smoking

	Smoking		**Total**
Isolated m o	yes	no	
Candida albicans	17	51	68
Candida catenulata	1	1	2
Candida dubliniensis	4	11	15
Candida glabrata	2	0	2
Candida krusei	0	2	2
Candida magnoliae	0	1	1
Candida pelliculosa	1	0	1
Cryptococcus humicola complex	0	1	1
Cryptococcus Laurentii	1	0	1
Geotrichum capitatum	0	1	1
Rhodotorula rubra	0	1	1
Trichosporon spp	1	4	5
Total	27	73	100

1.1.1. Systemic diseases of the patients with positive growths

The medical history of the patients with positive growths included a couple of medical conditions such as hypertension 4 patients, chronic sinusitis 7, diabetes mellitus 6, and anemia 3. While hypothyroidism, asthma, alcoholism reported by 1 patient each. Six diabetic patients with positive growths 5 of them have *candida albicans* alone and the other one has *candida dubliniensis* in addition to that. There were Four patients with hypertension in this sample with positive growths, the isolates included *candida albicans* in 2 cases, *candida dubliniensis* 1, and *Cryptococcus humicola complex* 1. Seven patients had chronic sinusitis, their isolates showed *candida albicans* in 5 cases and *Trichosporon spp* in 2 cases (Figure 24).

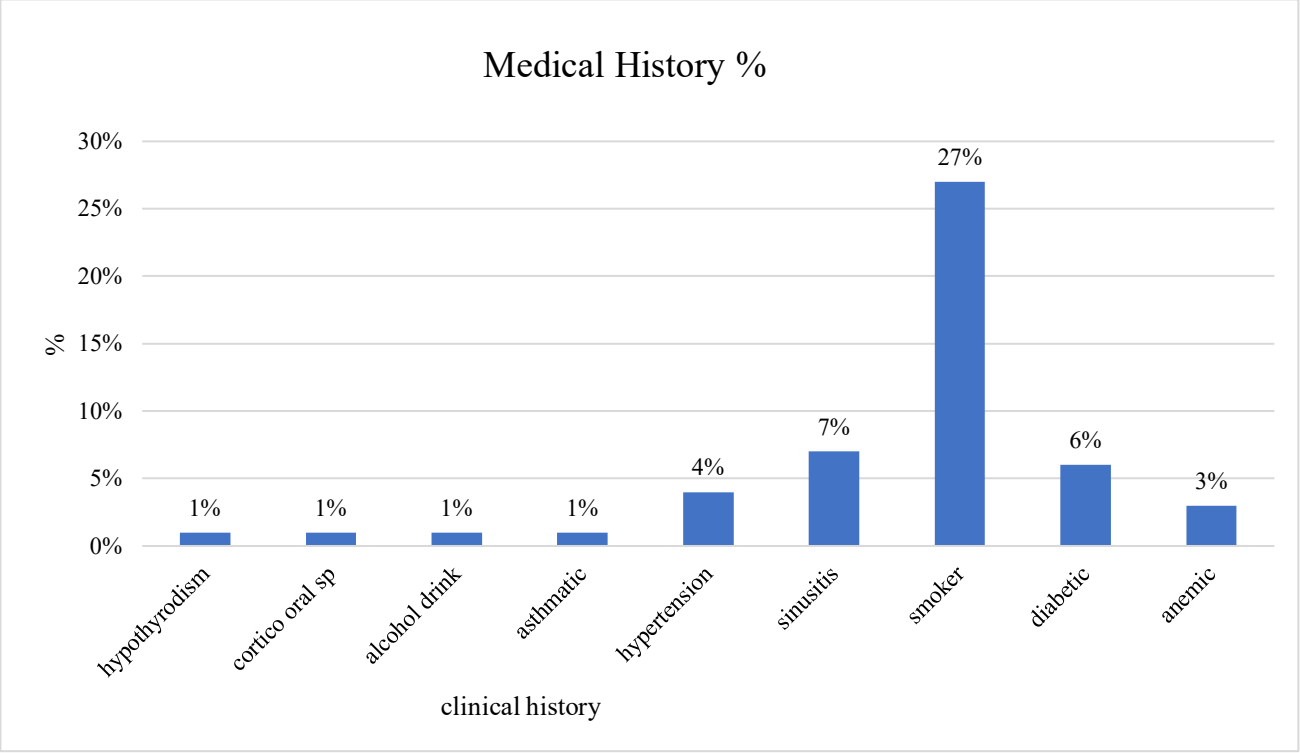

Figure24: Percentage of patients with systemic diseases in 100 patients

5.2.5. Dental problems detected in the group

As all patients in this sample came to this dental clinic seeking dental advice or treatment for at least one or more dental complaints, the type of dental complaints is listed in (Table 10). For the patients with positive growth, 3 of them were using complete dentures, 1 partial denture, 8 having fixed prosthesis (crown(s) or bridge). Interestingly, 86 patients had at least one carious tooth, 63 of them their carious lesions where simple. At the time of interview, there were 15 patients with acute periapical periodontitis, and 51 patients with chronic periapical periodontitis. Only 35 had at least 1 dental filling for their carious teeth. More than 86 patients had generalized mild gingivitis, and 6 patients with localized gingivitis, 4 patients had generalized chronic periodontitis, 30 patients had chronic localized periodontitis, 39 patients had more than 4 missing teeth, 1 patient had a decreased vertical dimension of the face as he is edentulous and didn't have prosthetic replacement. As shown in (Table 12).

Table 12: Correlation between the type of candida isolate and dental problems

Dental finding	No of cases with positive isolates	Pearson Chi-Square (Value)	P Value (2-sided), df=11
Complete denture	3	16.111[a]	0.137
Partial denture	3	33.293[a]	**0.000**
Crown o**r** bridges	8	29.374[a]	**0.002**
Simple dental caries	63	16.505[a]	0.123
APP	15	5.552[a]	0.902
CPP	51	13.491[a]	0.262
Badly decayed tooth	86	14.102[a]	0.227
Dental restoration	35	9.580[a]	0.569
Localized gingivitis	6	9.557[a]	0.571
Generalized chronic gingivitis	4	12.301[a]	0.341
Chronic periodontitis	30	6.438[a]	0.843
> 4 missing teeth	39	14.233[a]	0.220
Decreased vertical dimension	1	19.192[a]	0.058

5.2.6. Frequency of candida species among patients with periodontitis

The spectrum of the isolated fungi in 15 cases clinical diagnosed with acute periapical periodontitis (APP) included 9 cases with *candida albicans,* 4 cases of *candida dubliniensis* and 1 case of *Trichosporon spp*. There were 51 cases diagnosed with chronic periapical periodontitis (CPP), 35 cases of them carry *candida albicans,* 5 cases *candida dubliniensis,* 4 cases *Trichosporon spp,* 2 *candida catenulata,* 2 *candida krusei*, while there has been 1 case each for c*andida glabrata*, *Candida magnoliae,* and *Candida pelliculosa*. Six cases diagnosed with localized gingivitis, 3 cases carry *Candida albicans, 2 Candida dubliniensis,* 1 *Candida catenulata*).

5.2.7. Frequency candida species among patients with dental caries

Sixty-three cases had simple dental caries their isolates included *candida albicans* in 44 cases, *candida dubliniensis* 13, *Trichosporon spp* 2, and (1 case each) for *candida glabrata, candida krusei, Candida magnoliae,* and *Cryptococcus Laurentii.* But the patients with multiple badly decayed caries didn't show differences in type of candidal species in the isolates. Pearson Chi-Square=14.102, df=11, p-value=0.227 (Table 13).

Table 13: Prevalence of candida carriage in pts with badly decayed teeth

Isolated m o	**Presence of Carious teeth**		**Total**
	yes	no	
Candida albicans	59	9	68
Candida catenulata	2	0	2
Candida dubliniensis	13	2	15
Candida glabrata	2	0	2
Candida krusei	2	0	2
Candida magnoliae	1	0	1
Candida pelliculosa	1	0	1
Cryptococcus humicola complex	0	1	1
Cryptococcus Laurentii	1	0	1
Geotrichum capitatum	0	1	1
Rhodotorula rubra	1	0	1
Trichosporon spp	4	1	5
Total	86	14	100

5.2.8. Frequency of candida species among dental prosthesis users

Three cases use complete dentures, 2 of them had positive growth to *candida albicans*, 1 *candida glabrata*. Three cases use partial denture with (2 *candida albicans* isolates and 1 *Geotrichum capitatum).* Eight cases use crown(s) or bridges with *candida albicans* 4, and one case each of *candida dubliniensis*, *candida catenulata*, *Cryptococcus Humicola complex,* and *Rhodotorula rubra*.

5.2.9. Candida species among patients with multiple dental restorations

Thirty-five cases with dental restoration (28 *candida albicans*, 6 *candida dubliniensis)*. The type of candida species does not differ from those without dental restorations. Pearson Chi-Square=9.580, df=11, p-value=0.569 (Table 14) & (Figure 25).

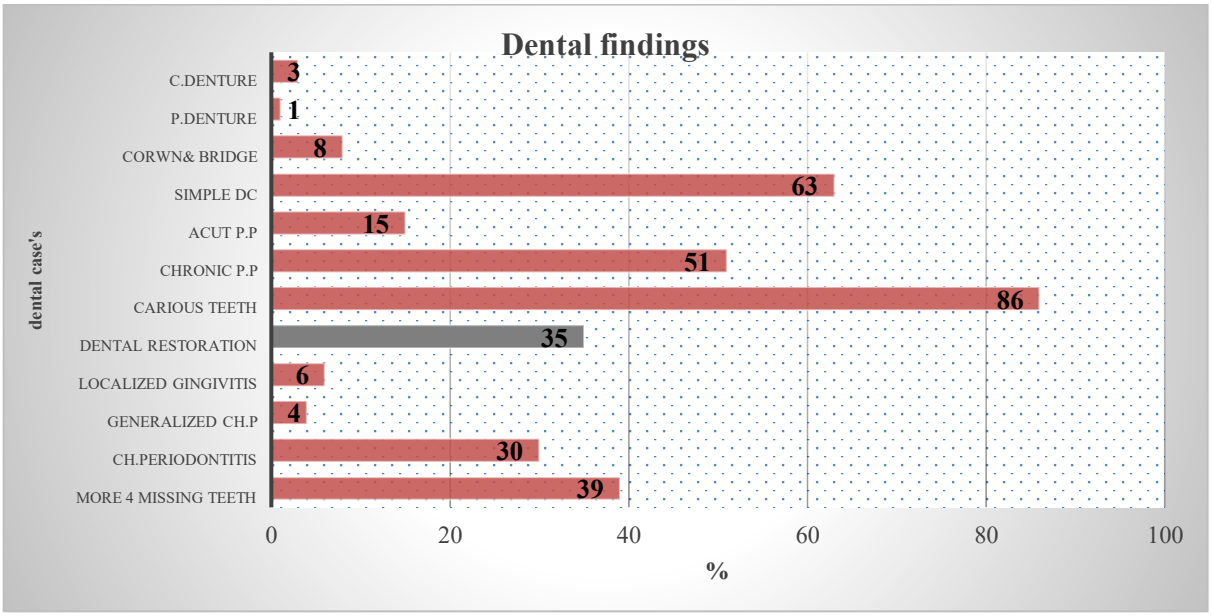

Figure25: Dental restorations and candidal species

Table 14: Distribution of isolated species in dental cases

Species Isolated	**acute pp.**	**Chronic pp.**	**carious teeth**	**Comp denture**	**partial denture**	**crown or bridge**	**simple D.C**	**dental resto**	**Local. gingiv**	**GC.G**	**ch. Period.**	**> 4 miss teeth**	**Decr V.d**
Candida albicans	9	35	59	2	2	4	44	28	3	3	21	28	0
Candida catenulate	0	2	2	0	0	1	0	0	1	1	1	2	0
Cand. Dubliniensis	4	5	13	0	0	1	13	6	2	0	5	2	0
Candida glabrata	1	1	2	1	0	0	1	0	0	0	1	1	0
Candida krusei	0	2	2	0	0	0	1	1	0	0	0	1	0
Candida magnoliae	0	1	1	0	0	0	1	0	0	0	0	0	0
Candida pelliculosa	0	1	1	0	0	0	0	0	0	0	0	1	0
Crypto. hum comp.	0	0	0	0	0	1	0	0	0	0	1	1	0
Cryptoc. Laurentii	0	0	1	0	0	0	1	0	0	0	0	0	0
Geotric. Capitatum	0	0	0	0	1	0	0	0	0	0	0	1	0
Rhodotorula rubra	0	0	1	0	0	1	0	0	0	0	0	0	0
Trichosporon spp	1	4	4	0	0	0	2	0	0	0	1	2	1
Total	15	51	86	3	3	8	63	35	6	4	30	39	1

5.3. Susceptibility to Antifungal agents

In the (Figure 26) the percentage of susceptibility of isolated microorganism to antifungal drug in general, the result showing that the high resistance was in (amphotericin, fluocytosine, Clotrimazole) 98%, 97% and 83% respectively, intermediate to (fluconazole 67% and Nystatin 60%) and highly sensitive to (voriconazole 91%, Itraconazole 71%). The grade of susceptibility is measured by the calibration machine (Figure 27).

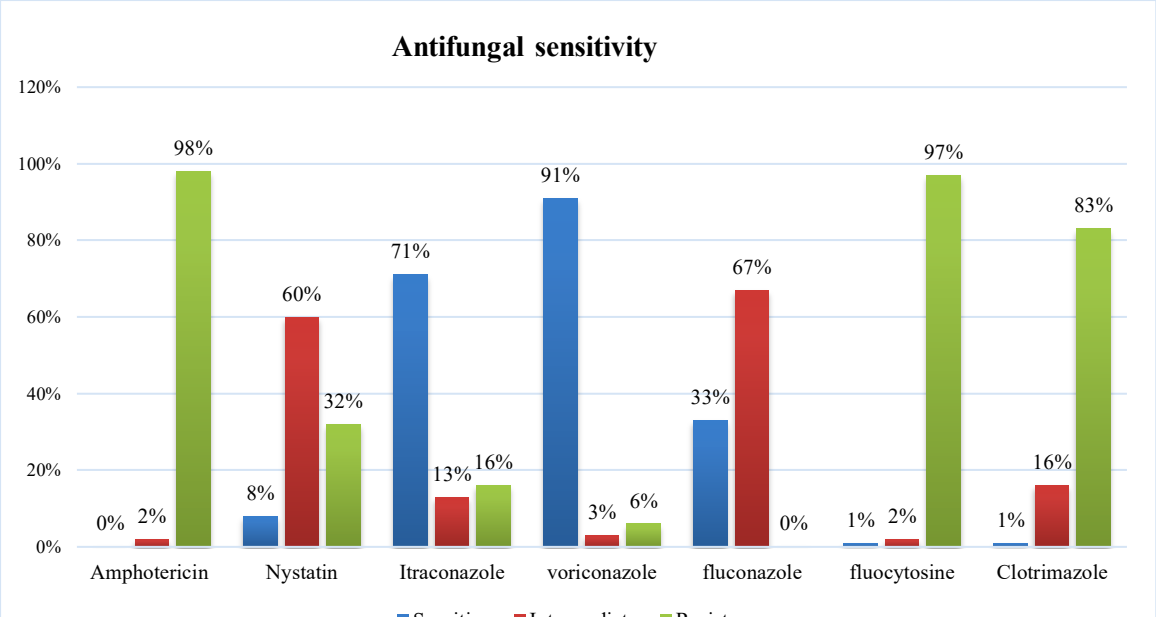

Figure26: Antifungal sensitivity

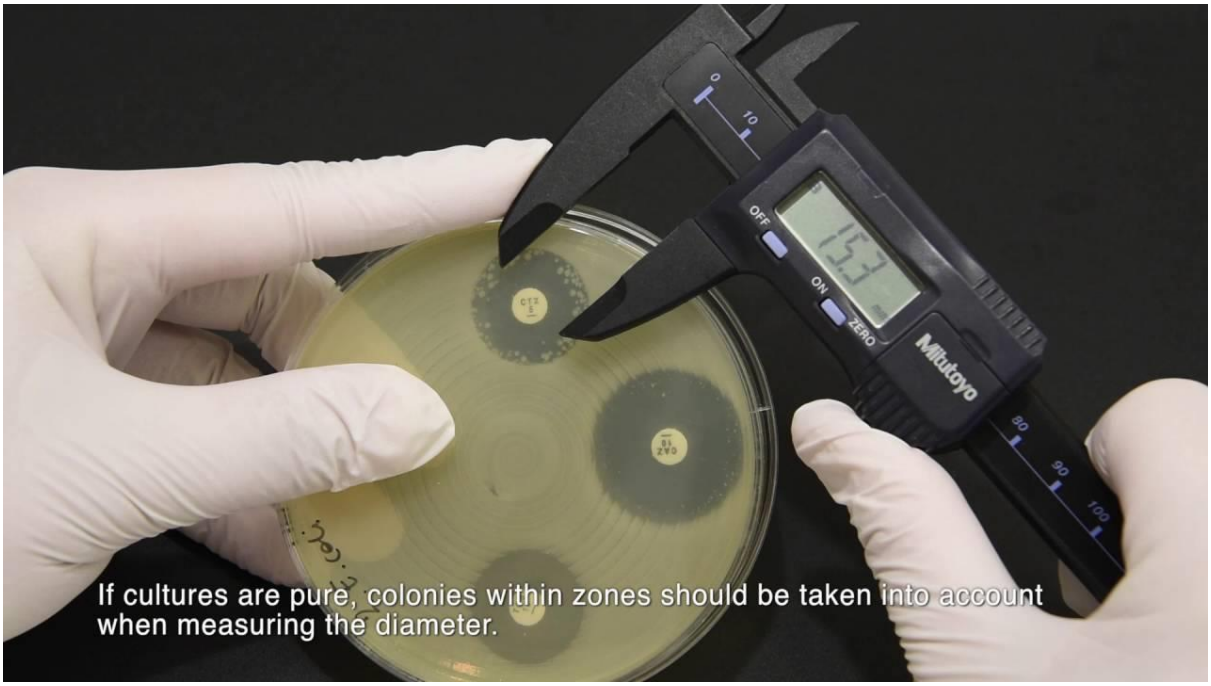

Figure27: Measuring the antifungal susceptibility by calibration machine.

Figure28: *Candida albicans* resistance to both amphotericin & fluocytosine

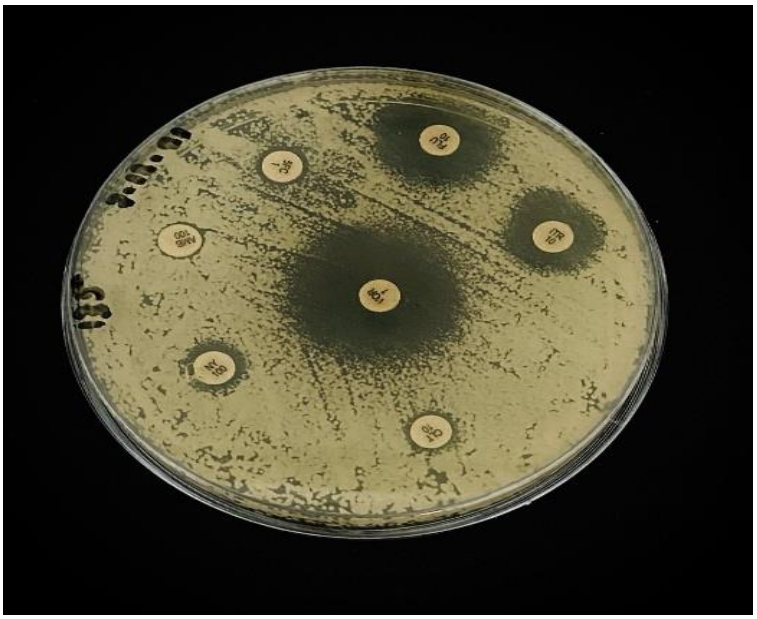

Figure29: *Candida albicans* sensitive to Itraconazole, fluconazole, voriconazole

Figure 30: *Candida dubliniensis* is sensitive to voriconazole, Itraconazole and fluconazole

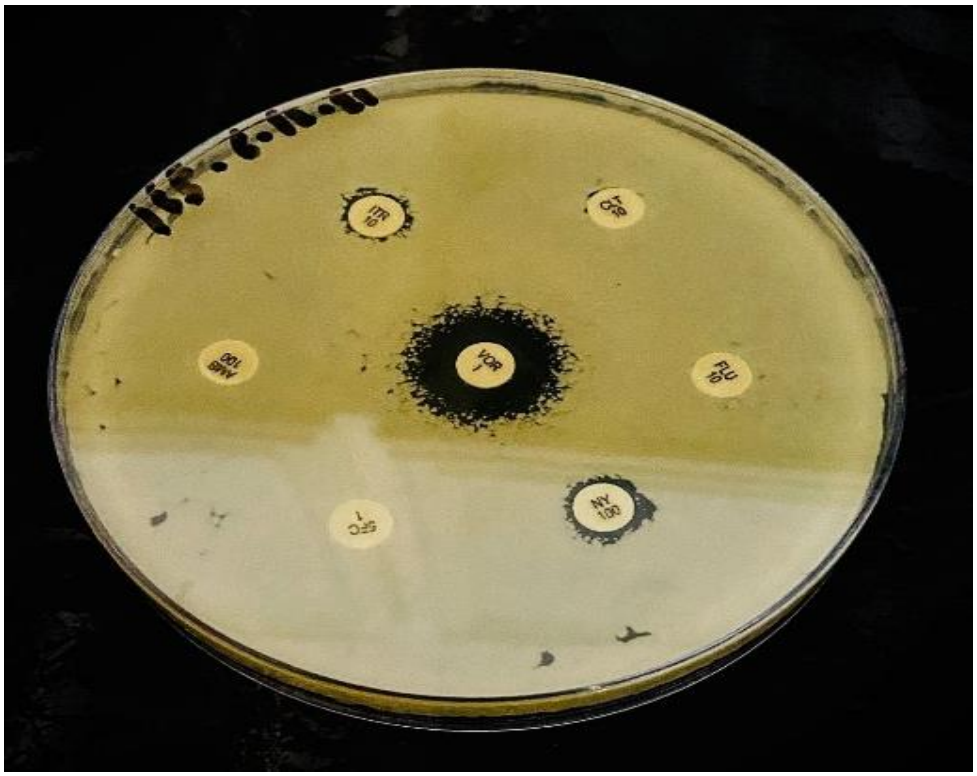

Figure 31: *C. Magnoliae* resistant to all antifungals & intermediate to voriconazole

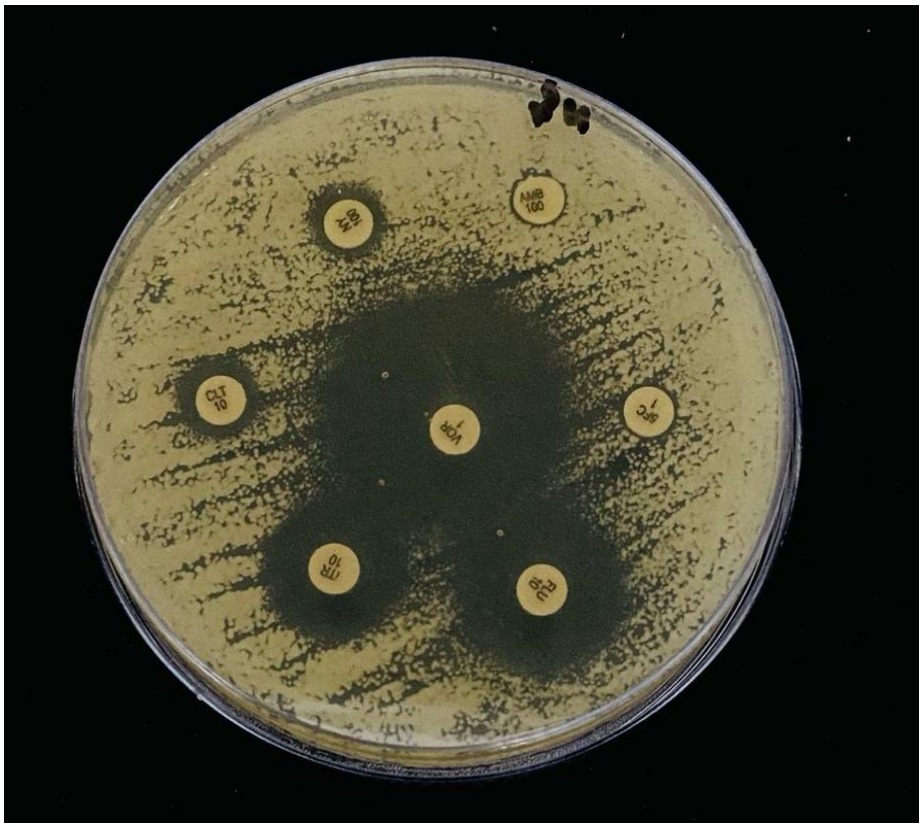

Figure 32: *Candida albicans* sensitive to voriconazole, Itraconazole, fluconazole

Figure 33: *Candida dubliniensis* sensitive to coltri., itracon., flucon., & voriconazole

Figure 34: Antifungal agents used in this study

5.3.1. Amphotericin (100 µg) -

The isolated microorganisms show high resistance to Amphotericin. Out of 68 *Candida albicans* 67 (99%) are resistant to amphotericin and only one (1%) had an intermediate sensitivity to it. two strains of *Candida catenulata* show resistance to amphotericin, out of 15 *Candida dubliniensis* there were 14 (93%) resistant and one (7%) intermediate, 2 *Candida glabrata* resistant, 2 *Candida krusei* resistant, the isolated fungi which were one from each species were resistant to amphotericin antimycotic (*Candida magnoliae Candida pelliculosa, Cryp. complex Cryp. Laurentii Geot. Capitatum Rhod. rubra*). *Tricho. spp* showed resistance to the agent for all the 5 microorganism, Pearson Chi-Square=2. 111, df =11, p-value= 0. 998 (Figure 35).

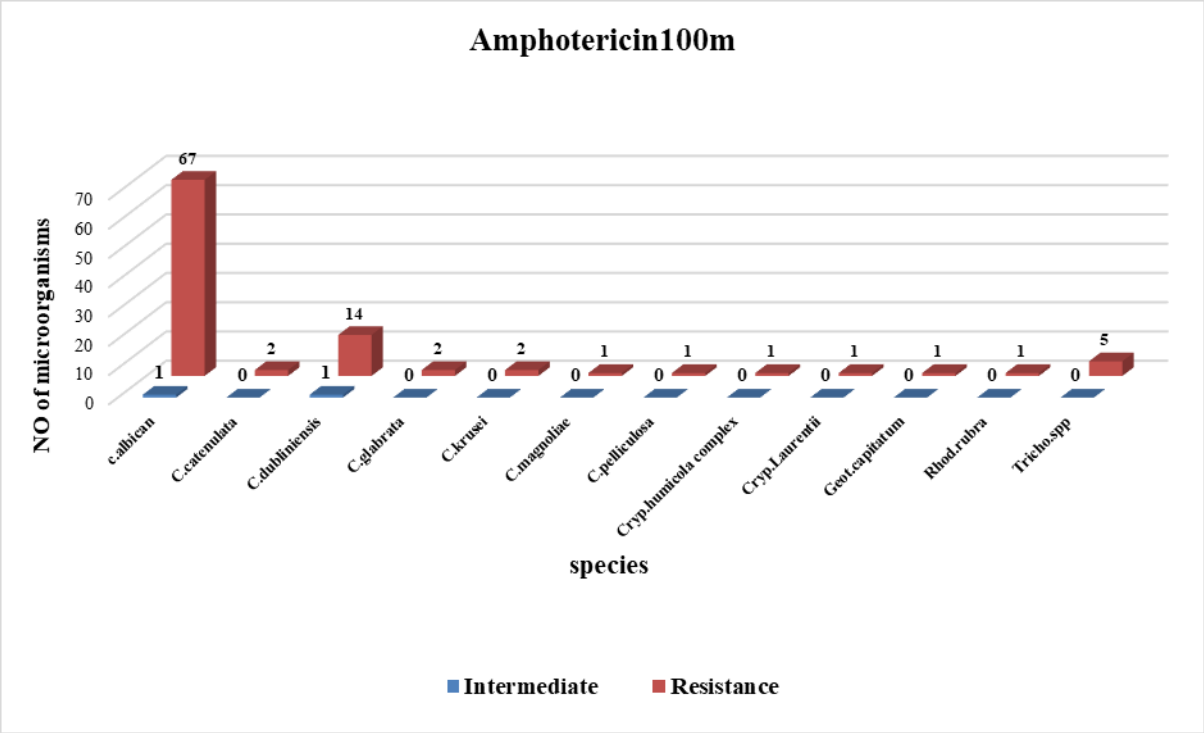

Figure 35: Susceptibility to amphotericin

5.3.2. Nystatin (100 IU)

Show from intermediate to resistance effect on isolated microorganism. Out of 68 microorganism of *Candida albicans* 48 intermediate (71%), 17 (25%) resistant and 3 (4%) sensitive to the agent. 2 *Candida catenulata* one intermediate and one resistant, 15 *Candida dubliniensis* 7 (47%) resistant, 6 (40%) intermediate and two (13%) sensitive, 2 *Candida glabrata* all intermediate, 2 *Candida krusei* resistant, *Candida magnoliae* resistant *Candida pelliculosa* intermediate, *Crypt. Humicola* complex sensitive, *Crypt. Laurentii* sensitive, *Geot. Capitatum* resistant, *Rhod. rubra* resistance and 5 microorganism of *Tricho. spp* one (20%) sensitive, 2 (40%) intermediate and 2 (40%) resistant to the agent. Pearson Chi square 43. 302, df (22), P-value 0. 004 (Figure 36).

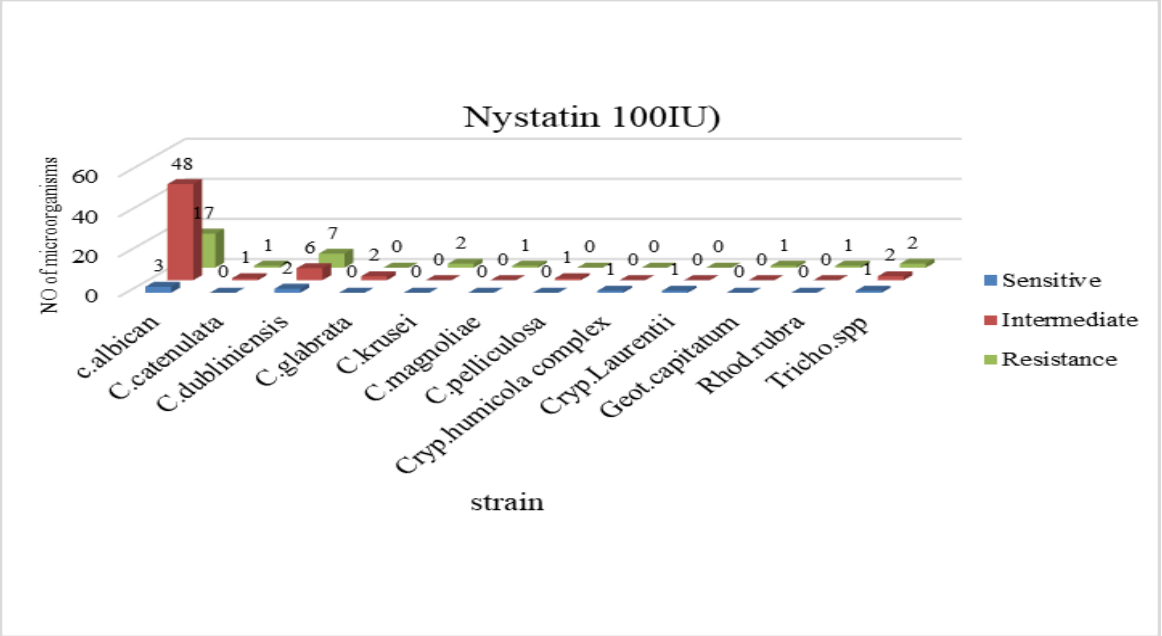

Figure 36: Susceptibility to Nystatin

5.3.3. Clotrimazole (10 mg) -

Show from intermediate to resistant effect on isolated microorganism, Out of 68 microorganism of *Candida albicans* 11 (16%) intermediate, 56 (82%) resistant and 1 (2%) sensitive to the agent. 2 *Candida catenulata* resistant, 15 *Candida dubliniensis* 10 (67%) resistant, 5 (33%) intermediate, 2 *Candida glabrata* all resistant, 2 *Candida krusei* resistant, *Candida magnoliae* resistant *Candida pelliculosa* resistant, *Cryp. complex* resistant, *Cryp. Laurentii* resistant, *Geot. Capitatum* resistant, *Rhod. rubra* resistant and all 5microorganism of *Tricho. spp* resistant to the agent. Pearson Chi-Square 7. 086, (df=22), p= 0. 999 (Figure 37).

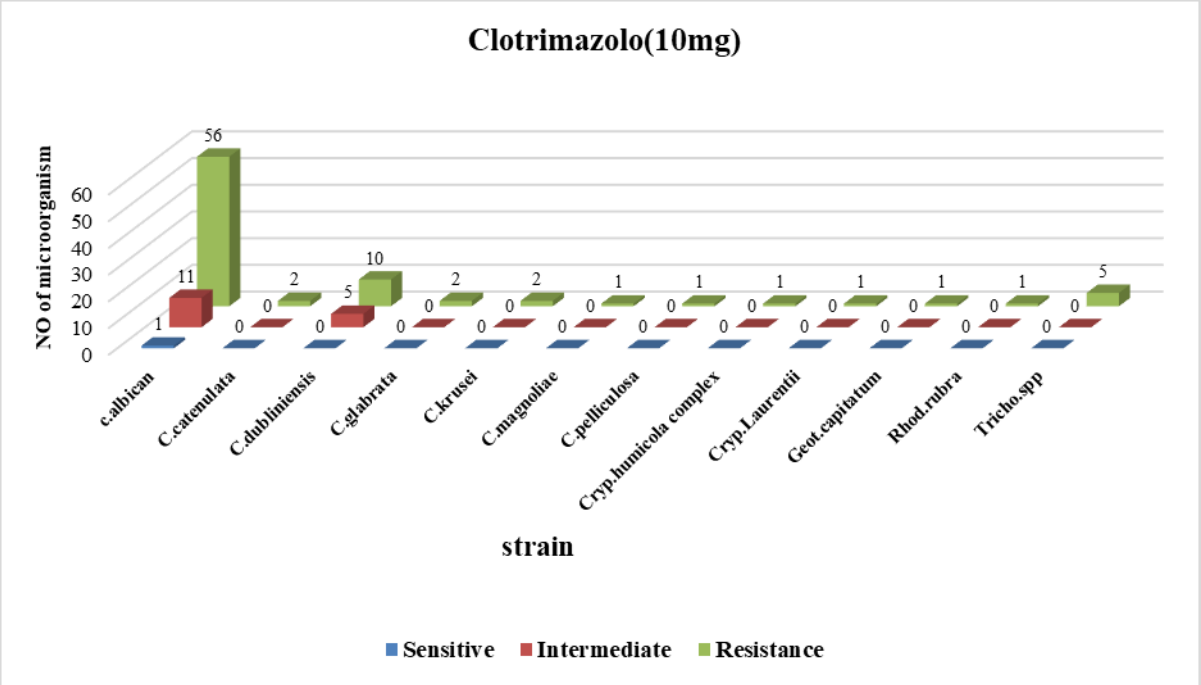

Figure 37: Susceptibility to Clotrimazole

5.3.4. Itraconazole (10 mcg)

Itraconazole (10mg): - Show from sensitive to resistant effect on isolated microorganism. Out of 68 microorganism of *Candida albicans* 9 (13%) intermediate, 6 (9%) resistant and 53sensitive (78%) to the agent. 2 *Candida catenulata* one sensitive and one intermediate. 15 *Candida dubliniensis* 12 (80%) sensitive and 3 (20%) intermediate. 2 *Candida glabrata* all resistant. 2 *Candida krusei* resistant, *Candida magnoliae* resistant *Candida pelliculosa* sensitive, *Cryp. complex* sensitive, *Cryp. Laurentii* resistant, *Geot. Capitatum* resistant, *Rhod. rubra* resistant and 5 microorganism of *Tricho. spp* 3 (60%) sensitive, 2 (40%) resistant to the agent. Pearson Chi-Square 53. 692, df= (22), p=0. 000 (Figure 38).

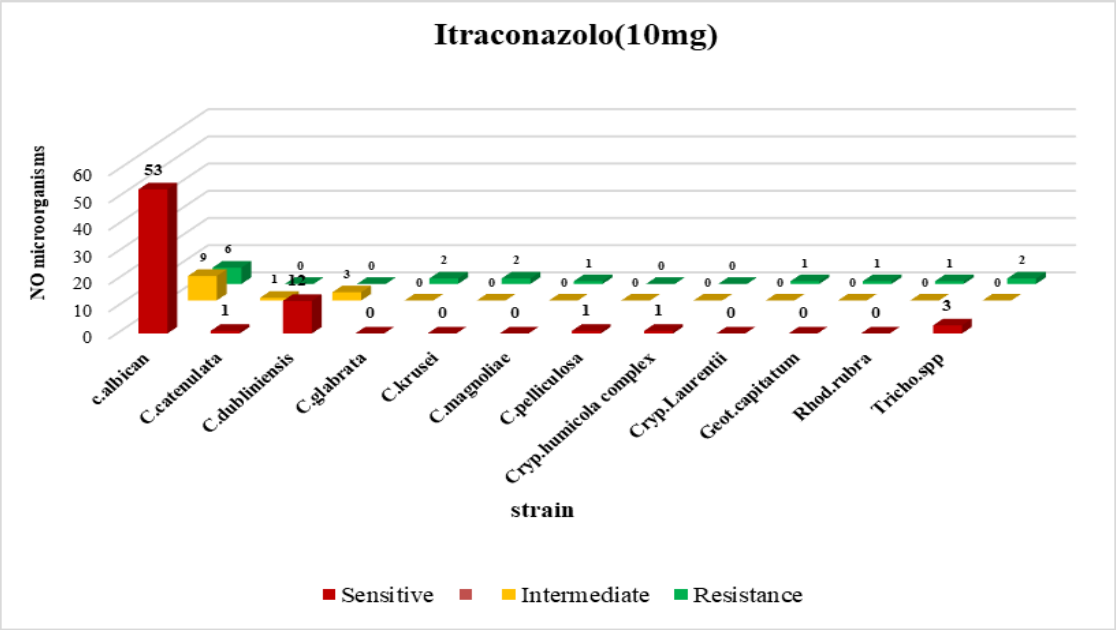

Figure 38: Susceptibility to Itraconazole

5.3.5. Voriconazole (1mcg) -

Show from sensitive to resistant effect on isolated microorganism, Out of 68 microorganism of *Candida albicans* 9 (13%) intermediate, 6 (9%) resistant and 53 (78%) sensitive to the agent. 2 *Candida catenulata* one sensitive and one intermediate, 15 *Candida dubliniensis* 12 (80) sensitive and 3 (20%) intermediate,2 *Candida glabrata* all resistant, 2 *Candida krusei* resistant, *C. magnoliae* resistant *Candida pelliculosa* sensitive, *Cryp.h. complex* sensitive, *Cryp. Laurentii* resistant, *Geot. Capitatum* resistant, *Rhod. rubra* resistant and 5 of *Tricho. spp* 3 (60%) sensitive, 2 (40%) resistant to the agent, Pearson Chi-Square 11.614, df (11), p= 0.393 (Figure 39).

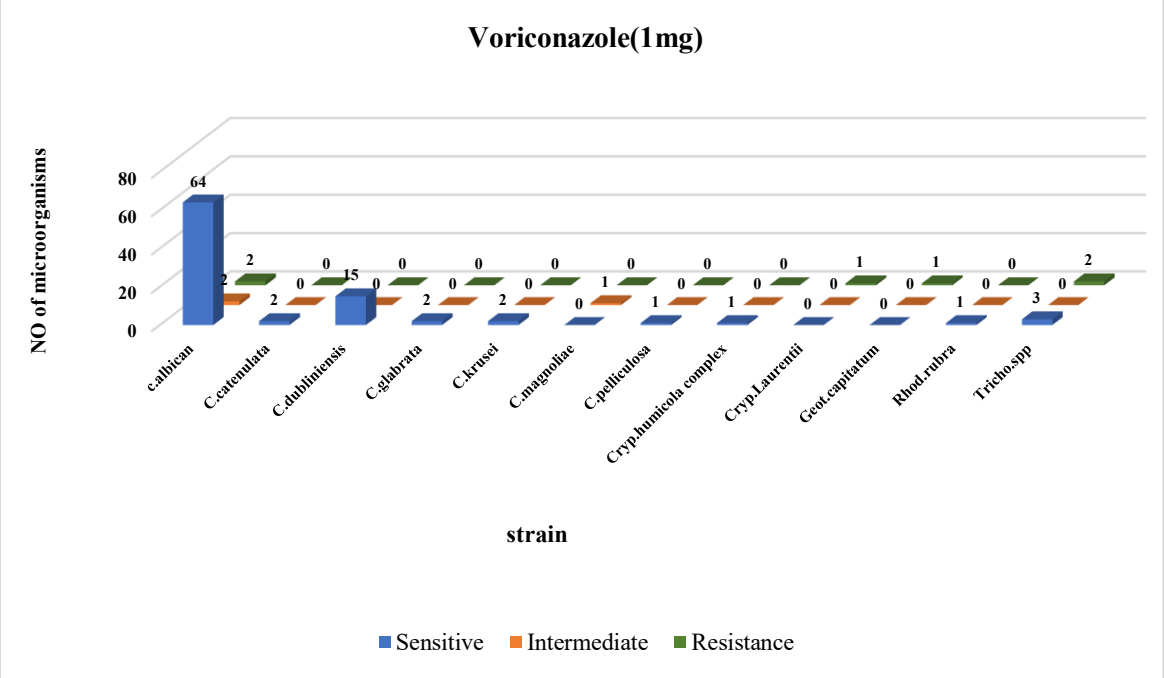

Figure 39: Susceptibility to Voriconazole

5.3.6. Fluconazole (10 mcg) -

The isolated microorganisms are sensitive to intermediately sensitive to fluconazole. Out of 68 microorganism of *Candida albicans* 43(63%) intermediate, 25(37%) sensitive to the agent. 2 *Candida catenulata* intermediate, 15 *Candida dubliniensis* 8(53%) sensitive and 7(47%) intermediate.2 *Candida glabrata* all intermediate, 2 *Candida krusei* intermediate, *Candida magnoliae* intermediate, *Candida pelliculosa* intermediate, *Cryp. h. complex* intermediate, *Cryp. Laurentii intermediate, Geot. capitatum* intermediate, *Rhod rubra* intermediate and 5microorganism of *Tricho. spp* intermediate to the agent. Pearson Chi-Square 11.614, df (11), p=0.393 (Figure 40).

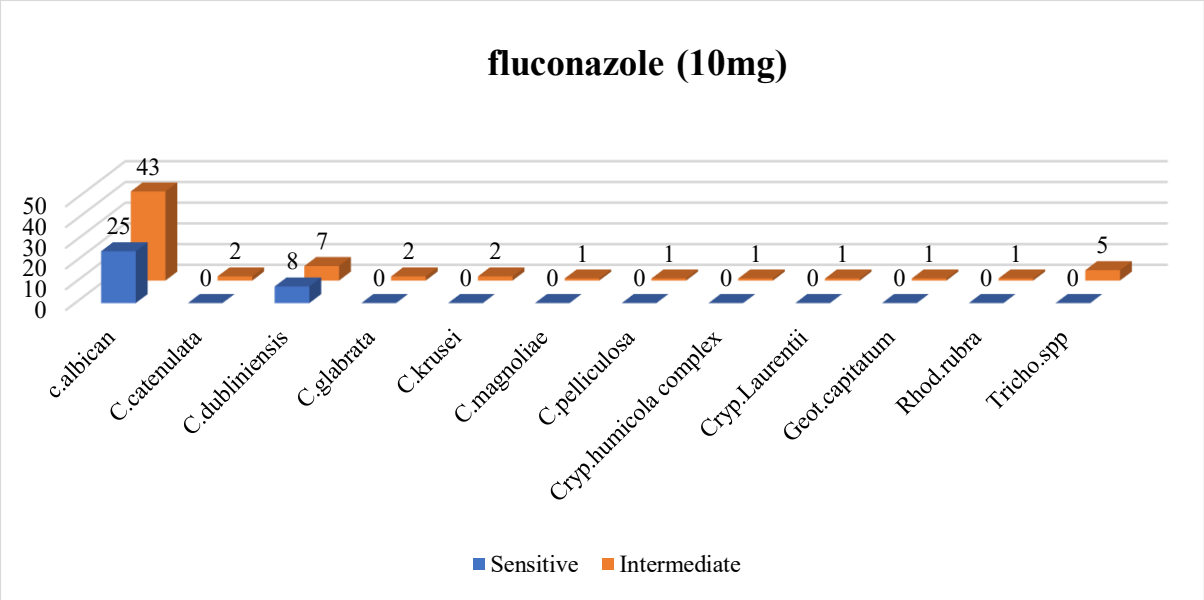

Figure 40: Susceptibility to fluconazole

5.3.7. Flucytosine-(10 mcg)

Show high resistance effect on isolated microorganism, Out of 68 microorganism of *Candida albicans* 66 (97%) resistant, one (1.5%) intermediate, one (1.5%) sensitive to the agent, 2 *Candida catenulata* resistant, 15 *Candida dubliniensis* resistant, 2 *Candida glabrata* resistant, 2 *Candida krusei* resistant, *Candida magnoliae* resistant, *Candida pelliculosa* resistant, *Cryp. h. complex* resistant, *Cryp. Laurentii* resistant, *Geot. Capitatum* resistant, *Rhod. rubra* resistant and 5microorganism of *Tricho. Spp* resistant to the agent. Chi-Square Tests were highly significant (P value 0.001) (Figure 41).

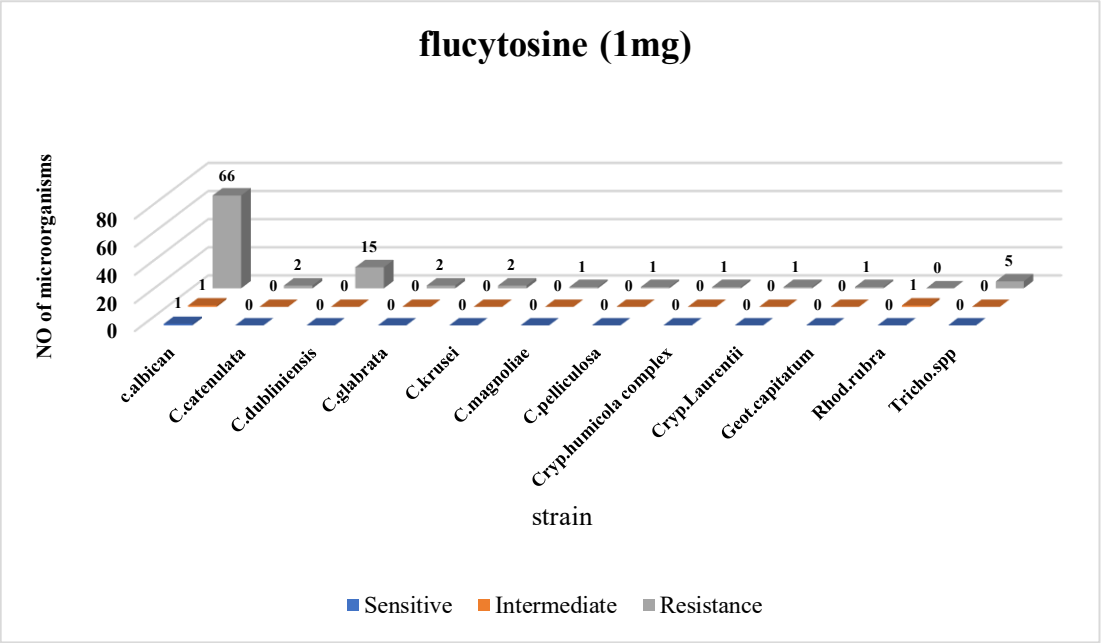

Figure 41: Susceptibility to fluocytosine

Table 15 Microorganism strains susceptibility to all antimycotic agents

Species	amphotericin			Nystatin			Clotrimazole			Itraconazole			Voriconazole			fluconazole			fluocytosine		
Grade ►	S	I	R	S	I	R	S	I	*R	***S	**I	R	S	I	R	S	I	R	S	I	R
Candida albicans	0	1	67	3	48	17	1	11	56	53	9	6	64	2	2	25	43	0	1	1	66
Candida catenulata	0	0	2	0	1	1	0	0	2	1	1	0	2	0	0	0	2	0	0	0	2
C. dubliniensi	0	1	14	2	6	7	0	5	10	12	3	0	15	0	0	8	7	0	0	0	15
C. glabrata	0	0	2	0	2	0	0	0	2	0	0	2	2	0	0	0	2	0	0	0	2
C. krusei	0	0	2	0	0	2	0	0	2	0	0	2	2	0	0	0	2	0	0	0	2
C. magnoliae	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	1
C. pelliculosa	0	0	1	0	1	0	0	0	1	1	0	0	1	0	0	0	1	0	0	0	1
Cryp. H. comp	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	1	0	0	0	1
Cryp. Laurent	0	0	1	1	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1
Geot. capitatu	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1
Rhod. rubra	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	1	0
Tricho. spp	0	0	5	1	2	2	0	0	5	3	0	2	3	0	2	0	5	0	0	0	5
Total	**0**	**2**	**98**	**8**	**60**	**32**	**1**	**16**	**83**	**71**	**13**	**16**	**91**	**3**	**6**	**33**	**67**	**0**	**1**	**2**	**97**

**R= Resistant* ***I= Intermediate* ****S=Sensitive*

DISCUSSION

Chapter: 6 Discussion

The aim of this study was to investigate the carriage rate and evaluate some systemic and dental factors underlying carriage of oral Candida species in-patient attending outpatient dental clinic in North East region of Libya, fortunately another similar study was published by (Krema et al., 2018) of a Libyan study group from in the western area of this country and reported slightly different figures which indicates the need for carrying out multiple studies in this regard to monitor the regional differences in fungal species and the antifungal susceptibility of the isolates due to the emergence of new pathogenic species through nosocomial spread. A rapid and accurate identification of the disease-causing species of Candida is crucial for clinical treatment of local or systemic candidiasis.

The findings of this study of 32% fungal carriage in the study group represent half the figure reported about the frequency of oral carriage of yeasts in the western part of the country (64 %) in a sample with almost similar age and the method of candida identification and antifungal susceptibility (Krema et al., 2018). This discrepancy might be due to differences sampling methods and sizes. However, chances exist of this is being a real difference which need to be investigated.

Previous studies had showed different figures of oral candida carriage in healthy people it has been reported as low as 18% in a Japanese study (Kama et al, 2014), but higher figures were reported particularly in the diabetics and the immunocompromised patients. In UAE 30.6% carriage rate had reported (Al-Amad et al., 2021), but higher oral candidal carriage rate (46%) has been reported in a study conducted in the nearby country (Kuwait) (Ellepola et al., 2011.

Candidal isolates could be cultivated from samples of different age groups from both genders with no correlation between the type of fungus species and age or gender. The results of the present study also showed no statistical difference from healthy individuals with comparable gender (Mun et al., 2016) .

This study failed to find any association between oral candidal carriage and the systemic diseases encountered in this group, may be due to the fewer number of cases with such systemic illness came to seek routine dental services and the small number of patients in this study as this study didn’t target specifically those patients with systemic diseases only as the case in most previous studies who reported an alleged association of certain systemic illness with an increased oral candida carriage were actually targeting specific group of patients with

those systemic diseases or the immunocompromised patients while this study included all routine consecutive patients. In this study there were no patients with HIV or other diagnosed immunosuppressive illnesses in which there were many reports of an increased oral fungal infection in those conditions.

The general characteristics of the studied sample indicate clearly that dental caries and periodontal disease (although avoidable), represent an enormous trouble in dental public health, since it affects approximately 90% of the examined population in this study, afflicts all ages and particularly the children and adolescents, compromising their quality of life and development Loesche et al, 1995). The reported high incidences of badly decayed teeth, advanced periodontitis and the loss of many teeth, simultaneously with the abundancy of untreated carious lesions and fewer patients having prosthetic replacement of their missing teeth clearly indicates the low standards of dental care in this region and the enormous difficulties in access to public and private dental services.

From the findings of this study it is difficult to correlate the oral fungal carriage with certain or one specified dental finding such as dental caries or periodontal disease or dental appliances alone, nevertheless there were many studies indicated an increased candida carriage with some of these dental findings.

Conventional sampling techniques from oral sites include swabbing, oral rinses, imprint cultures, and saliva sampling. Swab sampling is a commonly used method to recover Candida cells from palatal and denture surfaces, (Neppelenbroek et al, 2013). This method was chosen for sample collection in this study because as it is affordable, simple and with fewer pitfalls.

Currently, there are varieties of methods for identifying yeasts from clinical samples. These include traditional methods, e.g., the germ-tube test, morphology studies, and carbohydrate utilization; rapid methods, e.g., enzymatic and fluorogenic tests; commercially available methods; automated systems; and recently developed molecular typing techniques (Neppelenbroek et al, 2013).

Conventional methodologies have long been used for Candida species identification and are based on morphological and physiological attributes. However, these methods are laborious, time-consuming, and not reliable in identifying the broad spectrum of Candida species and usually require additional tests. The accurate identification of all isolates from clinical samples is often complex and time-consuming (Cooper et al, 1978; Cardenes et al, 2004). Hence, several manual and automated rapid commercial systems for identifying these organisms have been developed, some of which may have significant sensitivity issues (Pfaller et al, 1988; Shankland et al, 19 Candida species, either by polymerase chain reaction-

based (PCR) or non-PCR-base90). For molecular identification, several procedures have been proposed to detect and differentiate d methods (Neppelenbroek et al, 2006).

Germ tube test is considered a simple, economical, and efficient procedure for differentiating *Candida albicans* from other Candida species (Hoppe and Frey, 1999; Guzel *et al*, 2011). It is based on the observation that *C. albicans* produces tube-like structures (called germ-tubes) when incubated in serum within 2 to 4 h at 37º C (Hoppe and Frey, 1999; Cardenes et al, 2004). Germ-tube test provides a rapid identification test for *andida. albicans* that can be carried out on primary or purified cultures. This test was used to ensure the correct identification of both *Candida albicans* and *Candida dubliniensis* from other fungal species, its results are consistent with the findings of other studies.

Although *Candida spp* are distributed evenly throughout the mouth, the most common site of isolation is the buccal mucosa and the dorsum of tongue, or from dental plaque, or intraoral devices such as orthodontic appliances, acrylic dentures and other dental appliances.

On the distribution of different Candida species among the patients who yielded Candida in culture, this study results reveals that *Candida albicans* was the most dominant yeast species in this sample, accounting for 68% of all Candida isolates. Previous studies in Kuwaiti have also reported *Candia albicans* to be the dominant species isolated, accounting for up to 63.7% (Ellepola et al., 2011), & 55.6% in Saudi Arabia of all isolates tested (Alrayyes et al., 2019). *Candia glabrata*, *C. krusei* are considered as the commonest non-albicans oral Candida species isolated after *Candia albican*s. In contrast, in this study, among the non -albicans Candida species isolated, *Candia dubliniensis* was the most dominant species, accounting for 15% of all *Candida spp* tested.

Almost similar findings were obtained in another study carried out in the year (2018) in the western part of Libya as *Candida albicans* was the most prevalent species (41.7%), *C. glabrate* (27.1%) and *C. dubliniensis* (11.5%) (Kerma et al., 2018).

The isolation of *Candia. glabrata* and *Candida dubliniensis* in this study as the most important non albicans pathogens in the oral cavity were similar to the findings of other studies (Pelletier et al, 2005), (Cooper, 2011). This finding can be related to the fact that both species have been emerged as an important pathogen in the last few years among immunocompromised patient and oral candidiasis either due antifungal resistant as with *C. glabrata* or virulence factors in *Candida Dubliniensis* (Radosavljevic et al., 1999), (Ha et al., 2018)

In contrast to the findings of the study from western Libya sample, *Candida albicans* although found to be resistant to amphotericin B, Clotrimazole and fluocytosine, it is

sensitive or immediately sensitive to nystatin and azoles. *Candida dubliniensis* is also resistant to amphotericin B and Clotrimazole and sensitive or intermediately sensitive to the other agents. The susceptibility test in the above mentioned study showed that *Candida albicans* was highly resistant to most azole antifungal and *Candida dubliniensis* was highly resistant to fluocytosine. Other candida species show variable susceptibility to various antifungal drugs (Krema et al., 2018).

Amphotericin is the most antifungal agent in which most isolated species has resistance to it, followed by Clotrimazole then to nystatin. But in general, most fungal species has either intermediate or high susceptibility to the tested antifungal agents.

6.1.1. Study Limitations

The study samples should have been taken from multiple places to ensure representation of the population. Multicenter studies are preferable in this context as they can cover wider regions and apply the same methodology.

The microbiological techniques used in this study are the traditional methods, so it is better to carry out future studies with more advanced techniques such as PCR with good techniques. The forcible hurdle to that is the cost and expertise to apply such methods.

It is hoped that data from this study can contribute to decide on more effective strategies in antifungal treatments and to design an appropriate prophylaxis program for the benefit of such patients.

6.1.2. Recommendations

Further studies should be conducted using an appropriate methodology to monitor the emergence of the more pathogenic fungi and to evaluate the antifungal resistance of such fungi.

More revolutionized and modern laboratory techniques should be implied despite their costs to get more reliable results with lesser effort and labor.

Appendix

Chapter: 7 Appendix 1

Protocol of study

ORAL CANDIDA CARRIAGE AND FUNGI SPECIES PREVALENCE

انتشار حمولة المبيضات واصناف الفطريات الفموية

Protocol of Thesis

For

Master degree in ORAL MEDICINE

By

Logien Saleh Mustafa

(BDS, 2013)

Supervisor: Prof. Mohamed Saleh H. Ingafou

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(2022)

INTRODUCTION

Oral cavity is generally lacking significant microbial colonization at birth. However, microbes are continually introduced into the mouth from contaminated animate and inanimate objects. While the majority of these microbes are transients, successful oral colonizers will be obtained from exogenous saliva. After a few months, most mouths possess a microbiota consisting of recognizable oral organisms such as bacteria, fungi and actinomyces. Later on, the eruption of deciduous teeth at around 6 months of age provides hard non-shedding surfaces that allows further colonization of organisms that are exquisitely adapted to this environment. Significant proportion of these organisms are in dental plaque on tooth surfaces.

The oral microbiota continues to develop, changing with age in composition and overall activity. Hormonal changes during puberty can contribute to increased colonization by groups of gram-negative anaerobes and spirochetes, with some hormones possibly acting as nutritional sources. In adults, gradual age-related changes, physical exercise levels, and psychological stress can all influence the numbers or proportions of oral microbats, often through effects on immune function or salivary flow rate, lifestyle events such as smoking, frequency of carbohydrate consumption, or pregnancy can affect the microbial composition in the oral cavity. In later life, the decline in salivary flow rate and in general health status leads to changes in microbial colonization, such as increased carriage of the yeast *Candida albicans*, with subsequent higher risk of oral candidiasis (Lamont et al, 2014).

Fungi are normal, harmless commensals found in the mouth of approximately 40% of healthy individuals and can and do, however, cause oral mucosal diseases, particularly in immunocompromised individuals. The main fungal species are *Candida albicans, Candida glabrata, Candida tropicalis, Candida krusei, Candida parapsilosis, Candida guillermondi*. Although several other fungi can cause oral lesions, Candida albicans is the causative agent of the most common oral fungal infection (i.e. candidiasis), which has a variety of clinical presentations (Lamont et al, 2014).

Fungal cells have a diameter of approximately 3 to 6 $\mu m,$ and in general they are larger than bacteria and smaller than mammalian cells. Those fungi that exist predominantly in the unicellular state are usually ovoid and termed yeasts; while those grow as hyphae are commonly called molds. Hyphae consist of chains of individual cylindrical cells, each containing a nucleus

and divided from adjacent cells by walls called septa. The initial emergence of a hypha from a *Candida albicans* yeast cell is referred to as a germ tube.

Candida albicans is often referred to as a dimorphic fungus, as it exists mostly in either the yeast or hyphal morphological form. Macroscopically, yeast colonies on agar plates tend to be smooth with well-defined edges, whereas mold colonies are furry with individual hyphal threads visible at the edge of the colony, yeast can be cultured from the saliva of approximately 40% of healthy individuals.

Microscopic examination of clinical samples can be informative. Samples can be obtained by scraping mucosal surfaces with a wooden spatula, or tongue depressor, and transferring the material to a clean glass slide for wet-mount microscopy. Alternatively, a biopsy whereby the sample is stained before microscopic examination may be indicated for some lesions. However in some studies, phosphate-buffered saline in oral rinses is used. After that, fungi can be cultured from clinical specimens on Sabouraud's agar. Antibiotics such as chloramphenicol and gentamicin are to be included in the agar to inhibit the growth of bacteria from the samples. The agar plates are generally incubated aerobically at 30°C for 48 hours.

There are several methods for identifying fungi. Identification of fungi by growth characteristics is time-consuming. Techniques have been developed to utilize the differences in the nucleic acid sequences of different fungal species in fungal identification but they are humbled by the high false positive results as a result of specimen contamination. Hybridization of nucleic acids with labeled nucleic acid probes specific for a particular fungus can be used to detect that fungus in clinical samples. Candidal characterization studies had found that C. albicans is the most prevalent yeast species isolated then C. tropicalis (Akram et al., 2018).

Oral candidiasis is the most common oral fungal infection, its clinical presentations include pseudomembranous, erythematous, and plaque like/nodular candidiasis, angular cheilitis, and median rhomboid glossitis. The mere isolation of Candida from intraoral surfaces is not interpreted as a predictive signal for disease as 40% of healthy individuals from diverse populations may carry such yeasts without any symptom (Rosa, 2015).

Oral Candidiasis can be a manifestation to a number of underlying systemic conditions such as diabetes, hypertension, dehydration, malnutrition, and medicine intake to treat anxiety or depression resulted in severe reductions in the salivary production, incurring in converting the saprophytic yeasts into opportunistic pathogens.

Many studies showed increased candida carriage among diabetics (Zomorodian et al, 2016, Javed et al, 2017), cigarette- and water-pipe-smokers, electronic cigarette users, than in never smokers. (Mokeem et al, 2019, (Alaizari and Al-Anazi, 2020). Studies have also shown that asymptomatic oral colonization of Candida spp may lead to oral lesions or become a source of disseminated infections (Lourenço et al, 2017).

Other studies suggested a potential role of oral candidiasis in the development of dental caries (Xiao et al, 2019) and increases its severity (Moraga et al, 2017). Oral candidiasis has been suggested to play a role in the pathogenesis of other conditions such as OLP (Zeng et al, 2009).

REVIEW OF LITERATURE

Oral candidiasis is the most common mycosis occurring in human beings. Candida spp. are widely spread among people from different parts of the world. Differently from other microbes, the mere isolation of Candida from intraoral surfaces is not interpreted as a predictive signal for disease (Rosa, 2015). The commensal status of this fungal genus has been evaluated along the years and according to different authors, 54–71.4 % of healthy individuals from diverse populations may carry such yeasts without any symptom (Hauman et al. 1993; Drwazeh and al-Bashir 1995; Kindelan et al. 1998; Blignaut et al. 2002).

In a study to determine the presence and amount of oral yeast in the mouths of healthy patients without mucosal lesions, the oral yeast carriage was (48.3%) , which was not statistical different from individuals with a comparable gender, age, or presence of a removable prosthesis. However, both smoking and the presence of active carious lesions were found to be positively correlated with the carriage of oral Candida. Individuals who are current smokers are nearly seven times more likely to have oral Candida, and participants with high candidal colonization are more likely to be current smokers. Participants with active carious lesions were also more likely to carry oral Candida (Mun et al, 2016).

(Akpan and Morgan, 2002) have compiled data concerning to carrier status of individuals from different risk groups and stated that in the general population, carriage rates have been reported to range from 20 to 75 % without any symptoms. According to them, the incidence of Candida isolated from the oral cavity (not related to OC episodes) has been reported to be 45 % in neonates, 45–65 % of healthy children, 30–45 % of healthy adults, 50–65 % of people who wear removable dentures, 65–88 % in those residing in acute and long-term care facilities, 90 %

of patients with acute leukemia undergoing chemotherapy, and 95 % of patients with HIV (Rosa, 2015).

Investigators had tried to investigate the candida colonization in patients with diabetes and its relationship with factors such as Candida species, serum glucose level, and the susceptibility rate of isolated yeasts to antifungals in 113 patients with type 2 diabetes, 24 patients with type 1 diabetes, and 105 healthy control and concluded that significant association exists between the poor glycemic control and the higher prevalence rates of Candida carriage and density in diabetic patients. In addition, a high prevalence of *Candida dubliniensis* in diabetic patients was found, which might be misdiagnosed with its morphologically related species, *Candida albicans* (Zomorodian et al, 2016). Several studies reported that candida is significantly higher among diabetics than the non-diabetics, however, Candida albicans was the most prevalent species isolated from the diabetics and the non-diabetics denture-wearers (Javed et al, 2017).

Although, patients with clinical signs of xerostomia usually present with an increased carriage of Candida (Farah et al, 2018), there is no association between oral dysesthesia and the presence or load of oral Candida (Farah et al, 2018). In a recent study to establish a relationship between salivary glucose levels and Candida carriage rate in type 2 diabetes mellitus patients in 60 patients couldn't find any correlation between salivary PH levels and Candida carriage rate, despite that the increased salivary glucose level was associated with increased prevalence of oral Candida in diabetic subjects and the growth of Candida in saliva was accompanied by a rapid decline in PH, which in turn favored their growth (Balan et al, 2015).

In a study of 82 USA socioeconomically disadvantaged women (48 pregnant and 34 non-pregnant) it has been found that their oral Candida carriage is positively associated with hypertension and the number decayed teeth (Xiao et al, 2019). In a study of 79 patients diagnosed with oral dysesthesia the oral carriage of Candida was found in 63.3% (50 of 79) which was not higher than other oral conditions. So, this study concluded that there is no association between oral dysesthesia and the presence or load of oral candida (Farah et al, 2018).

Oral *Candida albicans* carriage (but not the other Candida species) in one study was significantly higher among cigarette and waterpipe-smokers and E-Cig users than never-smokers (Mokeem et al, 2019). Periodontal disease is associated with increased Candida species carriage in HIV-infected patients and may be a predisposing factor to clinical manifestations of candidiasis (Lourenço et al, 2017).

Higher Candida carriage rate, is associated with the highest level of caries severity among school children and high salivary Candida carriage rate is associated with presence of specific species of this fungus (such as *Candida albicans* and *Candida dubliniensis*) which appear to be related to the severity of caries experienced by preschool children (Moraga et al, 2017).

The association between smoking and smokeless tobacco with oral Candida carriage has been studied by meta-analysis including 14 studies until April 2020, and concluded that there was a significant relationship between smoking/smokeless tobacco users and oral Candida carriage. However, observational studies cannot clarify whether the observed epidemiologic association is a causal effect or the result of some unmeasured confounding variables (Alaizari and Al-Anazi, 2020). More detailed study included the daily frequency of smoking and its duration and reasons plus daily oral hygiene maintenance habits have conclude the same findings (Akram et al., 2018).

Another study was carried out to identify the oral carriage of Candida spp in 246 patients infected by human immunodeficiency virus (HIV) and the possible correlation with clinical characteristics found that Candida yeasts were present in 41.87% of the samples, and *Candida albicans* was the most prevalent (32.52%). Other identified Candida species were C tropicalis (4.88%), *C parapsilosis* (2.85%), *C dubliniensis* (0.81%), and *C famata* (0.81%). There was low rate of oral Candida carriage in patients infected by HIV who were on highly active antiretroviral therapy. A greater prevalence of *C albicans* than non-albicans Candida species was found at the species level. Prior candidiasis predicted the oral carriage of *C albicans*; however, it did not influence the carriage of non-albicans species. This is the first report of oral carriage of C famata in patients with HIV infection (Ribeiro Ribeiro et al, 2015).

AIMS OF THIS STUDY

To determine the oral Candida carriage and species prevalence in both healthy individuals

To determine candidal carriage in some oral conditions by laboratory methods

MATERIALS AND METHODS

Study design

A prospective analysis of mycological investigations of candida species in patients with clinically diagnosed certain oral conditions to determine candidal carriage among:

1. Non-smokers- healthy persons (without oral mucosal or dental diseases).
2. Smokers, healthy dental persons.

3. Patients with aphthous stomatitis
4. Patients with oral lichen planus

Denture wearers.

Patients with orthodontics appliances

Setting:

Oral Medicine and Diagnosis department, Faculty of dentistry, and El-slmany polyclinic and certain dental clinics with good flow of patients.

The laboratory work will be conducted mainly in the faculty of Public health of University of Benghazi.

Sample size:

Target to examine 300 patients from consecutive patients with different oral diseases.

Study registration:

This clinical study to be registered into the registry of the postgraduate studies office and an ethical approval to be obtained from the ethical committee of the dental faculty which authorized for that according to the roles.

Inclusion criteria:

Consecutive patients attending oral medicine and diagnosis department faculty of dentistry, or other appointed dental clinics.

1. Patients aged 18 years or above.
2. All of those persons born or permanently resident in Libya for at least 10 years.
3. Both genders are included.
4. Exclusion criteria:
5. Patient younger than 13 years.
6. Patient, who do not permanently resident in the country, tourists & immigrants.
7. Patient received chemo, radiotherapy, or treatment of malignancy.
8. Patients currently taking antifungal o antibiotics.
9. Study conduct:

Patients will be registered at reception desk, interviewed for their general health and case history to be taken on the scene.

Explaining the purpose of the study.

Obtaining a signed written consent from patient.

Patient be seated on dental chair to conduct clinical examination.

To confirm any anticipated underlying systemic illness further investigation (hematological, histopathological or radiological) may be ordered.

A smear sample to be obtained from the indicated oral site.

Clinical data:

Patient interview and case history.

Clinical examination according to WHO criteria 2017.

Tools used in clinical examination :

Dental mirror, dental probe, tongue spatula, condescend chair light, gauze, cotton roll and tweezers.

Digital camera for documentation of the cases.

Data analysis

Information to be recorded in a specially designed clinical audit, and then tabulated in a Microsoft excel spreadsheet.

Information specifically obtained for this study In the clinical setting, the personal data (age, gender occupation and smoking habits) will be collected as well as drug hypersensitivity. and information regarding the status of general health of the patient and the current systemic diseases such as bronchial asthma, cardiovascular diseases, endocrine disorders such as diabetes mellitus and thyroid diseases, gastrointestinal diseases, hepatitis, hematological disease, infectious disease, kidney disorders and further diseases which known to predispose to oral candidiasis.

Samples collection

To evaluate the oral carriage of yeasts sample swabs to be collected from specified sites, namely the buccal mucosa, palate and oral Commissures and immediately transferred to laboratory for cultivation.

Laboratory study

The obtained samples will be cultured on Sabouraud agar plates to assess the Candida carriage rates. The collected samples will be processed in the lab according to standardized methods for:

Presence of *Candida albicans*

Presence of other types of fungi.

The obtained results to be recoded and photographed whenever it deemed necessary.

Data analysis

All obtained information will be tabulated in a Microsoft excel spreadsheet and, the validated data will be exported into an SPSS format for statistical analysis. The obtained information will be analyzed by descriptive analysis for the mean, median, mode, SD & range.

Comparison according to gender &age will be undertaken by Chi square static. Any suspected correlation will be explored. All obtained data will be critically discussed & contrasted against the available information from other worldwide studies.

Appendix

Benghazi University
Dental faculty
Department of Oral medicine, Pathology, Diagnosis & Radiology
Form for the study of candidal carriage

Date: …/…/……

General information

Case No: …… Pt. name (optional): …… Diagnosis: ……

Age: Sex Nationality: ……..

Occupation: …… Telephone:

Medical history: ….……

Times visited dentist… tooth brushing: ……. Reason of attendance (now):

Smoker □ Yes □ No: Other habits: ……..

History

Heavy smoker	Cardiac disease
Diabetes mellitus	Decreased vertical demission of the face
Anemia	Generalized chronic gingivitis
Aphthous stomatitis	Chronic periodontitis
Oral lichen planus	More than 4 missing teeth
The use of corticosteroids oral spray	With orthodontics appliances
The use of immune suppressive agents	

Clinical examination:

Wearing complete Dentures

Wearing partial dentures

Using fixed orthodontics appliances

Having less than 3 dental restorations

Having more than 2 dental restorations

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Appendix II

Benghazi University
Dental faculty
Department of Oral medicine, Pathology, Diagnosis & Radiology

Form for the study of candidal carriage

Date: …/…/……

General information

Case No: …… Pt. name (optional): …… Diagnosis: ……

Age: Sex Nationality: ……..

Occupation: …… Telephone:

Medical history: ….……

Times visited dentist… tooth brushing: ……. Reason of attendance (now):

Smoker □ Yes □ No: Other habits: ……..

History

Heavy smoker

Diabetes mellitus

Anemia

Aphthous stomatitis

Oral lichen planus

The use of corticosteroids oral spray

The use of immune suppressive agents

Clinical examination:

Wearing complete Dentures

Wearing partial dentures

Using fixed orthodontics appliances

Having less than 3 dental restorations

Having more than 2 dental restorations

Cardiac disease

Decreased vertical demission of the face

Generalized chronic gingivitis

Chronic periodontitis

More than 4 missing teeth

With orthodontics appliances

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الملخص العربي

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انتشار حمولة المبيضات واصناف الفطريات الفموية

اعداد: لجين صالح مصطفى

اشراف الأستاذ الدكتور: محمد صالح حماد انقافو

والمشرف المساعد الدكتور: محمد عبد الحميد غانم رمضان

أهداف الدراسة: تهدف هذه الدراسة إلى تحديد انتشار الأنواع الفطرية الفموية في مرضى الأسنان الروتينيين من خلال الزراعة المختبرية للعزلات وتحديدها ببرنامج تحديد هوية الخمائر إربا بالإضافة إلى تقييم نمط القابلية المضادة للفطريات المعزولة عن طريق طريقة انتشار الأجار باستخدام نيو سينسي تابس.

الموضوعات والأساليب: تم فحص ما مجموعه 310 مريض أسنانٍ في العيادة السلماني المركزية للأسنان ببنغازي خلال 9 أشهر في العام 2021 في ظل مراعاة كاملة للتدابير المفروضة لمكافحة جائحة كوفيد-19. اشتملت هذه الدراسة على مرضى متعاقبين تراوحت أعمارهم من 18 عاما أو أكثر، جاءوا بشكاوى روتينية مختلفة من أمراض الأسنان مثل (تسوس الأسنان، التهاب اللثة المزمن، أمراض حول الاسنان، أو المشاكل المتعلقة باستعمال أطقم الأسنان الاصطناعية المتحركة أو الثابتة). أدخل هؤلاء المرضى هذه الدراسة شريطة أن يكونوا أما أن يكونوا مولودين بليبيا أو مقيمين فيها بشكل دائم لمدة 10 سنوات على الأقل. وجد أن بعض المرضى يعانون من أمراض طبية عامة مثل (مرض السكري أو ارتفاع ضغط الدم أو الربو أو التهاب الجيوب الأنفية أو فرط نشاط الغدة الدرقية أو قصور الغدة الدرقية أو فقر الدم) وتلك الامراض قد تؤثر على معدل حمل الفطريات الفموية والأخيرة قد تتأثر أيضاً ببعض العادات الشخصية مثل التدخين أو إدمان الكحول. تم تسجيل كل هذه العوامل قبل أخذ مسحة عن طريق الفم ونقلها على الفور إلى المختبر لزراعتها باستخدام وسائط سكر الذرة سابو رادس آجار (دي أيه إس) طبقاً للطرق القياسية لزراعة واستنبات الفطريات معملياً.

ثم أجراء اختبار نمو الفطريات أولًا عن طريق اختبار صبغة غرام ومن بعده اختبار الأنبوب الجرثومي، كما لوحظت خصائص نمو المستعمرات وطريقة ظهورها للعيان. تم استخدام اختبار كانديدا21 بالتوازي مع اختبار فاعلية المواد المضادة للفطريات بطريقة قابلية الانتشار القرصي. كما تم اختبار الحساسية لسبعة عوامل مضادة للفطريات وهي (فلوكونازول، أمفوتريسين ب، نيستاتين، فلوسيتوزين، كلوتريمازول، إيتراكونازول، فلوكونازول).

النتائج: تم تسجيل حمل فموي موجب للخمائر لدى 100 (32%) عينة ذات نمو إيجابي من إجمالي 310 حالة اخذت لهم عينات في هذه الدراسة وقد أمكن عزل اثنتي عشرة سلالة من الفطريات بالفم خضعت جميعها لسبعة أنواع من العوامل المضادة للفطريات لتقييم نشاطها. وقد لوحظ الحمل الفطري لدى 54 أنثى و46 ذكراً.

لا يمكن استثارة أي علاقة بين المتغيرات السريرية المدروسة ونقل المبيضات في هذه الدراسة باستثناء الزيادة الطفيفة في ميل لنقل الفطريات الفموية لدى المرضى الذين يستخدمون الأطقم الاصطناعية للأسنان القابلة للإزالة أو الثابتة منها.

المبيضات البيضاء أكثر الأنواع شيوعا ووجدت في 68٪ من العزلات، في حين كانت *مبيضات دوبلينيسيس* ثاني الأنواع المعزولة في 15 حالة، تريكوسبورون في 5 حالات، مبيضات كاتينولاتا في حالتين، والمبيضات كروسي في حالتين أيضاً، وحالة واحدة لكل من مبيضات ماغنوليا، مبيضات بيليكولوسا، كريبتوكوكوس هيميكولا المعقد، كريبتوكوكوس لورينتي، جيوتريكوم كابيتاتوم، ورودوتورولا روبرا.

أظهرت جميع العزلات مقاومةً عالية لكلٍ من (أمفوتريسين، فلوسيتوزين، كلوتريمازول) 98٪ و97٪ و83٪ على التوالي، في حين كانت المقاومة متوسطة لكلٍ من (فلوكونازول ونيستا تين) و67٪ و60٪ على التوالي وسجلت حساسية عالية لكلٍ من (فلوكونازول إيتراكونازول) 91٪ و71٪ على التوالي. ومن المثير للاهتمام

أن الفلوكونازول أدى بشكل فعال في هذه الدراسة، حيث أن جميع العزلات إما حساسة له بشدة (67٪) أو حساسة له بشكل متوسط (33٪) ، ولم يتم عزل أي مقاومٍ واحد له.

الاستنتاجات: كانت المبيضات البيضاء أكثر الأنواع المعزولة في هذه الدراسة، تلتها *مبيضات دوبلينيسيس* في توافق تام مع معظم الدراسات العالمية، في حين ظهرت أرقاما مغايرة للأنواع الأخرى والتي هي أقل شيوعاً منهما. أما مقاومة الفطريات المعزولة للعوامل المضادة للفطريات فكانت عالية لبعض العوامل مثل الأمفوتريسين، وفلوسيتوزين، كلوتريمازول وأقل شدة لعوامل أخرى مثل فلوكونازول إيتراكونازول ولم يتسنى العثور على سلالات مقاومة للفلوكونازول بهذه الدراسة.

انتشار حمولة المبيضات واصناف الفطريات الفموية

اعداد
لجين صالح مصطفى

هذا البحث مقدم للاستكمال الجزئي لمتطلبات درجة الماجستير
في
طـــــب الـفـــم

تحت اشراف: الأستاذ الدكتور محمد صالح حماد انقافو
المشرف المساعد: الدكتور محمد عبد الحميد غانم رمضان

جـــامعة بنغازي
كلية طب الاسنان
يونيو, 2022