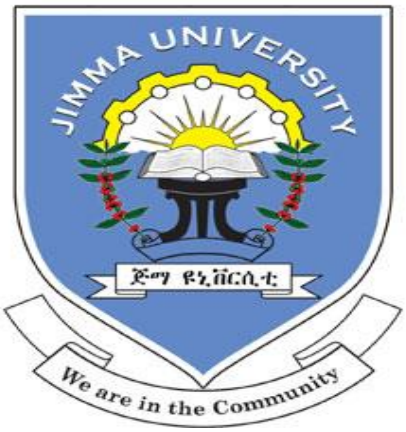

DIVERSITY, DISTRIBUTION AND DRUG RESISTANT PATTERNS OF *CANDIDA* SPECIES ISOLATED AMONG PATIENTS ADMITTED AT TEPI GENERAL HOSPITAL

BY

MOHAMMED SEID MUHE

MASTER THESIS

SUBMITTED TO THE DEPARTMENT OF BIOLOGY FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY

ADVISOR
ANBESSA DABASSA (PhD)

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JIMMA, ETHIOPIA

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THESIS APPROVAL SHEET

JIMMA UNIVERSITY, POST GRADUATE STUDIES

As thesis research advisers, we hereby certify that we have read and evaluated the thesis prepared, under our guidance by Mohammed Seid, which is entitled "**Diversity, Distribution and Drug Resistant Patterns of *Candida* species Among Patients Admitted at Tepi General Hospital in South western Ethiopia**". We recommend that the thesis be accepted as it fulfills the requirements.

Anbessa Dabassa (PhD)	_____	_____
Major Adviser	Signature	Date

As members of the Board of Examiners of the MSc. Thesis Open Defense Examination, we certify that we have read and evaluated the thesis prepared by Mohammed Seid and examined the candidate. We recommend that the thesis be accepted as it fulfills the requirements for the Degree of Master of Science in Biology.

_____	_____	_____
Chair person	Signature	Date
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DEDICATION

This thesis is dedicated to my family for their support for the successful completion of this work in particular and for the success in my life in general.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is the result of my work and all other sources of material and information used for writing it have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc degree at Jimma University and is deposited at the university's library to be made available to borrowers under the rules and regulations of the library. I solemnly declare that this thesis has not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author was born on 1984 at Tepi, Southwestern Ethiopia from his father Seid Muhe and his mother Zehara Issa. He attended primary education (grades one to eight) at Shayi Elementary School from 1993 to 2000 and secondary school at Tepi secondary and preparatory school from 2001 to 2004. In 2005, He entered to Wollo University and attended Biology. He have graduated in 2007 in Biology. He have versatile teaching background.

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LIST OF ACRONYMS/ABERRATIONS

AIDS	Acquired Immuno Deficiency Syndrome
ANOVA	Analysis of variance
BSI	Bloodstream Infection
CD4	Cluster differentiation
ELISA	Enzyme Linked Immuno Sorbent Assay
FIs	Fungal infections
FISH PNA	Fluorescence in situ hybridization with peptide nucleic acid method
GMS	Gomori's methenamine silver stains
HIV	Human Immuno Virus
ICI	Invasive *Candida* infection
ICUs	Intensive care unit
OPC	Oropharyngeal candidiasis
PFGE	Pulsed Field Gel Electrophoresis
PAS	Periodic Acid-Schiff
PCR	Polymerase Chain Reaction
PH	Power of hydrogen
RAPD	Random Amplified Polymorphic DNA
RT-PCR	Real-Time Polymerase Chain Reaction
RFLPs	Restriction Fragment Length Polymorphisms
SDA	Sabouraud Dextrose Agar
SPSS	Statistical Package for Social Science
UV	Ultraviolet
VVC	Volvo Vaginal Candidiasis

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ABSTRACT

Candida species are becoming an important cause of opportunistic infections worldwide due to their ability to adapt to various environmental changes. However, in Ethiopia, due to scarcity of data, much has not been documented regarding the diversity and distribution of *Candida* species. *Hospital-based cross sectional study was conducted from September 2022 to June 2023 to investigate the diversity, distribution and drug resistant patterns of Candida species. For the present study 120 admitted patients were selected by using purpusive sampling techniques based on characteristics of infection. Clinical samples including surgical wound swab, blood, oropharengial swab, urine specimens, sputum, stool, and vaginal swabs were collected from patients with signs and symptoms of infections and referred to the study site for culture and susceptibility testing. The results of present study showed that from the total of 120 collected clinical sample, 92/120(76.7%) were positive for Candida infection. Candida prevalence was significantly higher in females than males (P= 0.0254). The most common risk factors was HIV/AIDS which accounts for (90%), followed by pregnancy (86.7%), diabetic mellitus (83.3%), prolonged antibiotic therapy (73.3%) of patients with Candida colonization. A total of 5 species of Candida were identified based on the colony color characteristics on Sabouraud Dextrose Agar, BiGGY agar test and matrix assisted laser desorption/ionization time of flight (MALDI-TOF MS) identification. Among identified 5 Candida species, the predominant Candida species was Candida albicans (55.4%), which was followed by Candida krusei (17.4%) and Candida tropicalis (14.1%), Candida parapsilosis (8.7%) Candida glabrata (4.3%) were the commonest isolates among non-albican Candidia species. Of the total of 92 Candida isolates, (71.2%), (7.3%) and (21.5%) Candida isolates were susceptible (affected), susceptible dose dependent (depending on dose and resistant (not affected) to the drug, respectively. Amphotericin-B was the most effective drug. The results showed that, 82.6% (76/92) of the Candida isolates were found to be susceptible to Amphotericin-B. Therefore, Candida species distribution is changing, the emergence of non albicans Candida other than Candida albicans has increased and antifungal drug resistance is also increasing. Eventually, it is recommended that a national surveillance be conducted to study the epidemiology and susceptibility pattern of Candida species isolates to antifungal drugs.*

***Key words**: Candida albicans, Candida krusei, Candida tropicalis, Candida parapsilosis and Candida glabrata*

1. INTRODUCTION

1.1. Background

The genus *Candida* is the largest genus of yeast and is closely related to human life. Candidiasis is a fungal infection caused by species of the genus *Candida*. It affects more than 4 billion people *worldwide each year (Segal, 2004). Candida species of medical importance include Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis, Candida krusei, Candida kefyr, Candida guilliermondii, Candida lusitaniae, Candida stellatoidea, and Candida dubliniensis. Candida albicans* is a widespread opportunistic pathogen of growing interest in both clinical medicine and basic biology (McManus and Coleman, 2014). It is present as a harmless commensal in the gastrointestinal and genitourinary tract in about 70% of people, and about 75% of women experience *Candida* infection at least once in their lifetime (Sarvtin *et al*., 2014).

In Africa, the most common species causing invasive *Candida* infections were *C. albicans* (55.18%), *C. parapsilosis* (17.40%), *C. tropicalis* (14.63%), *C. glabrata* (12.44%), *C. dubliniensis* (6.64%), *C. guilliermondii* (6.52%), and *C. krusei* (4.62%). However, *C. albicans* predominated in superficial *Candida* infections (76.92%), followed by *C. parapsilosis* (28.83%), *C. glabrata* (11.27%), and *C. tropicalis* (9.00%) (Ali *et al*., 2014). Sub-Saharan and Central Africa had the highest prevalence of *C. albicans* superficial and invasive infections. Northern Africa had the highest prevalence of non-albicans *Candida* species superficial and invasive infections (Ali *et al*., 2014). In Ethiopia, approximately 8% of the population suffers from FIs each year, the majority of whom are schoolchildren with tineacapitis. The most common causes of fungal death are *invasive aspergillosis* (IA), *Cryptococcus meningitus* (CM), and *Pneumocystis pneumonia* (PCP). Approximately 50% of newly HIV-diagnosed patients with low CD4 T-cell count have oral candidiasis as their first symptom of HIV infection (Mulu *et al*., 2013). Cases of oesophageal candidiasis were estimated to be 55,900 per year, at a rate of 53.3 per 100,000 people (Tafese *et al*., 2019). *Candida albicans*, non *albicans Candida* species, and other yeasts were found in 43.1 percent, 15%, and 7.2% of the *Candida* species in Ethiopia, respectively. *Candida krusei* was the most common isolate among non-*Albicans Candida* species (Seyoum *et al*., 2020). The study conducted at Debre Markos Referral Hospital, indicated that the predominant *Candida* species was

Candida albicans (56.25%) followed by *Candida krusei* (21.9%), *Candida glabrata* (17.7%), *Candida tropicalis* (1%) and (3.1%) were other *Candida* species (Tsega and Mekonnen, 2019).

Under normal circumstances, the yeast *Candida* is controlled by the body's normal defenses as well as by other members of the normal flora. For example, the acidity of the vagina is maintained at a pH level of 4.0-4.5. This level of acidity prevents certain vaginal pathogens from establishing themselves. However, physiological changes in the balance of body systems will affect both yeast, bacteria and other beneficial and harmful organisms in the body. This will therefore alter the acidity of the vagina by reducing the pH to between 5.0 and 6.5, thus allowing the establishment of pathogenic organisms such as *Candida.* Vaginal pH may increase with age, phase of the menstrual cycle, sexual activity, contraceptive choice, and pregnancy, presence of necrotic tissue or foreign body, and use of products. Hygiene products or antibiotics (Nyirjesy, 2008; Akinbiyi *et al*., 2008).

Candida species are the most common cause of fungal infections, leading to a wide range of life-threatening invasive diseases from candidaemia to non-fatal mucocutaneous candidiasis such as genital-secreting candidiasis. Urinary tract infections, vulvovaginal and oropharyngeal candidiasis (Zaoutis *et al*., 2005). They are also an important cause of superficial fungal diseases such as onychomycosis. Among fungal infections, invasive candidiasis is often associated with high morbidity and mortality. For example, *Candida* species are among the top 10 pathogens causing bacteremia (Zaoutis *et al*., 2005), resulting in increased mortality, hospitalizations and medical costs (Colombo *et al*. associates, 2006). Mucocutaneous candidiasis is one of the indirect markers of cell-mediated immunodeficiency and is estimated to have a positive predictive value of over 90% for invasive candidiasis (Wilcox *et al*., 1995).

Until recently, *C. albicans* was recognized as the commonest species causing most of the cases of candidiasis. However, in the last few decades, several studies reported that there has been a progressive shift from a predominance of *C. albicans* to non-*albicans Candida* species (NAC) such as *C. tropicalis, C. glabrata* and *C. krusei* (Snydman*et al.,* 2003; Latha*et al.,* 2011). An increase in opportunistic fungal infections is the result of an increase in the number of immune-compromised patients. Excessive use of broad-spectrum antibiotics, metabolic disorders, and the emergence of AIDS are among the various contributing factors for an increase in opportunistic fungal infections (Upton *et al.*, 2006; Sievert *et al*., 2011). Development of resistance to azoles,

the treatment of choice for fungal infections, mainly by NAC species, differences in drug susceptibility profile among yeast isolates, and frequent isolation of emerging yeasts (i.e., NAC species) in clinical samples initiated the use of accurate species identification and in vitro susceptibility testing methods (Pfaller *et al.*, 2001). Therefore, the present study aimed to investigate the diversity, distribution and resistance patterns of *Candida* species in hospitalized patients at Tepi General Hospital in Southwest Ethiopia.

1.2. Statement of the Problem

Globally, more than one billion people of all ages suffer from fungal infections (FIs) annually (Vos *et al*., 2012), causing more than 1.6 million deaths and contributing to the poor and fatal outcomes of many other diseases (Denning, 2015). However, it is still a neglected topic by public health authorities, even though most deaths from fungal diseases are preventable. Serious fungal infections occur as a result of other health problems, including asthma, AIDS, cancer, organ transplants, and corticosteroid therapy (Bongomin *et al*., 2017).

In recent years, with the widespread use and even abuse of hormones and broad-spectrum antibiotics, cancer patients with radiotherapy and chemotherapy, organ and bone marrow transplants, a large number of immunosuppressants caused by immunosuppressive disorders; and an increase in AIDS immunodeficiency patients, especially deep infections. The incidence of *Candida* infection is on the rise.

There are also sporadic reports of epidemiological testing of fungal infections in Ethiopia, and most of them are limited to a small area such as a ward or hospital. Clinicians agree that the number of patients with fungal infections, especially deep ones, has increased significantly in recent years, but extensive statistics are still scarce. *Candida* is an opportunistic pathogen and its main target is people with low immunity. Ethiopia is currently in a period of rapid increase in HIV infection. As AIDS patients increase, *Candida* infection rates will also show a significant upward trend. The workers posed serious problems.

Although invasive FI is life-threatening, it does not receive enough attention from many global health organizations or governments. Especially in sub-Saharan African countries, there is a lack of data on the incidence and prevalence of these infections, and limited diagnostic facilities, few

evidence-based case management protocols, and the unavailability of appropriate antifungal drugs and specialists in the field compound the problems Brown *et al*. , 2012; Bongomin *et al*., 2017).

Therefore, the changing epidemiology, increasing rates of resistance and narrow availability of antifungals may further underscore the required attention to resistant fungal infections caused by *Candida* and other yeasts (Jensen *et al*., 2016). The growing trend of antifungal resistance and the emergence of new *Candida* species raises the need for regional monitoring of antifungal susceptibility profiles, as in vitro susceptibility patterns are associated with therapeutic outcome. Whereas there are limited studies of mild fungal or life-threatening invasive fungal infections in Ethiopia, the absence of population-based or hospital-based surveys as a source of epidemiologic data, and the lack of surveillance data, combined with the unavailability of any fungal diagnostic test other than microscopy, reinforces the feeling that these infections are rare or non-existent. Data on the incidence and prevalence of fungal diseases in Ethiopia are lacking. To this end, one of the highest priorities and in a timely manner in Ethiopia in general and in the southwestern region of Tepi General Hospital in particular is to determine the diversity and distribution of the *Candida* species profile of the yeasts that cause candidiasis and their drug sensitivity patterns. To date, no research and development work has been carried out on the diversity, distribution and resistance patterns of *Candida* species in the study area. Therefore, this study was designed to answer the following research questions.

1.3. Research Questions

- What is the diversity of *Candida* species in different samples taken from patients admitted at Tepi General Hospital?
- What is *Candida* species distribution in different samples taken from patients admitted at Tepi General Hospital?
- What are the antifungal susceptibility profiles of *Candida* species in the study area?
- What are the risk factors of candidiasis infection among patients in the study area?

1.4. Objectives

The present study was designed for the following general and specific objectives.

1.4.1. General objective

- To investigate the diversity, distribution, and Drug resistant patterns of *Candida* species among patients admitted at Tepi General Hospital in South western Ethiopia.

1.4.2. Specific objectives

- To identify the *Candida* species among patients admitted at Tepi hospital.
- To determine the antifungal susceptibility of *Candida* species and
- To determine the risk factors of candidiasis infection among patients attending at Tepi General Hospital.

1.5. Significance of the study

The information obtained from this study provided baseline data for future epidemiological studies on *Candida* species. The results obtained from this study helped the authorities concerned to identify the species diversity and distribution of *Candida* causing the infection and provided complementary information for infection control planning and surveillance. Thus, policy makers can use this information to recommend and implement fungal infection control in hospital-acquired infections as well as in the community to prevent treatment failure in order to control nosocomial infections in Ethiopia. This information can also be used to create awareness or engage general practitioners due to the ignorance of fungal infections and government health capacity in Ethiopia have considered any fungal disease and fungal diagnostic test can support the development of different strategies to deal with these emerging diseases.

2. LITERATURE REVIEW

2.1. Microbiology and Environment of *Candida*

*Candida i*s a genus of yeast and the most common cause of fungal infections worldwide. Fungi are eukaryotic organisms that take the form of yeasts, molds, or dimorphs. (Manorakaki *et al*., 2010). *Candida* species are yeast (that is, they are predominantly single-celled in form). They are small, 4–6 μm in size, have a thin-walled, oval appearance, and are called sporospores (Dadar *et al.*, 2018). They reproduce by buds. Under the microscope, these yeasts can be seen in the form of pseudohyphae, undissociated budding cells, or true hyphae (multicellular organisms). The genus

Candida belongs to the phylum Ascomycota, class Saccharomycetes, order Saccharomycetales, family Saccharomyceteceae (Howell *et al*., 2015).

There are about 200 species of *Candida.* However, only a limited number are pathogenic in humans (Howell *et al*., 2015). Many species are harmless commensal or endosymbiotic hosts, including humans. It is always present in the skin, mouth, throat, intestines, vagina, etc. and does not cause any problems. However, when the mucosal barrier is disrupted or the immune system is compromised, they can enter and cause diseases known as opportunistic infections. *Candida* can cause infections if it gets out of control or penetrates deep into the body. Kourkoumpetis *et al*., 2011). Candidiasis most commonly occurs as a secondary infection in immunocompromised patients. These are common residents of the oral cavity, gastrointestinal tract, vagina, penis or other parts. They become pathogenic only under favorable conditions. It can affect the oral cavity, vagina, penis, or other parts of the body (Raesi Vanani *et al*., 2019). (Kourkoumpetis et al., 2011).

2.2. Epidemiology of *Candida*

Presently, more than 150 *Candida* species exist in nature, and more than 17 Candida species are regarded to be etiological retailers of human contamination (Yapar, 2014; Pfaller *et al*., 2007), however extra than 90% of invasive diseases are caused by these five. The most not unusual pathogens are: *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis and C. krusie*. Every of these organisms has precise virulence capacity, antifungal susceptibility, and epidemiology, however severe infections commonly resulting from those organisms are usually known as invasive candidiasis (Pappas *et al*., 2016). For the past two decades; modifications were found in the share of *Candida* species remoted from patients with candidiasis. *Candida albicans* reduced and *Candida* non-*albicans* improved. This could consist of new antifungals and new treatment strategies including antifungal prophylaxis, secondary prophylaxis and prophylaxis (Yapar, 2014). Oropharyngeal candidiasis and esophageal candidiasis arise in association with HIV contamination and their prevalence is taken into consideration a trademark of immune dysfunction. In HIV-infected individuals, oropharyngeal candidiasis is maximum common while the CD4 matter is <2 hundred cells/μl (Bodhade *et al*., 2011).

Due to the fact *C. albicans* is a natural organism of the human gastrointestinal tract, candidiasis is ubiquitous. Greater than 30% of the world's population is expected to be inflamed, in particular women over the age of 12 (Tang *et al*., 2016). At the least 70% of all human *Candida* infections

are as a result of *C. albicans*, with the the rest being *C. parapsilosis, C. tropicalis, C. guillermondii, C. kruzei*, and many different rare *Candida* species. Globally full-size, albicans had been isolated from soil, animals, hospitals, inanimate objects and food (Edwards, 2009).

Oropharyngeal candidiasis had a high prevalence in hospitalized AIDS patients (83%), and the most prevalent species was *Candida albicans* (56%) (Terças*et al.,* 2017). Distributions and antifungal Susceptibility of *Candida* Species from Mucosal Sites in HIV Positive Patients Iran studied by Badieeetalin in 2010.Three hundred and nine samples from mucosal sites which consisted of 273 oral and 86 vaginal were collected and evaluated for *Candida* species distributions and their corresponding susceptibility patterns. The most commonly isolated species were: *C.albicans* (50%) followed by *C. glabrata* (21.4%), *C. dubliniensis* (13.3%), *C. Krusei* (9.8%), *C. kefyr* (3.1%), *C. parapsilosis* (1.6%), and *C. tropicalis* (0.8%) (Badiee*et al.,* 2010). Likely, *Candida albicans i*s by far the most prevalent etiological agent, particularly for the most severe chronic condition known as recurrent vulvovaginal candidiasis (Cassone, 2015).

A look at at the conduct of volvovaginal candidiasis by Ali *et al*., 2016 discovered that cultures have been effective in 34 (28.3%) vaginal specimens and in 3 *Candida* species which includes; C. albicans (88.2%), C. glabrata (8.eight%) and C. kefir (2.9%) (Rezaei-Matehkolaei *et al.*, 2016). Any other examine, carried out in tertiary care hospitals in Peshawar, published in 2018, established 108 *Candida* species isolated from vaginal swabs; there have been forty five (41..7%) *Candida albicans*, 18 (16.7%) *Candida tropicalis*, 18 (16.7%) *Candida krusei*, sixteen (14.8%) C. *glabrata* and eleven (10.2%) *Candida dubliniensis* (Khan *et al*., 2018).

Hamza *et al.* studied the species distribution and in vitro antifungal susceptibility of oral yeast isolates from HIV-infected Tanzanian patients with primary and recurrent or pharyngeal candidiasis. The result of this observe confirmed that *Candida albicans* turned into the maximum often remoted species from 250 (84.5%) patients, observed with the aid of *C. glabrata* from 20 (6.8%) sufferers and *C. krusei* from 10 (3.4%) patients. No massive difference in species distribution became observed among patients with number one and recurrent oropharyngeal candidiasis (Hamza *et al*., 2008).

2.3. *Candida* albicans outbreak

Healthcare-associated infections are a significant cause of morbidity and mortality in hospitalized patients worldwide. Most nosocomial infections are caused by multidrug-resistant (MDR) bacteria such as methicillin-resistant Staphylococcus aureus (MRSA), carbapenem-resistant Enterobacteriaceae and Acinetobacterbaumannii, with outbreaks commonly reported (Qin *et al.*, 2014). However, nosocomial fungal infections, including outbreaks, have been reported less frequently. Candidemia is increasingly recognized as an important cause of morbidity and mortality in critically ill patients (Horn *et al*., 2009). It is generally accepted that candidemia is an endogenous infection arising from autoinfection after previous colonization of the gastrointestinal tract, skin, or vagina. However, nosocomial origin can have commensalism with subsequent colonization, which usually precedes the spread of the organism (Fu, 2010). Most nosocomially acquired candidemia cases are caused by *C. albicans*, although *C. tropicalis*, *C. glabrata,* and *C. parapsilosis* have also been reported. *Candida parapsilosis* is now recognized as an important fungal pathogen that is the predominant *Candida* species in bloodstream infections (Horn *et al*., 2009).

The first documented outbreak of systemic candidiasis demonstrated to result from cross-infection with a specific strain of *Candida albicans* has been reported. During nine months in the intensive care unit, 13 patients developed definite and one probable systemic candidiasis. Another twenty-five patients had superficial *Candidal* infections. The strain responsible for the outbreak was serotyping a, morphotype Al, biotype O/15 5/7). Forty-four percent and 17% of superficial *Candida* infections were inside and outside the ward, but in the same hospital. The strain was also isolated from oral swabs taken from four nurses working on the unit and from the hands of one of these nurses. Two of 17 nurses were shown to have strained their hands when examined immediately after caring for systemically infected patients (Burnie *et al*., 1985).

A recent surveillance in Turkey to investigate an outbreak of *C. albicans* associated with total parenteral nutrition in a neonatal unit believed that the *C. albicans* outbreak occurred in a neonatal pediatric unit due to contamination of the total parenteral nutrition solution (Guducuoglu *et al*., 2016).

2.4. Pathogenesis and Clinical Pictures

Candida species belonging to the microbiota of healthy individuals can be found scattered in the environment. The main transmission mechanism is endogenous candidemia, in which *Candida*, which form the microflora of various anatomical sites in conditions of host impairment, behave as opportunistic pathogens (Colombo and Guimaraes, 2003). Another mechanism of transmission is exogenous, and this occurs primarily through the hands of healthcare professionals who care for patients. Rarely, person-to-person transmission can occur between family members or between patients. Medical supplies such as contaminated catheters and intravenous fluids are also indicated when the infection spreads (Odds, 2010; Ingham *et al*., 2012). Major risk factors for candidiasis include immunocompromised, extreme age, pregnancy, diabetes mellitus, long-term antibacterial and aggressive cancer chemotherapy, or undergoing invasive surgery and organ transplantation (Tang *et al*., 2016).

Decades ago, it was believed that yeast passively participates in the pathogenesis of a fungal infection. Thus, being immunocompromised was considered to be the only mechanism responsible for the development of opportunistic infection. Today, this concept is modified. The current consensus is that these organisms are actively involved in the pathophysiology of the disease process using host defense evasion mechanisms called virulence factors (Tamura *et al*., 2007).

2.5. Virulence factors

The ability of *C. albicans* to infect such diverse host niches is supported by a wide variety of virulence factors. A number of attributes are thought to be virulence factors, including cell surface expression of adhesins and invasins, polymorphism, biofilm formation, secretion of hydrolytic enzymes, and phenotypic switching (Deorukhkar *et al*., 2012).

2.5.1. Adhesion to host surfaces

Adherence to the host surface is a primary factor in the colonization of human tissues by fungi; this process is controlled and induced by several cellular signaling cascades both in the fungus and in the environment. In addition, *Candida* can adhere to medical device surfaces and form biofilms (Zordan and Cormack, 2012). The phenomenon of adhesion is manifested by specialized surface proteins, called adhesins (agglutinin sequences), which specifically bind to amino acids and sugars on the surface of other cells or promote adherence to biotic surfaces (Nobile *et al*., 2008).

2.5.2. Invasion

Candida albicans is a remarkable pathogen because it can use two different mechanisms to invade host cells: induced endocytosis and active penetration (Naglik *et al*., 2011). For induced endocytosis, the fungus expresses specialized proteins on the cell surface (invasins) that trigger engulfment of the fungal cell into the host cell. It is still unclear exactly which factors mediate this second route of host cell invasion. Fungal adhesion and physical forces are considered key. Secreted asparagine proteases (Saps) have also been suggested to contribute to active penetration (Dalle *et al.*, 2010).

2.5.3. Polymorphism

C. albicans is a polymorphic fungus that can grow either as an ovoid budding yeast, as elongated ellipsoidal cells with constrictions at the septa (pseudohyphae), or as true hyphae with parallel walls. The morphology of *C. albicans* is influenced by a number of environmental stimuli. For example, at low pH (< 6), *C. albicans* cells grow predominantly in the yeast form, while at high pH (> 7), hyphal growth is induced. Indeed, hyphal formation is promoted by a number of conditions, including starvation, the presence of serum or N-acetylglucosamine, physiological temperature, and CO2.

2.5.4. Biofilm formation

Another important virulence factor of *C. albicans* is its ability to form biofilms on abiotic or biotic surfaces. The most common substrates are catheters, dentures (abiotic) and mucosal cell surfaces (biotic). Mature biofilms are much more resistant to antimicrobial agents and host immune factors compared to planktonic cells. Factors responsible for increased resistance include complex biofilm architecture, biofilm matrix, increased expression of drug efflux pumps, and metabolic plasticity (Fanning and Mitchell, 2012).

Dispersal of yeast cells from a mature biofilm has been shown to directly contribute to virulence, as dispersed cells were more virulent in a mouse model of disseminated infection (Uppuluri *et al*., 2010). The major heat shock protein Hsp90 was recently identified as a key regulator of dispersion in *C. albicans* biofilms. In addition, Hsp90 was also required for biofilm antifungal drug resistance (Robbins *et al.*, 2011).

2.5.5. Secretion of hydrolytic enzymes

Extracellular hydrolytic enzymes appear to play an important role in adherence, tissue penetration, invasion and destruction of host tissues (Silva *et al*., 2011). The most important hydrolytic enzymes are proteases, lipases and phospholipases. The family of secreted aspartate proteases (Saps) includes ten members, Sap1–10. Sap1–8 are secreted and released into the surrounding medium, while Sap9 and Sap10 remain bound to the cell surface. Saps 1–3 have been shown to be required for injury to reconstituted human epithelium in vitro and for virulence in a murine model of systemic infection (Naglik *et al*., 2003).

Putative roles of microbial extracellular lipases include digestion of lipids for nutrient acquisition, adhesion to host cells and tissues, nonspecific initiation of inflammatory processes by influencing immune cells, and self-defense by lysis of competing microflora (Gacser *et al*., 2007).

Phospholipase is another enzyme secreted by *C. albicans*. Seven phospholipase genes (PLA, PLB1, PLB2, PLC1, PLC2, PLC3 and PLD1) were identified; however, the role of the enzymes encoded by these remains unclear (Samaranayake *et al.*, 2006).

2.5.6. Phenotypic switching

The transition between yeast and hyphal growth forms is called dimorphism, and it has been suggested that both growth forms are important for pathogenicity. The hyphal form has been shown to be more invasive than the yeast form and plays an important function in tissue invasion and resistance to phagocytosis. On the other hand, the smaller yeast form is thought to represent the form primarily involved in propagation (Jayatilake *et al*., 2006).

2.5.7. Host defects

As with most fungal infections, host defects also play a significant role in the development of *Candida* infections. Host defense mechanisms against *Candida* infection and associated defects that allow infection include: Intact mucocutaneous barriers: wounds, intravenous catheters, burns, ulcerations; Phagocytic cells: granulocytopenia; Polymorphonuclear leukocytes: Chronic granulomatous disease; Monocytic cells: myeloperoxidase deficiency; Complement: hypocomplementemia; Immunoglobulins: hypogammaglobulinemia; Cell-mediated immunity:

Chronic mucocutaneous candidiasis, diabetes mellitus, cyclosporin A, corticosteroids, HIV infection, and mucocutaneous protective bacterial flora: Broad-spectrum antibiotics (Yang, 2003).

2.5.8. Immunity against *Candida albicans*

Candida albicans isn't a microorganism in sound people, yet can cause serious fundamental candidiasis in safe compromised patients. It has different destructiveness factors and initiates the inborn insusceptible framework. In particular, *C. albicans* actuates supportive of provocative cytokine creation in different cell types through numerous receptors, for example, Cost like receptors (TLRs) and C-type lectin receptors (CLRs). This microorganism likewise advances phagocytosis by means of CLRs on macrophages. *Candida albicans* first connects with epithelial cells that assume an essential part in safeguarding the body against intrusion by pathogenic life forms and in directing the cytokine net-work. *C. albicans* is perceived by numerous receptors including Cost like receptors (TLRs) and C-type lectin receptors. Cost like receptors for *Candida albicans* is a record factor, which is expected for favorable to fiery cytokine creation C-type lectin receptors and can prompt supportive of provocative cytokine and chemokine creation in different cell types including epithelial cells, which act as obstructions to oral candidiasis (Weindl et al., 2010).

Phagocytosis is quite possibly the earliest course of inborn resistance. Macrophages, neutrophils, and dendritic cells immerse *C. albicans*. The cell wall parts of *C. albicans* (mannans, glucans, and chitins) are harmfulness factors and actuate phagocytosis (Mama *et al*., 2012).

Antimicrobial peptides are likewise blended and discharged by different cells including epithelial cells, neutrophils, and safe cells. Human LL-37 is an antimicrobial peptide that is chemotactic for neutrophils and monocytes. The peptide hinders *C. albicans* bond to plastic surfaces and kills the microorganism. LL-37 additionally upsets the cell layer of *C. albicans* and causes an efflux of nucleotides and proteins with atomic masses of up to 40 kDa (Tsai *et al*., 2011).

Histatins are likewise essential histidine-rich proteins discharged in human parotid and submandibular-sublingual spit in people. Among histatins, histatin 5 is emitted by human salivary organs and has the strongest fungicidal action against *C. albicans*. The antimicrobial peptide causes little layer surrenders a lot more modest than those prompted by LL-37and nucleotide spillage from *C. albicans* (Strijbis *et al*., 2008).

2.6. Major types of candidiasis

Classifications of Candidiasis can be assembled under three principal types; superficial, mucocutaneous and invasive. Superficial and mucocutaneous candidiasis is the two most common type of candidiasis that generally affect the immune-competent or the healthy host (Dabas, 2013; Pfaller *et al*., 2006; Vazques& Sobel, 2011). Mycoses brought about by *Candida* show a wide range of clinical introductions and can be named shallow, mucosal and intrusive candidiasis. *Candida albicans* is a significant human yeast microorganism that records for most of shallow and fundamental diseases brought about by the *Candida* sort (Sobel, 2015).

2.6.1. Cutaneous candidiasis

Mucocutaneous candidiasis is the most well-known type of disease influencing a for the most part enormous number of sound or immunocompetent hosts (Vazquez and Sobel, 2011; Sobel, 2006). Vulvovaginal candidiasis (VVC) and oropharyngeal candidiasis (OPC) are the two fundamental sorts of mucocutaneous candidiasis experienced in centers around the world (Akpan and Morgan, 2010; Calderone, 2002; Dangi *et al*., 2010). OPC is normal among individuals who wear false teeth, the old and children (Akpan and Morgan, 2010; Dangi *et al*., 2010; Terezhalmy&Huble, 2011).

Cutaneous candidiasis is a sort of *Candida* disease that happens because of maceration and injury to the skin. It generally happens in a warm, sodden and creased region of the skin, like frill folds, inguinal or intergluteal regions, and at times settle all alone with next to no antifungal mediation (Xiao-dong *et al.*, 2008).

Cutaneous candidiasis is normally an optional disease of the skin and nails in inclined patients. It happens as a subacute or constant disease. The inclusion of the illness can be limited or summed up on the skin or nails. The range of cutaneous candidiasis incorporates diaper rash, intertrigo candidiasis, candidal folliculitis, otomycosis, onychia, and paronychia. It normally happens in warm, sodden and badly crumpled regions like the axillary folds, inguinal or intergluteal regions. It is a somewhat considered normal pioneering illness and for the most part prompts maceration and skin injury. It is regularly tracked down in diabetics and fat individuals. Other inclining factors are the mistaken utilization of anti-toxins and the utilization of oral contraceptives (Xiao-dong *et al.*, 2008).

2.6.2. Oropharyngeal candidiasis (OPC)

Oropharyngeal and esophageal candidiasis likewise includes unmistakably as a typical contagious contamination in HIV-Helps patients (Dangi *et al*., 2010; Vazquez and Sobel, 2011). OPC presents as a few types of clinical sores, including: pseudomembranous candidiasis (oral aphthous), erythematous and hyperplastic candidiasis, and rakish cheilitis (Dangi *et al*., 2010; Akpan& Morgan, 2010, Terezhalmy&Huble, 2011)

Numerous different microorganisms fill in solid mucous films in the body. Be that as it may, when different creatures are drained, yeast can seek restricted assets. At the point when *Candida* relocates to the mouth or throat, it is frequently called "thrush" and causes an irritated throat alongside a white covering in the mouth (WebMD, 2013). There are three general factors that can prompt clinically apparent oral candidiasis; the insusceptible status of the host, the oral mucosal climate and the specific kind of *C. albicans* (the hyphal structure is generally connected with pathogenic disease) (Ugun *et al.*, 2007). Oral candidiasis is one of the most well-known contaminations of the oral mucosa in individuals with human immunodeficiency infection (HIV) (Dangi and Soni, 2010). The event of OPC in HIV-positive patients proclaims the beginning of AIDS (Helps), which compares to a diminishing in the quantity of CD4+ T-lymphocytes under 200/µl and a plasma viral heap of in excess of 100,000 duplicates/ml (Reiss *et al.* , 2012). Neighborhood inclining states of the host incorporate; diminished salivary discharge, epithelial changes and nearby mucosal sickness, changes in commensal vegetation, high sugar diet and wearing false teeth. There are various sorts of oropharyngeal candidiasis including intense pseudomembranous, intense atrophic, persistent hyper-plastic, ongoing atrophic, middle rhomboid glossitis, dental replacement stomatitis and precise cheilitis. The most discrete injury addresses transformation from harmless colonization to obsessive excess (Akpan and Morgan, 2010).

2.6.3. Vulvovaginal candidiasis

Vaginal candidiasis is the most common reason for gynecological consultations in primary health care services (Gonzalez *et al*., 2011). When the infection is in the female genital areas, it is called vulvovaginitis and can cause extreme itching and burning along with a possible white discharge (Centers for Disease Control and Prevention, 2012). *Candida albicans* is the most common colonizer and also responsible for the majority of VVC cases. It has the ability to survive and proliferate at physiological extremes of pH, osmolality, nutrient availability, and temperature. This

versatility may explain its successful behavior as a commensal colonizer of the vagina as well as a pathogen (Hube, 2004).

2.6.4. Invasive or systemic candidiasis

Invasive or life-threatening candidiasis is typically associated with immunocompromised and weakened hosts (Arora *et al*., 2011; Pfaller *et al*., 2006). Among the different forms of candidiasis, the most frequently discussed types are superficial candidiasis (cutaneous candidiasis); mucocutaneous candidiasis (oropharyngeal and vulvovaginal, gastrointestinal (GT) candidiasis); and invasive candidiasis (candiduria (urinary tract candidiasis), candidemia (bloodstream candidiasis) (Pfaller *et al*., 2006; Vazquez & Sobel, 2011; Arora *et al*., 2011, Akpan& Morgan, 2010).

The pathogenicity of *Candida albicans* is dependent on its ability to survive in multiple microenvironments within the host, including organs, mucosa, and bloodstream. Severe organ-invasive or systemic hematogenously disseminated candidiasis is characterized by the spread of *Candida* cells almost throughout the body with a tendency to form abscesses in vital organs, which leads to their failure, leading to mortality in ~ 50% of all cases, regardless of the administration of intensive antifungal therapy (Zaoutis *et al*., 2005). Clinical symptoms of ongoing systemic candidiasis are hyper- and/or hypothermia, tachycardia, hypotension, high white blood cell count, need for vasopressor, etc. It occurs mainly as a result of some invasive medical procedures, immunosuppressive therapy and aging. Major predisposing factors are severe neutropenia, and a variety of protections are associated with the patient's ability to produce *Candida*-specific antibodies, as evidenced by those who have recovered from systemic candidiasis (Kalkanci *et al.*, 2005).

2.7. Laboratory Diagnosis of Candidiasis

Conventional methods of phenotypical detection of *C. albicans* include microscopy, blood cultures, and biochemical identification (Alam *et al.*, 2014). However, it is less sensitive, time consuming and labor intensive. The long waiting time required for diagnosis of *C. albicans* infection often delays initiation of antifungal treatment. Additionally, several molecular biological methods such as polymerase chain reaction (PCR) have been applied to detect *C. albicans*

(Vahidnia *et al*., 2015), real-time PCR (RT-PCR), mass spectrometry (Zehm *et al*., 2012) and immunoassays (Gunasekera *et al.*, 2015).

2.7.1. Direct Microscopy

Direct microscopy is an inexpensive and rapid method of diagnosing candidiasis. Less expertise required. This is done by wet mount preparation. Potassium hydroxide (KOH) facilitates detection of fungal elements. The KOH content varies between 10-40% depending on the sample type. KOH digests voluminous sediments in the sample and clarifies the visibility of fungal elements. The addition of 36% dimethylsulfoxide to KOH quickly removes bulky debris and eliminates the need for him to heat KOH preparations prior to microscopy. Calcofluor White in KOH binds to chitin and cellulose in fungal cell walls and fluoresces when excited by long-wave ultraviolet (UV) light or short-wave visible light. Addition of Parker's ink or lactophenol cotton blue also enhances detection of fungal elements (Baveja, 2010).

On Gram staining, *Candida* fungi are Gram-positive, oval or round, showing short elements of budding yeast cells, pseudohyphae, and true hyphae (Fenn, 2007). Histological stains such as methenamine silver stain (GMS) are used to retrospectively detect invasive *Candida* in biopsies of lesions in cases of chronic hyperplastic candidiasis. Fungal components within tissues are detected because they are deeply stained by these stains. The presence of spores and hyphae or pseudohyphae allows the histopathologist to identify the fungus as a *Candida* species and, given the presence of other histopathological features, make a diagnosis of chronic hyperplastic candidiasis. (Nassar *et al*., 2006).

2.7.2. Culture

Sabouraud Dextrose Agar (SDA) is used for fungal culture. Incorporating antibiotics into SDA further enhances its selectivity (Marsh and Martin, 2009). SDA is typically incubated aerobically at 37°C for 24-48 hours. *Candida* occurs as off-white, smooth, pasty, convex colonies on the SDA, making it almost impossible to distinguish between species (Baveja, 2010).

Recently, other differentiation media have been developed that allow identification of specific *Candida* species based on colony appearance and color after primary culture. The advantage of such vehicles is that they can detect the presence of multiple *Candida* in a single infection. This may be important in selecting subsequent treatment options (Marsh and Martin, 2009).

CHROMagar™ *Candida* is an effective and rapid screening method that can be used for speciation of *Candida.*

We can distinguish *C. albicans* from other clinically important strains, and we can also distinguish *Candida* strains from other yeast strains based on the color change produced by *Candida* colonies. This can be determined using pH indicators and fermentation of specific or chromogenic compound substrates. *C. albicans, C. pseudotropicalis, C. tropicalis, C.parapsilosis and C. guilliermondii* (Golia *et al*., 2013).

Several advances in blood culture technology have improved the detection of candidaemia. This includes the development of lysis centrifuge tubes and automated monitoring of blood culture bottles.

Dissolution centrifugation systems increase *Candida* yields. Obtained from blood using surfactants to release fungi trapped in host phagocytic cells. The obtained precipitates are plated on five different agar plates. This procedure reduces the time from inoculation to detection of growth. This system is expensive and labor intensive for routine use (Ellepola and Morrison, 2005).

2.7.3. Germ tube examination

When *C. albicans* and *C. dubliniensis* are incubated in serum at 37°C for 2 hours, they produce short, slender tube-like structures called germ tubes, allowing them to be identified quickly. Many clinical microbiological laboratories have begun using non-human serum media for testing germ tube production due to the time required to prepare human serum and the inherent safety issues associated with its use. Egg white, saliva, tissue culture medium, sheep serum, trypticase soya broth, and various peptone media are among them. Trypticase soya broth has been found to be more stable, effective, and safe than other media for germ tube production (Deorukhkar *et al*., 2012).

2.7.4. Chlamydospore Formation

Candida albicans and *C. dubliniensis* can also be distinguished from other species by their ability to produce a morphological feature known as chlamydospores. Chlamydospores are refractile globular structures produced at the ends of hyphae after culturing isolates on nutrient-deficient

media such as cornmeal agar. Seed the isolates in a crosshatch pattern on the agar and cover with a sterile coverslip. Incubate the agar at 37°C for 24–48 hours and examine microscopically for the presence of chlamydospores (Marsh and Martin, 2009).

2.7.5. Physiological Criteria/Biochemical Identification

The biochemical identification of *Candida* species is based primarily on carbohydrate utilization. Traditional testing required culturing test isolates on basal agar media without a carbon source. The carbohydrate solution is then placed in wells of inoculated agar or in filter paper discs placed on the agar surface. Growth near carbon sources indicates utilization (Ellepola and Morrison, 2005).

2.7.6. Matrix –Assisted Laser Desorption /Ionization Time of Flight (MALDI –TOF MS)

MALDI- TOF MS has recently become an effective instrument for rapid microbiological diagnostics.In microbiology, it is used as arapid accurate, and cost effective method for identifying microorganisms (bacteria, fungi and viruses).Identification of the organisms by MALDI-TOF MS is based on assessing protein profiles and database comparsion.

2.7.7. Serology

Serology is widely used to determine the clinical significance of isolates of *Candida* species. Increased titers of IgG antibodies against *C.albicans* may reflect invasive candidiasis in immunocompetent individuals. Detection of IgA and IgM antibodies is important for the detection of acute infections. Immunocompromised people often show fluctuations in antibody production, and the use of antigen detection tests is recommended in such cases. Dissociation of antigen-antibody complexes is critical for the detection of mannan, a major component of the *Candida* cell wall, in serum. This is because these immune complexes mask antigenic sites and reduce the sensitivity of the test. Mannan can be detected in serum and other body fluids by a number of serological reactions such as enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, and latex agglutination (Ahmad and Khan, 2012).

2.7.8. Molecular-Based Identification Methods

Identification by analysis of genetic variants is a more robust approach than using methods based on phenotypic criteria. Electrophoretic karyotypic differences and restriction fragment length

polymorphisms (RFLP), gel electrophoresis or deoxy-ribonucleic acid-deoxy-ribonucleic acid (DNA-DNA) hybridization have been used to identify *Candida* based on genetic variation.

2.7.9. Polymerase Chain Reaction (PCR)

PCR methods for diagnosing *Candida* bloodstream infections are not yet internationally standardized, and testing using different parameters may yield different results. There is a nature. Indeed, the choice of biological material to be tested (whole blood, plasma or serum), the different protocols for DNA extraction, the gene target to be analyzed, the PCR assay used (conventional, nested, PCR-RFLP or real-time). , cycle number, limit of detection, and parameters used for sequence analysis. 'Internal' PCR-based methods are still used worldwide for the diagnosis of candidaemia, in the absence of a commercial system that has been extensively validated in multicenter studies (Xafranski *et al*., 2013).

A. species-specific PCR approach was also used to identify *Candida* species. Several target genes for identifying *Candida* species have been described, but the most commonly amplified sequences are from the ribosomal ribonucleic acid (RNA) operon. Identification is based on the size of the PCR products obtained after resolution by gel electrophoresis, or sequence variation of the PCR products determined using direct sequencing or restriction fragment analysis after cutting the PCR sequences with restriction endonucleases. (Marsh and Martin, 2009).

Real-time PCR shows that *Candida* biofilms can confer resistance to many antifungal drugs commonly used in clinical settings. RT-PCR can be used to detect amplification in reactions and has advantages over conventional PCR, which measures kinetics and uses agarose gels to detect PCR amplification at the final endpoint stage of the reaction (Seneviratne et al. et al., 2008). A gene from *Candida albicans* confirmed that it regulates signaling in oral epithelial cells and vascular endothelial cells (Park *et al*., 2009).

Fluorescent in situ hybridization by peptide nucleic acid method (FISH PNA) is a novel detection technique that targets highly conserved species-specific sequences in abundant rRNA of living *C. albicans.* Single cells can be detected directly without amplification (Shepard *et al*., 2008). A sensitivity of 98 is achieved with this technique.

It can distinguish *C. albicans* from phenotypically similar *C. dubliniensis* with 7-100%, 100% specificity (Trnovsky *et al*., 2008). Molecular-based techniques can also be used to identify strains of *Candida* spp., but the use of techniques such as PFGE, random amplified polymorphic DNA (RAPD) analysis, and repetitive sequence amplification PCR (REP) is not recommended for epidemiological studies. Widely used in Reserved for oral studies is candidiasis (Marsh and Martin, 2009).

2.8. Candidiasis Treatment

The majority of *Candida* infections occur on epithelial surfaces such as the mouth, nails, vaginal area, and skin. These superficial infections are easily treated with antifungal creams or pills (Segal, 2004). Because *Candida* spp. have different resistance patterns to common antifungals, it is necessary to distinguish *C. albicans* from other *Candida* species for appropriate preventive and antimicrobial therapy (Kim and Brehm-Stecher, 2015). Furthermore, the number of clinical isolates of *Candida albicans* that are resistant to antifungals is increasing (Liu *et al*., 2014).

2.8.1. Cutaneous candidiasis

Cutaneous candidiasis can be treated with a variety of topical antifungals (clotrimazole, econazole, miconazole, ketoconazole, ciclopiroxolamine, sulconazole, and oxiconazole). For pulse therapy, itraconazole is taken orally. Initial diaper rash treatment should promote local dryness and avoid occlusion and provide good hygiene (Mistiaen and van Halm-Walters, 2010).

2.8.2. Oropharyngeal candidiasis (OPC)

The goal of antifungal therapy in OPC is rapid symptom relief, prevention of complications, and early relapse following therapy discontinuation. Topical azoles (clotrimazole troches), oral azoles (fluconazole, ketoconazole, or itraconazole), or oral polyenes (such as nystatin or oral amphotericin B) can all be used to treat oropharyngeal candidiasis (Shokohi et al., 2010). OPC infections tend to respond more slowly in HIV-positive patients, with approximately 60% of patients experiencing a recurrence within 6 months of the initial episode (Vazquez *et al*., 2011).

2.8.3. Vulvovaginal candidiasis

Topical agents such as azoles (clotrimazole, butoconazole, miconazole, tioconazole, terconazole, nystatin (100,000 U per day for 7 to 14 days), oral azoles (ketoconazole 400 mg for five days),

itraconazole (200 mg for one day, or 200 mg per day for three days), and fluconazole (150 mg) are effective options (Nyirjesy, 2008).

2.8.4. Invasive candidiasis

Fluconazole (6 mg/kg daily) is generally preferred for clinically stable patients. Amphotericin B deoxycholate (0.6-0.7 mg/kg/day) or lipid-related preparations of amphotericin B (3-5 mg/kg/day) can be used for patients with acute or refractory disease. Treatment of chronic disseminated candidiasis in such patients continues through chemotherapy.

Adjunctive glucocorticoids may play a role in rapidly resolving fever, abdominal pain, and inflammatory reactions in patients unresponsive to antifungal therapy, although long-term antifungal therapy is still required (Legrand, 2008).

2.8.5. Candidemia

As treatment, fluconazole (400 mg/day) and amphotericin B deoxycholate (0.5-0.6 mg/kg each day) are powerful. An enormous randomized preliminary showed that caspofungin (70 mg on the main day followed by 50 mg/day) is identical to amphotericin B deoxycholate (0.6 to 1.0 mg/kg each day) in obtrusive candidiasis (for the most part candidemia) (Mora-Duarte *et al*., 2002).

2.8.6. Antifungal resistance

An expansion in the quantity of yeasts that are impervious to antifungal medications is right now perceived around the world; accordingly, the utilization of in vitro lab tests can help the clinician in choosing proper treatment (Ingham *et al*., 2012). The capacity of *Candida* species to frame drug-safe biofilms is a significant calculate their commitment to human infection. Similarly as with by far most of microbial biofilms (Rajendran *et al*., 2010), sessile cells in *C. albicans* biofilms are less delicate to antimicrobials than planktonic cells (Kuhn and Ghannoum 2004). The movement of medication obstruction inside *Candida* biofilms has been related with an equal expansion in the development cycle (Sardi *et al*., 2011).

Ongoing examinations in Ethiopia affirm that HIV/Helps patients are orally colonized with at least one albic and non-albic *Candida* species, which are frequently impervious to azoles and sometimes to amphotericin B, 5-fluorocytosine and micafungin (Nasir *et al*, 2011; Mullu *et al*, ., 2013). These

feature the requirement for public reconnaissance to research *Candida* the study of disease transmission and antifungal opposition.

The antifungal vulnerability example of *Candida albicans* clinical detaches segregated from pee and vaginal swab was examined by Doughari Peter in Nigeria in 2009. Among the separates, 3 (37.5%) were defenseless while the leftover 11 (78.57%) were impervious to fluconazole, ketoconazole and nystatin Doughari and Peter, 2009). Similarly, the Burkinafaso review showed generally high protection from regularly and broadly utilized azoles (fluconazole, ketoconazole) for an in vitro antifungal weakness trial of C. albicans (Zida *et al*., 2017). A cross-sectional review was directed from November 2015 to December 2016 in the Family Guiding Relationship of Ethiopia and shown that all *Candida* disconnects were 100 percent powerless to voriconazole, caspofungin, and micafungin. *C. albicans* was 100 percent vulnerable to all medications tried aside from fluconazole and flycytosine with an opposition pace of 2% for each medication. *C. krusei* was 100 percent impervious to fluconazole and 33.3% to flu cytosine (Bitew and Abebaw, 2018).

In vitro antifungal vulnerability of *Candida albicans* detaches from the oral cavity of human immunodeficiency infection tainted patients in Ethiopia was performed by Wubie *et al* of 42 segregates, 41 (97.7%) of all separates were completely helpless to amphotericin B, 40 (95.3)% to nystatin and 39 (92.9%) to ketoconazole and miconazole. Then again, thedisengages showed the most elevated pace of fluconazole obstruction (11.9%). They likewise detailed that there was little distinction in the antifungal helplessness of *C. albicans* disengaged from patients who had a past filled with earlier antifungal treatment contrasted with the individuals who didn't get an antifungal. The review didn't record other *Candida* species (Webe *et al.,* 2011). One more concentrate in northwestern Ethiopia was directed by Mulu *et al.* name incessant recognition of 'azole' safe *Candida* species among patients with later introducing Helps results showed paying little mind to *Candida* species distinguished 12.2% (11/90), 7.7% (7/90) and 4.7 % (4) of the confines were impervious to fluconazole, F ketoconazole and itraconazole, individually. Conversely, protection from micafungin, amphotericin B and 5-luorocytosine was not normal (Mulu *et al*., 2013).

2.9. Prevention of candidiasis

Since most antifungals have a significant amount of toxic effect on human cells, it will be better to prevent the development of the disease than to treat it. The best preventive measures that can be

taken are keeping the skin clean and dry, adjusting food preferences, avoiding frequent use of antibacterial soaps, taking medications as directed by the doctor, and leading a healthy lifestyle. Although the number of antifungals is increasing rapidly and they are used to treat *Candida* infections for both mucosal and invasive infections, the result is not yet satisfactory. (Cassone *et al.*, 2007).

Although the concept of antibody protection has long been controversial, a large body of data is emerging in favor of its use to prevent as well as treat disease. This alternative method is gaining importance in the context of the growing number of immunocompromised patients sensitive to the toxic effect of conventional drugs. Antibodies against cell wall polysaccharides, heat shock protein, secreted proteins and peptides have been developed for the treatment of Candida infections. A synthetic glycopeptide vaccine against disseminated candidiasis has been found to be quite effective in mice. (Xin *et al.,* 2008).

3. MATERIALS AND METHODS

3.1. Description of the Study area

The study was conducted in Tepi General Hospital, which is located in Tepi town and 611 km far from Addis Ababa, the capital city of Ethiopia (fig 1). Tepi town is found in Southwest Ethiopian Peoples Regional State, Sheka Zone. The total area of Yeki Woreda is about 59000 km^2 which composed of 22 *Kebeles*. The minimum and maximum monthly temperature of the area varies from 15°C to 30°C, respectively. The area receives annual rainfall of 1591 millimeter. The area is located South West Ethiopia between the geographical coordinates $7^0 3'$ North Longitude and $35^0 0'$ East Latitude with altitude of 1200 meter above sea level. The hospital has eight outpatients departments and four wards namely medical, surgical, pediatrics, and obstetrics/gynecology wards.

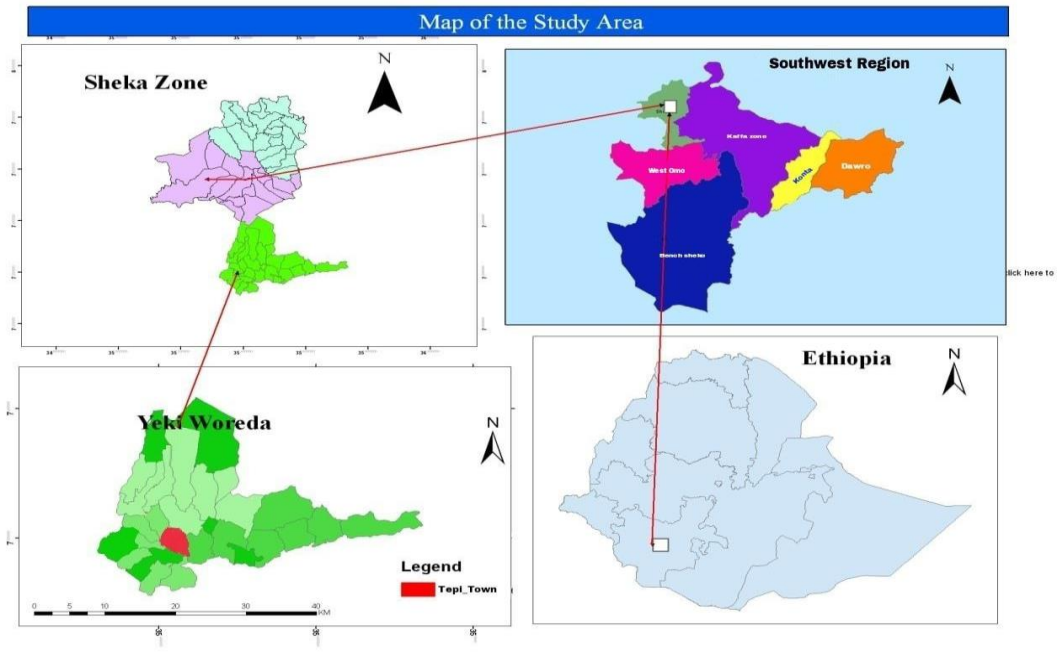


Fig 1. Map of study area

3.2. Study Design

For the current study hospital-based cross-sectional study was conducted from July 2022 to June 2023 to investigate the diversity, distribution, and drug resistant patterns of *Candida* species. Among 120 patients admitted for surgical, systemic, intestinal infection selected by purposive sampling techniques based on characteristics of infection. Structured questionnaires were also used to collect information about socio demographic characteristics and risk factors of *Candida.*

3.3. Inclusion and Exclusion Criteria

All patients admitted at Tepi General Hospital and clinically suspected of candidiasis infection were included for the current study. Patient were not willingness to participate and severely ill were excluded from the study.

3.4. Sampling Techniques and Data Collection Methods

For the present study 120 patients who have sign and symptoms of infection of *Candida* species were selected by purposive sampling techniques based on characteristics of infection. Specimen was collected from each participant using a sterile cotton swab moisten by physiological saline

then inserted and rotated gently to pick up the specimen. For cross-sectional data, structured questionnaire was used to collect information about socio demographic characteristics and risk factors related to *Candidiasis* from patients who attending in Tepi General Hospital during the study period. Questionnaires data was employed through face to face interview after informed consent.

3.5. Collection of Specimens and Identification of *Candida* Species

All samples were collected according to standardized work procedures. Clinical specimens including surgical wound swabs, blood, oropharyngeal swabs, urine, stool, sputum, and vaginal swabs were collected from patients with signs and symptoms of infection by medical professionals and sent to the study site for culture and susceptibility testing. The collected swab and sample were marked with a unique sample number and the date and time of collection and immediately delivered to the microbiology laboratory of Jimma University. A sample was collected from each participant using a sterile cotton swab moistened with saline, then inserted and gently rotated to collect the sample. A portion of each clinical swab was initially inoculated onto Sabouraud dextrose agar (Oxoid, UK) and then incubated at 37°C for 24 to 48 hours. White cream colonies were then identified.

In addition, A fungal suspension inoculated onto selective BiGGY agar based on the manufacturer. BiGGY agar (Oxoid Company, Wade Road, Basingstoke, and Hampshire, UK) is a chromogenic medium, it contains bismuth sulphite and the growth on this medium produces brown to black colonies because of the extra cellular reduction of bismuth sulphite to bismuth sulphide. *Candida albicans* species showed light brown while *Candida tropicalis* isolates produced light and dark brown color when grown on BiGGY agar. It was hard to differentiate the colors of these two species. In this study two species showed typical, distinctive appearance on BiGGY agar. One was *Candida krusei* which produced typical large, rough, dark brown colonies with surrounding yellow zone and the other was *Candida parapsilosis* which grew as light brown-geenish, gray, cream colored colonies. *Candida glabrata* strains grew weakly on BiGGY agar after 48 hours. Brown color was only observed at the first streaks of the cultures, where the colonies were very crowded. Other areas especially single colonies were very small and colorless.

Germ tubes production in serum has been used to identify *Candida* species (Deorukhkar *et al*., 2012). To distinguish *C. albicans* from non-*albicans*, a yeast colony is added to a sterile tube containing 0.5 ml of human serum and incubated at 37°C for 3 hours, after which the loop-full sample is placed on a microscope slide and covered with a cover glass. Finally, germ tube formation was observed under a microscope (Donbraye *et al*., 2010).

In addition, all isolates were processed for matrix assisted laser desorption/ionization time of flight (MALDI –TOF MS) EXS3000 (Zybio, China) .The recognition results were scored according to the manufacturer criteria.In this regard, the log score values of >2.0, 1.7 – 2, and <1.7 indicated correct species identification and no reliable identification respectively.

3.6. Antifungal Susceptibility Test

Antifungal susceptibility testing was performed on all *Candida* isolates using the disk diffusion method according to Clinical Laboratory Standard Institute guidelines by adding 2% glucose and 0.5 μg/ml methylene blue dye to Mueller-Hinton agar. A suspension was prepared using normal saline by adding five different colonies and incubating overnight in Sabouraud dextrose agar. The suspension was then compared with 0.5 McFarland standards (this was prepared by mixing 1% $BaCl_2$ and 1% H_2SO_4 at various ratios and determining the concentration, estimated to be equivalent to about 10^4 to 10^6 organisms/ml). It was inoculated by dipping a sterile cotton swab into it and blotting it with Muller Hinton agar according to the standard procedure (CLSI), and then antifungal discs were firmly placed on it and the plates were incubated at 37°C for 24 h. Antifungal discs including amphotericin B 100 μg, clotrimazole 10 μg, fluconazole 25 μg, and ketoconazole 10 μg (Oxoid, UK) were placed on Mueller-Hinton agar using disk dispenser, and the plates were incubated at 37°C for 24 hr. Finally, the zones of inhibition (zone averages) around each disc were measured using a caliper, and the results were interpreted as sensitive, intermediate, and resistant using the standard CLSI (M44-A2) zone interpretation chart.

3.7. Confirmation of Identified *Candida* species

A presumptive positive for *Candida* species was the presence of brown or black colonies. *Candida* colony morphology was presented as follows: *C. albicans* – Smooth, circular brown-black colonies; slight mycelial fringe; no color diffusion into surrounding medium; no sheen. *C. tropicalis* – Smooth, discrete brown-black colonies with black centers; slight mycelial fringe;

diffuse blackening of medium after 72 hours; sheen. *C. krusei* – Large, flat, wrinkled silvery brown-black colonies with brown edge; yellow halo. *C. parapsilosis* grows as light brown-greenish, grey- cream colored colonies in BiGGY agar. *C. glabrata* strains grew weakly on BiGGY agar after 48 hours. Brown color was only observed at the first streaks of the cultures, where the colonies were very crowded. Other areas especially single colonies were very small and colorless. *Candida albicans* was found to be germ tube positive. Non *albicans Candida*, on the other hand, were not positive for germ tube formation.

3.8. Data Analysis

The collected data were coded and properly managed by using Excel computer software. Descriptive statistics were analyzed by using SPSS software (Statistical Package for the Social Sciences, version 20, SPSS Inc, and Chicago, Ill, USA). The result was presented in form of table and text. Comparisons between the study groups were made with the Chi square (χ^2) test. The statistical significance differences between *Candida* species in different samples among patients were analyzed by using ANOVA single factors test at 95% confidence level. Finally, p-value < 0.05 were considered statistically significant.

3.9. Ethical Considerations

This study was conducted in accordance with the Declaration of Helsinki and a formal letter was received from Jimma University, Department of Biology to conduct the study. The administrative office of Tepi General Hospital was informed about the purpose of the study to obtain permission. Respondent confidentiality was ensured by excluding respondent identifiers such as name from the data collection format. Informed written consent was obtained from the respondents before conducting the study.

4. RESULTS

4.1. Socio Demographic Characteristics of Study Patients

The socio- demographic characteristics of participants were presented in Table 1. A total of 120 respondents were included in the present study, the percentage of male and female were (56.7%) and (43.3%), respectively. Regarding to age, majority (45%) of respondents were aged 21-30 years, (33.3%) from those between the ages of 31-40 years, and (14.2%) and (7.5%) were aged above 41 years and below 20 years old, respectively. The majority (65.6%) of the respondents were rural residents. Whereas, (34.4%) of patients were urban residents. Regarding to marital status, (82.5%) of patients were married, (12.5%) were single and (1.7%) of were divorced, and (3.3%) widowed. About (35%) of the study participants were illiterate, while, (65%) are literates.

Table 1: Socio-demographic characteristics of patients (n=120)

Characteristics	Frequency	Percentages
Gender		
Male	68	56.7
Female	52	43.3
Age		
Below 20 years	9	7.5
21-30	54	45
31-40	40	33.3
Above 41 years old	17	14.2
Residence		
Urban	75	34.4
Rural	45	65.6
Marital status		
Single	15	12.5
Married	99	82.5
Widowed	4	3.3
Divorced	2	1.7
Educational status		
Literates	78	65
Illiterates	42	35

n=number of respondents

4.2. Isolation rate of Candidiasis by Socio Demographics among Patients

Of the total 120 sample collected for identification of diversity and distribution of *Candida* species, 92 (76.7%) were positive for *Candida* infection (Table 2). In terms of sex, the result of present study revealed that *Candida* prevalence was significantly higher in females 45(86.5%) than males 47(69.3%) (P= 0.0293). Of the total positive patients the isolation rate of candidiasis was (86.5%) in female's participants. From the total of 52 samples from female patients, 45 (86.5%) and 68 samples from males 47 69.3(%) were positive, respectively. The rate of infection was high (81.5%) in the age groups of 21-30 years old, followed by 31- 40 years old (75%), and (70.6%) of *Candida* infected the age groups above 40 years old years old. The least (66.7%) incidence rate was observed in the age groups below 20 years old.

Table 2. Isolation rate of Candidiasis among patient admitted at Tepi General Hospital (n=120)

Demographic Characteristics	Total examined	Positive	Negative	P-value
Gender				
Male	68(56.25%)	47(69.3%)	21(31.7%)	
Female	52(43.75%)	45(86.5%)	7(13.5%)	0.0254*
Age				
Below 20 years	9(11.2%)	6(66.7%)	3(33.3%)	
21-30	54(45.8%)	44(81.5)	10(18.5%)	
31-40	40(29.7)	30(75%)	10(25%)	0.9497
Above 41 years old	17(13.3%)	12(70.6%)	5(29.4)	
Residence				
Urban	45(34.5%)	30(66.7%)	15(30.3%)	
Rural	75(65.6%)	62(82.7%)	13(17.2%)	0.0448*
Marital status				
Single	15(12.5%)	8(53.3%)	7(46.7%)	
Married	99(82.5%)	80(80.8%)	19(19.2%)	
Widowed	4(3.3%)	3(75%)	1(25%)	0.0972
Divorced	2(1.7%)	1(50%)	1(50%)	
Educational status				
Literates	78(75.5%)	55(70.5%)	23(29.5)	
Illiterates	42(24.5%)	37(88.1%)	5(12.9%)	0.0299*
Symptoms				
Symptomatic	85(68%)	77(90.6%)	8(9.4%)	
Asymptomatic	35(32%)	15(35%)	20(65%)	0.0001*

Where; n=number of participants, * = Chi squares (X^2) significant at 95% of confidence interval

4.3. Predisposing factors for *Candida* Infection among Patients

In the current study, out of the total 120 patient samples were collected, the majority of patients (76.7%) were *Candida* positive due to various risk factors. The most common risk factors for these patients was HIV/AIDS which accounts (90%), followed by pregnancy (86.7%), diabetic mellitus (83.3%), prolonged antibiotic therapy (73.3%), Tuberculosis (66.7%), Trauma/wound (40%) of patients with *Candida* colonization.

The current study showed that 30 clinical specimens were obtained from HIV/AIDS patients. Of these specimens, 27 (90%) were positive for *Candida*, indicating that HIV/AIDS is a significant risk factor associated with *Candida* infection.

Regarding to pregnancy, 15 clinical specimens was collected from pregnant women, *Candida*-positive result was 13 (86.7%); Current results indicated that pregnancy was a significant risk factor associated with *Candida* infection.

Of the 30 samples collected from diabetic patients, 25 (83.3%) were *Candida* positive. Among the clinical sample (73.3%) of antibiotic users were affected by *Candida* infection.

Table 3. Frequency of isolation of *Candida* among patients having predisposing factors

Risk factors	Total isolation	*Candida* Positive		*Candida* Negative	
		number	%	number	%
HIV/AIDS	30	27	90	3	10
Diabetic mellitus	30	25	83.3	5	16.7
Trauma/wound	15	6	40.0	9	60.0
Tuberculosis	15	10	66.7	5	33.3
Pregnant women	15	13	86.7	2	13.3
Antibiotics therapy	15	11	73.3	4	26.7
Total	120	92	76.7	28	23.3

Chi square test (P = 0.005; Calculated Chi sq. Value = 16.7702; Critical Chi sq. Value = 11.0705)

4.4. Isolation rate of *Candida* per Clinical Samples

A total of 92 *Candida* were isolated from 120 different clinical samples (Table 4). Clinical samples were isolated for the present study including 15 (12.5%), 20 (16.7%), 15 (12.5%), 20 (16.7%), 20 (16.7%), 15 (12.5%) and 15 (12.5%) were from Surgical wound swab, Oropharengial swab, Blood, Urine, Stool, Sputum and Vaginal swabs, respectively (Table 4).

Table 4. Distribution of *Candida* isolates per clinical samples (n=120)

Clinical Samples	Status		Total
	Positive	Negative	
Surgical wound swab	9 (60%)	6 (40%)	15 (12.5%)
Oropharengial swab	16 (80%)	4 (20%)	20 (16.7%)
Blood	11 (73%)	4 (27%)	15 (12.5%)
Urine	17 (85%)	3 (15%)	20 (16.7%)
Stool	16 (80%)	4 (20%)	20 (16.7%)
Sputum	10 (66.7%)	5 (33.3%)	15 (12.5%)
Vaginal swabs	13 (86.7%)	2 (13.3%)	15 (12.5%)
Total	**92 (76.7%)**	**28 (23.3%)**	**120 (1001)**

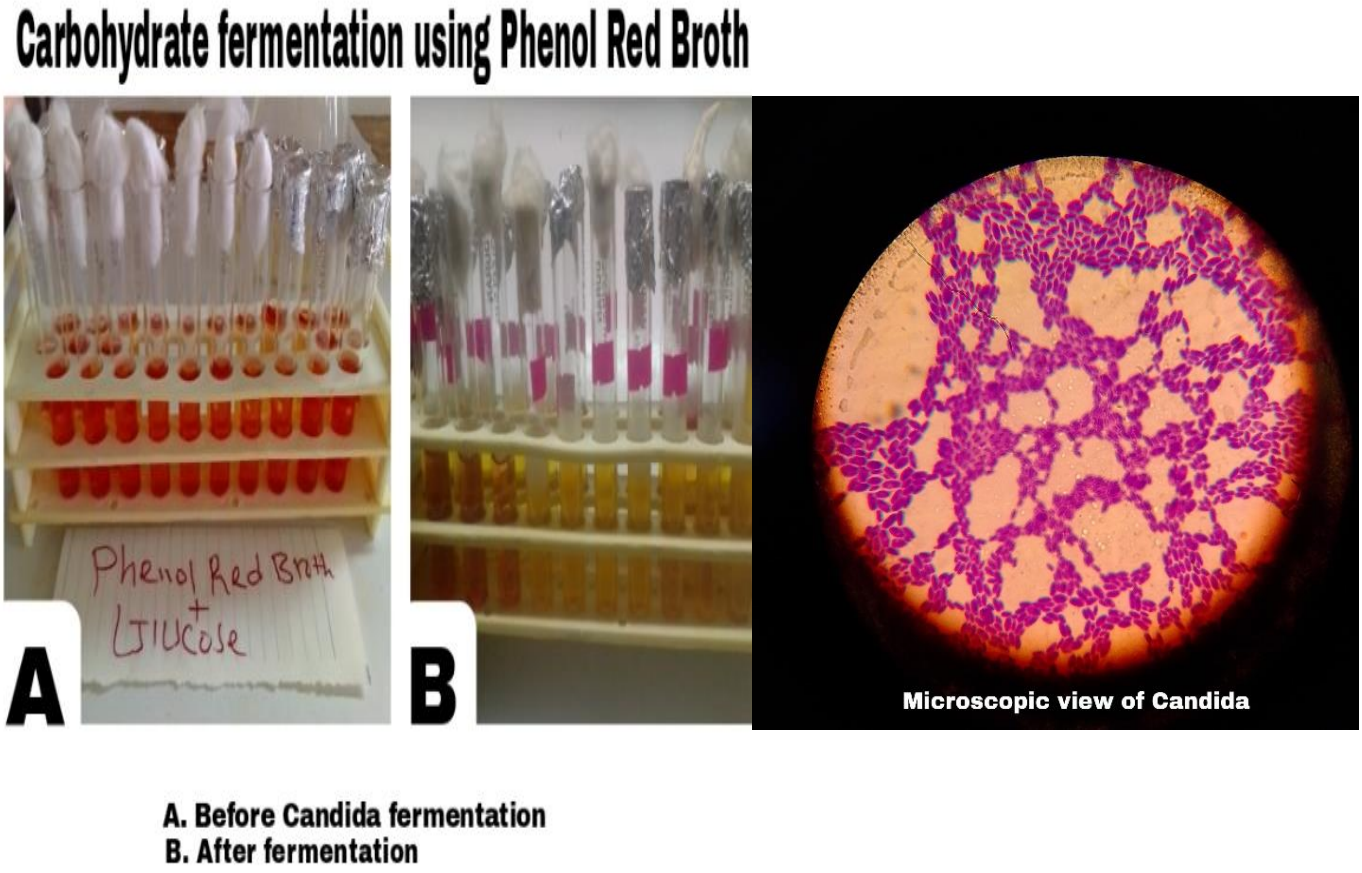
4.4. *Candida* Species Diversity and Distribution in Tepi General Hospital

4.4.1. *Candida* Species Diversity among Clinical Isolates

A total of 92 isolates of *Candida* species were recovered from 120 diverse clinical sources. A total of 5 species of *Candida* were identified based on the *Candida* species colony color characteristics on Sabouraud Dextrose Agar, BiGGY agar test and matrix assisted laser desorption/ionization time of flight(MALDI-TOF MS). The *Candida* species type was described using colonial morphological features and the different colors produced by the isolates. Colony morphology on Sabouraud Dextrose Agar was found to aid in the identification of *Candida* species. BiGGY agar contains bismuth sulphite, which results in brown to black colonies due to the extracellular reduction of bismuth sulphite to bismuth sulphide. On Sabouraud Dextrose Agar, all *Candida* albicans isolates grew as convex dome-shaped pearl-like colonies. By matrix assisted laser desorption time of flight (MALDI –TOF MS) five species were identified in the presence of other coinfecting microorganisms.

The carbohydrate fermentation test was used to determine the *Candida's* ability to acidify carbohydrate substrate detected by a pH indicator in the medium (phenol red). The fermentation test principle is based on the detection of acid and gas produced as a result of sugar fermentation.

Five isolates showed positive reactions to glucose in the carbohydrate fermentation test. According to Staudacher and Whelan, the pH indicator changed from red to yellow when the acid produced by fermentation exceeded the alkaline.



. Fig 2. Carbohydrate fermentation and microscopic image of *Candida*

According to the manufacturer, *Candida albicans, Candida tropicalis* and *Candida krusei, Candida parapsilosis,* and *Candida glabrata* were reported to be identifiable on BiGGY agar. When grown on BiGGY agar, all *Candida albicans* species produced light brown color, while *Candida tropicalis* isolates produced light and dark brown color. On BiGGY agar, two species displayed typical, distinct appearances. *Candida krusei* produced large, rough, dark brown colonies with a surrounding yellow zone, whereas *Candida parapsilosis* produced light brown-geenish, gray, cream colored colonies. After 48 hours, *Candida glabrata* strains grew slowly on BiGGY agar. Brown color was only seen in the cultures' first streaks, where the colonies were densely packed. Other areas, particularly single colonies, were extremely small and colorless.

On Gram staining, *Candida* species were Gram-positive, oval or round, showed short elements of budding yeast cells, pseudohyphae, and true hyphae. *Candida* occurs as off-white, smooth, pasty, convex colonies on the SDA, making it almost impossible to distinguish between species.

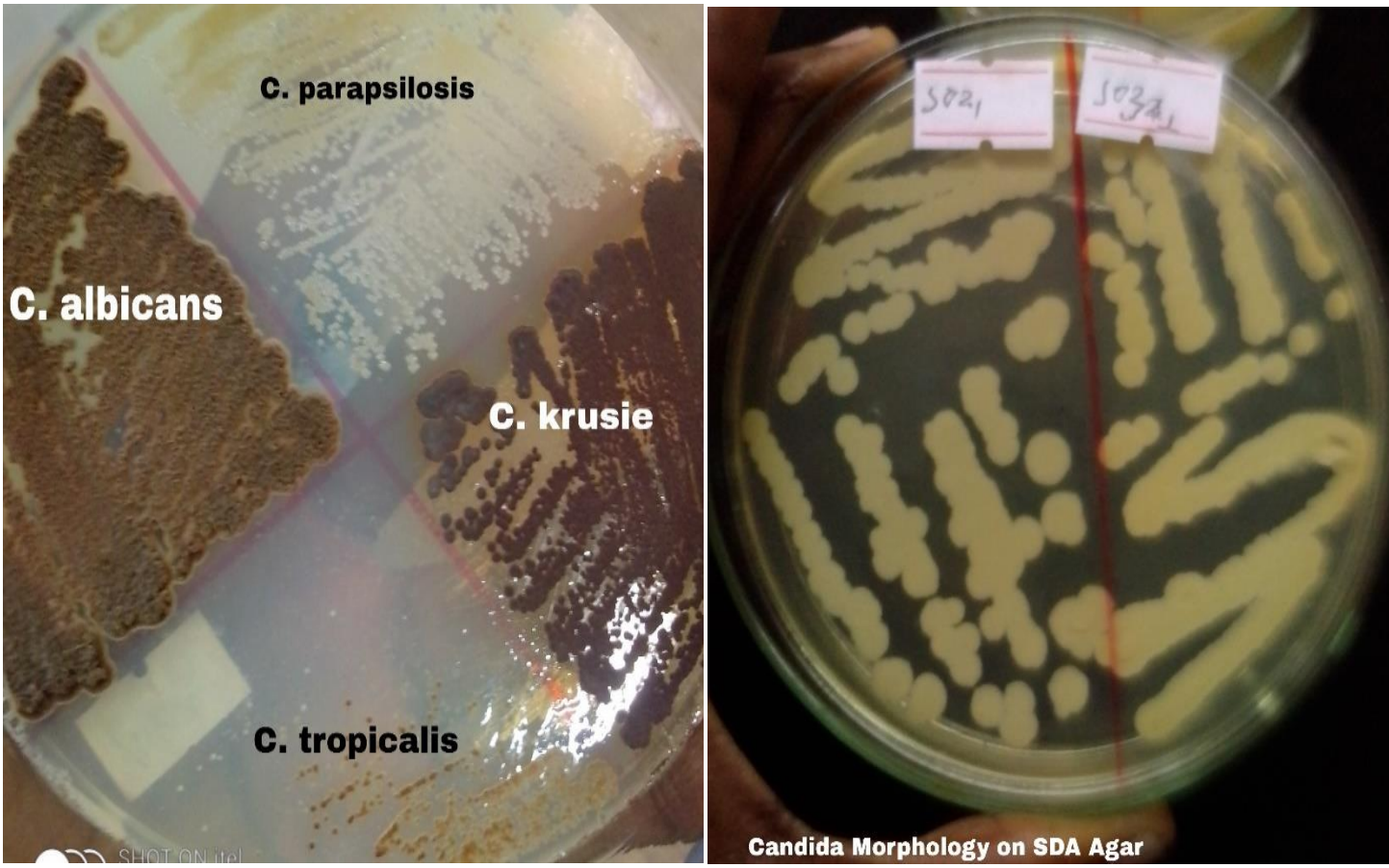


Figure 3. *Candida* on BiGGY agar (Representative colonies color) and *Candida* on SDA Agar

Table 5. *Candida* species Distribution among Clinical samples in Tepi General Hospital

***Candida* Species**	**Clinical Isolates**							**Total**	**Percent (%)**
	SWS	**OS**	**Blood**	**Sputum**	**Stool**	**Urine**	**VS**		
C. albicans	6	11	6	4	7	7	10	51	55.4
C. krusei	1	1	2	3	4	3	2	16	17.4
C. tropicalis	2	2	1	2	2	3	1	13	14.1
C. parapsilosis	0	2	1	1	2	2	0	8	8.7
C. glabrata	0	0	1	0	1	2	0	4	4.3
Total	9	16	11	10	16	17	13	92	100

One way ANOVA test highly significant differences ($p<0.01$) Where; SWS= surgical wound swab, OS= oropharengial swab, VS= vaginal swabs

4.4.2. Distribution of *Candida* Species in Relation to Sex

The distribution of the *Candida* species in relation to sex was presented in Table 6. The results of current study revealed that from *Candida* isolates, (*Candida albicans, Candida krusie* and *Candida tropicalis*) were three most common *Candida* species from 92 total positive samples at Tepi General Hospital.

Table 6. Distribution of the *Candida* species in relation to sex (n=120)

Candida Species	Sex		Total
	Male (n=47)	Females (n=45)	
Candida albicans	21 (41.7%)	30 (58.9%)	51
Candida krusei	6 (87.5%)	10 (62.5%)	16
Candida tropicalis	10 (77%)	3 (23%)	13
Candida parapsilosis	7 (87.5%)	1 (12.5%)	8
Candida glabrata	3 (75%)	1 (2.5%)	4
Total	**47**	**45**	**92**

Significant resuts of Chi Square (X^2) = ($P = 0.0187$)

4.4.3. *Candida* Species Distribution by Age

Candida albicans was more frequently (68.2%) in the age group of (21-30) which was closely followed by the age group below 20 years old incidence rate of (66.7%). *Candida krusie* and *Candida tropicalis* were higher in age group of above 41 years old, accounting (33.3%) and (16.7%), respectively. It was followed by the age group (31-40) years old, accounting of (16.7%) and (16.7%) of colonization rate respectively.

Table.7. *Candida* Species Distribution by Age

***Candida* Species**	**Age Groups**				**Total**
	Below 20	**21-30**	**31-40**	**Above 41**	
Candida albicans	4 (66.7%)	30 (68.2%)	14 (46.7%)	3 (25%)	51 (55.4%)
Candida tropicalis	1 (16.7%)	5 (11.4%)	5 (16.7%)	2 (16.7%)	13 (14.1%)
Candida krusei	1 (16.7%)	6 (13.6%)	5 (16.7%)	4 (33.3%)	16 (17.4%)
Candida glabrata	0 (0%)	1 (2.3%)	2 (6.7%)	1 (8.3%)	4 (4.3%)
Candida parapsilosis	0 (0%)	2 (4.5%)	4 (13.3%)	2 (16.7%)	8 (8.7%)
Total	**6 (100%)**	**44 (100%)**	**30 (100%)**	**12 (100%)**	**92 (100%)**

4.5. Drug Resistant Patterns of *Candida* Species in Tepi General Hospital

Of the total of 92 *Candida* isolates, (71.2%), (7.3%) and (21.5%) *Candida* isolates were susceptible, susceptible dose dependent and resistant to the drug, respectively. High resistant rate was observed for Fluconazole 26/92 (28.3%) and Clotrimazole 22/92 (23.9%). Whereas, 76/92 (82.6%) and 65/92 (70.7%) *Candida* isolates were more susceptible to Amphotericin-B and Clotrimazole, respectively.

From 51 *Candida albicans* isolates, (15.7%), (13.7%), (11.8%), (7.8%), were resistant to Fluconazole, Clotrimazole, Ketoconazole, Amphotericin-B, respectively. Whereas, *Candida albicans* was more sensitive to Amphotericin-B, Clotrimazole with (90.2%), (84.3%), respectively. Out of 13 *Candida tropicalis*, (53.8%), (7.7%), and (38.5%), were susceptible, susceptible dose dependent and resistant, respectively. *Candida tropicalis* was (69.2%), were sensitive to Amphotericin-B. But it was (53.8) of them were resistant to Fluconazole, respectively.

Regarding to *Candida krusie* drug resistant patterns of the current study shows that, (51.6%), (10.9%) and (37.5%) of *Candida krusie* were susceptible, susceptible dose dependent and resistant. The majority, 7/16 (43.8%) and 7/16 (43.8%) of *Candida krusei* were resistant to Fluconazole and Ketoconazole, respectively. Among 8 isolates of *Candida parapsillosis*, 3/8 (37.5%), 2/8 (25.5%) were resistant to Clotrimazole and fluconazole, respectively. Whereas, 7/8(87.5%) of the isolates were sensetive against Amphotericin B.

Fluconazole is the first-line (first-choice) drug in the treatment of candidiasis according to the Ethiopian Ministry of Health's current guidelines. In the current study, the percentage of *Candida* species with a drug resistance profile against fluconazole was higher (28.3%). *Candida* species' susceptibility to fluconazole, on the other hand, varied between species.

Table 8. Drug resistant patterns of *Candida* species in Tepi General Hospital, 2023 (n=92)

Candida spp	Patterns	Anti Fungal Agents								Total
		AmphotericinB		Clotrimazole		Fluconazole		Ketoconazole		
		N	%	N	%	N	%	N	%	N
C. albicans (51)	S	46	90.2	43	84.3	40	78.4	42	82.4	171
	SDD	1	2.0	1	2.0	3	5.9	3	5.9	8
	R	4	7.8	7	13.7	8	15.7	6	11.8	25
C. tropicalis (13)	S	9	69.2	7	53.8	5	38.5	7	53.8	28
	SDD	0	0.0	1	7.7	1	7.7	2	15.4	4
	R	4	30.8	5	38.5	7	53.8	4	30.8	20
C. krusie (16)	S	11	68.8	9	56.3	7	43.8	6	37.5	33
	SDD	1	6.3	1	6.3	2	12.5	3	18.8	7
	R	4	25.0	6	37.5	7	43.8	7	43.8	24
C. glabrata (4)	S	3	75.0	2	50.0	2	50.0	3	75.0	10
	SDD	1	25.0	2	50.0	0	0.0	1	25.0	4
	R	0	0.0	0	0.0	2	50.0	0	0.0	2
C. parapsilosis (8)	S	7	87.5	4	50.0	4	50.0	5	62.5	20
	SDD	0	0.0	1	12.5	2	25.0	1	12.5	4
	R	1	12.5	3	37.5	2	25.0	2	25.0	8
All isolates (92)	**S**	**76**	**82.6**	**65**	**70.7**	**58**	**63.0**	**63**	**68.5**	**262**
	SDD	**3**	**3.3**	**6**	**6.5**	**8**	**8.7**	**10**	**10.9**	**27**
	R	**13**	**14.1**	**21**	**22.8**	**26**	**28.3**	**19**	**20.7**	**79**

5. DISCUSSION

Of the total 120 isolates collected for identification of diversity and distribution of *Candida* species, 92 (76.7%) were positive for *Candida* infection (Table 2). The current results of *Candida* incidence rate was lower than 82.3% (177/215) which was reported by (Mulu et al. 2013), but higher than 69.2% (155/224) colonization rate reported by (Birhan Moges et al. 2016) in Ethiopia.

In terms of sex, *Candida* prevalence was significantly higher in females than males (P= 0.0293). Similarly, (Mulu et al. 2013), found that females were at a significantly higher risk compared to male counter-parts.

As indicated in Table 2, prevalence of *Candida* species was no significant differences (p=0.9497) among different age groups. The rate of infection was high (81.5%) in the age groups of 21-30 years old, followed by 31- 40 years old (75%) and (70.6%) of *Candida* infected the age groups

above 40 years old years old. This may be due to the use of contraceptives, drug abuse or even due to sexual promiscuity. The current results was agreed with (Taura *et al.*, 2013) reported that the overall frequency distribution of *Candida* Species was found to be higher among 21-30 years old.

Prevalence of *Candida* was significantly higher in patients who were rural residents (82.7%) than urban residents (66.7%) (p= 0.0448). The current findings showed that there was significant differences between literates and illiterates in *Candida* colonization. The prevalence of candidiasis also higher in illiterates (88.1%) than literates (70.5%) (p = 0.0299). Education was also closey linked to the infection. The current results shows that patients with higher education were unlikely to be infected by *Candida* species. The possible explanation for this outcome maybe that the high educated peoples mastered the knowledge of *Candida* infection and discerned how to protect themselves from infection.

The current study showed that 30 clinical specimens were obtained from HIV/AIDS patients. Of these 27 (90%) were positive for *Candida*, indicating that HIV/AIDS is a significant risk factor associated with *Candida* infection. Similar to the current study (Arya and Naureen, 2022), more than 90% of HIV patients were reported to have candidiasis. Likewise, the high risk of obtaining *Candida*-positive results in samples submitted by HIV/AIDS patients in the current study concurs with a study that revealed intestinal *Candida* infection primarily affects immunocompromised and those infected with HIV/AIDS among other patient sub populations (Gupta, 2012)

The overuse of immunosuppressive drugs for life threatening health situations occasioned by a rise in Human Immuno deficiency Virus (HIV) infections has led to more cases of severely immunosuppressed patients (Enrique and Puebla, 2012) and in whom the principal etiological agent of fungal infection is *Candida* (Enrique and Puebla, 2012). HIV pandemicity and invasive procedures for critically ill patients require the use of prolonged prophylactic antifungal treatments since such patients are more at risk of developing fungal infections including candidiasis (Loeffler and Stevens, 2003).

Regarding *Candida* isolation associated with pregnancy, out of 15 clinical specimens collected from pregnant women, *Candida*-positive was 13 (86.7%); Current results indicated that pregnancy was a significant risk factor associated with *Candida* infection. This was mainly due to their relatively weakened immunity (Nnaemeka, 2010) and (Mukasa *et al.*, 2015). Although *Candida*

albicans frequently colonizes the vagina without causing disease (Apalata *et al*., 2014); (Schelenz *et al*., 2016), *Candida* infections are primarily seen as opportunistic infections due to changes in host conditions (Apalata *et al*., 2014). Some of the altered conditions that occur during pregnancy are changes in pH (Apalata *et al.*, 2014) of the vagina promoting the flourishing of the *Candida* organism. During pregnancy, the pH of vaginal secretion is acidified to around 4 or 5, which unfortunately favors the growth of *Candida albicans* (Kamath and Nayak, 2013). Reduces vaginal acidity to pH 5.0-6.5, provide an environment conducive to colonization and infection by *Candida* (Nnaemeka, 2010).

During pregnancy, elevated levels of estrogen and glycogen in vaginal secretions increase the risk of vulvovaginal candidiasis in women (Kamath et al., 2013, Plitteri Adele, 2007, Soong and Einarson, 2009). Similarly, (Apalata *et al*., 2014) list pregnancy, among other conditions, as a risk factor associated with vulvovaginal candidiasis.

Regarding diabetes, in this study, 30 of the samples tested were collected from diabetic patients, of whom the probability of obtaining a *Candida*-positive result in patient-submitted samples was 25 (83.3%) of the prevalence. It became clear that this is because diabetics have high levels of glucose in their tissues. This is in line with other studies reporting diabetes as an essential carbon source that *Candida* requires for its active metabolism, colonization, and infection with it, and as an associated and recognized important risk factor. Diabetes mellitus is one of several risk factors for vaginal candidiasis, with *Candida albicans* being the most commonly isolated pathogen (Saporiti *et al*., 2001).

Regarding the relationship between *Candida* isolation and antibiotic therapy, (73.3%) of antibiotic users were affected by *Candida* infection.This result is consistent with studies by (Alem and Mekonnen, 2019) and (Babin *et al.*, 2013). The reason may be that antibiotics disrupt (eliminate) the body's normal flora, which are important in defending against pathogens, resulting in higher *Candida* colonization (Babin *et al.*, 2013). Similarly, Sahni *et al*., 2005 reported that use of antibiotics was significantly associated with candidaemia (Sahni et al., 2005). High colonization rates have been reported in critically ill surgical patients (Arslankoylu *et al*., 2011).

The occurrence of *Candida* species among various clinical isolates shows no significant differences among clinical samples (P=0.528). A comparison of the occurrence of *Candida* species

was higher (86.7%), (85%), (80%) and (80%) in vaginal swabs, urine specimens, Oropharengial swab and stool, respectively. The lowest incidence rate were observed in surgical wound swabs (60%) and sputum (66.7%).

The current study revealed that among 5 *Candida* species identified, the predominant *Candida* species was *Candida albicans* (55.4%), which was followed by *Candida krusei* (17.4%) and *Candida tropicalis* (14.1%), *Candida parapsilosis* (8.7%) and *Candida glabrata* (4.3%), were the commonest isolates among non-*albican candidia* species. *Candida albicans* the main isolated in vaginal swabs isolate, oropharengeal swabs and urine while *Candida krusie* were from stool swabs and urine specimens.

Among the NAC species, the most prevalent species were *C. Krusie, C. tropicalis,* and *C. glabrata* were observed in the present study was similar, Elias Seyoum *et al.,* 2020 reported that 49.8% were *Candida albicans,* 43.1% were non-*albicans Candida* species. Among NAC species, *C. krusei* 15.6%, *C. famata* 14.4%, *C. rugosa,* 11.1% were the commonest isolates. Furthermore, Tsega and Mekonnen, 2019) reported that the predominant *Candida* species was *C.albicans*; it accounts 56.3%, which was followed by *Candida krusie* 21.9%. Likewise, the result was agreed with the report by, Badiee *et al.,* 2011, showed that, *Candida albicans* (48%) was the most frequently isolated species, followed by *Candida kruzei* (16.1%), *Candida glabrata* (13.5%), *Candida kefyr* (7.4%), *Candida parapsilosis* (4.8%), *Candida tropicalis* (1.7%) and other species (8.5%). Amita and Naimshree, 2020 found that the predominant of *C. albicans* (51.6%) and non *albican Candida* (48.4%). Generally, similar results also reported by (Terças *et al.,* 2017; Colombo *et al.,* 2006; Hamza *et al.,* 2008,; Kwamin *et al.,* 2013).

In the present study, *Candida krusei* was the second dominant species. The current result was in line with that of Bitew and Abebaw, 2018; Elias Seyoum *et al.,* 2020 and Mohndas and Ballal, 2011, while in contradiction with many other studies where *C. glabrata* or *C. tropicalis* was reported as a 2nd predominant NAC species (Mulu *et al.,* 2013; ElFeky DS *et al.,* 2016 and Das KH *et al.,* 2016).

There was statistically significant association observed between sex and the species of *Candida* (P = 0.0187). The dominant species in females were *Candida krusie* and *Candida albicans* which accounting (62.5%) and (58.9%), respectively. Whereas, in males, *Candida tropicalis* and *Candida*

glabrata and *parapsilosis* were the dominant species with (77%) (75%) and (87.5%), respectively. The current results was almost agreed with Birhan Moges (2014), who reported that the two common yeast isolated from male patients were *C. albicans* and *C. tropicalis* amounting 66.0% and 16.0 % respectively. Whereas, in females *Candida albicans* was the most common species of yeast.

The finding of present study was agreed with (Pfaller and Diekema, 2002; Pfaller *et al.*, 2002) who reported *C. albicans* is more frequent in patients aged up to 18 years. The frequency of cases caused by azole-resistant species such as *C. glabrata* and *C. krusei* is much lower in neonates (3% vs. 23% and 0% vs. 2%), probably reflecting scant use of azoles in neonatology.

According to the data in table 8, it was revealed that Amphotericin-B was the most effective antifungal drugs. Whereas, Fluconazole and Ketoconazole were the poorest activity against all species of *Candida* to inhibit the growth of *Candida* isolated in this study.

According to the current findings, fluconazole was effective against (78.4%) of *Candida albicans.* Similarly, the previous study conducted in Ethiopia by (Elias Seyoum, 2019) found that fluconazole susceptible, susceptible dose dependent, and resistant rates are (85.6%), (10.5%), and (3.9%), respectively. On the other hand *Candida* isolates such as *Candida krusei, Candida tropicalis,* and *Candida glabrata,* were resistant to the drug in proportions of 50%, 53.8 %, and 50%, respectively.

The most likely reason is that fluconazole antifungal drugs are the most commonly used drugs to treat *Candida* infection because they are less toxic, less expensive, and can be administered orally. Long-term or repeated exposure to low-dose fluconazole has been linked to the emergence of fluconazole resistance in *Candida albicans* strains (Lopez *et al*., 2001) and the potential selection of non-*albicans Candida* species such as *Candida kruse* (Sobel *et al*., 2001)

The current study found that of the four antifungal drugs tested for susceptibility, Amphotericin-B was the most effective. The findings revealed that most 82.6% (76/92) of the *Candida* isolates were susceptible to Amphotericin-B. This finding was consistent with the findings of a previous study conducted in Ethiopia by Alem Tsega and Feleke Mekonnen, who found that (100%) *Candida albicans* and (71.43%) *Candida krusie* were susceptible to Amphotericin-B. Similarly, Arul Sheeba et al., 2012 found 90% Susceptible *Candida* in India and 87.2% in Ghana (Abruquah,

2012). The reason for amphotericin-higher *Candida* sensitivity compared to others is that it is not commonly prescribed or widely used due to its high cost, difficulty in administration, and lack of availability.

6. CONCLUSION

Candida is one of the most common opportunistic fungi in humans. *Candida* species cause a wide range of diseases. However, due to a lack of data, much about the diversity and distribution of *Candida* species in Ethiopia has not been documented. In Ethiopia, data on fungal disease diversity, distribution, and drug resistance are scarce.To that end, discovering the diversity and distribution of *Candida* species profiles of yeast implicated in candidiasis and their drug susceptibility pattern is a top priority in Ethiopia in general, and in the Southwest Region at Tepi Hospital in particular.

Of the total of 120 collected samples for identification of diversity and distribution of *Candida* species, 92 (76.7%) were positive for *Candida* infection. In terms of sex, of the total positive patients the prevalence of candidiasis was (86.5%) of incidence rate in females participants. The results revealed that *Candida* infection was reported in all age groups in Tepi General Hospital. However, the rate of infection was high (81.5%) in the age groups of 21-30 years old, followed by 31- 40 years old (75%).The prevalence of candidiasis also higher in illiterates (88.1%) than literates (70.5%) ($p = 0.0299$). The most common risk factors for these patients was HIV/AIDS which accounts (90%), followed by pregnancy (86.7%), diabetic mellitus (83.3%), prolonged antibiotic therapy (73.3%), Tuberculosis (66.7%), Trauma/wound (40%) of patients with *Candida* colonization. A total of 92 candida were isolated from 120 different clinical samples. Clinical samples were isolated for the present study including 15 (12.5%), 20 (16.7%), 15 (12.5%), 20 (16.7%), 20 (16.7%), 15 (12.5%) and 15 (12.5%) were from Surgical wound swab, Oropharengial swab, Blood, Urine, Stool, Sputum and Vaginal swabs, respectively.

A comparison of the occurrence of candida species was higher (86.7%), (85%), (80%) and (80%) in vaginal swabs, urine specimens, Oropharengial swab and stool, respectively. The lowest incidence rate were observed in surgical wound swabs (60%) and sputum (66.7%).

A total of 5 species of *Candida* were identified based on the *Candida* species colony color characteristics on Sabouraud Dextrose Agar, BiGGY agar test and matrix assisted laser desorption /ionization time of flight(MALDI-TOF MS).

The results of analysis of one way ANOVA test for *Candida* species distribution and occurrence among clinical isolates shows highly significant differences ($p<0.01$).

Among 5 *Candida* species identified, the predominant *Candida* species was *Candida albicans* (55.4%), which was followed by *Candida krusei* (17.4%) and *Candida tropicalis* (14.1%), *Candida parapsilosis* (8.7%) and *Candida glabrata* (4.3%), were the commonest isolates among non-albican *Candidia* species. *Candida albicans* the main isolated in vaginal swabs isolate, oropharengeal swabs and urine while *Candida krusie* were from stool swabs and urine specimens.

The dominant species in females were *Candida krusie* and *Candida albicans* which accounting (62.5%) and (58.9%), respectively. Whereas, in males, *Candida tropicalis* and *Candida glabrata* and *parapsilosis* were the dominant species with (77%) (75%) and (87.5%), respectively.

Candida albicans was more frequently (68.2%) in the age group of (21-30) which was closely followed by the age group below 20 years old incidence rate of (66.7%). The frequency of cases caused by azole-resistant species such as *Candida krusie* and *Candida tropicalis* were decreases with age decreases. *Candida krusie* and *Candida tropicalis* were higher in age group of above 41 years old, accounting (33.3%) and (16.7%), respectively.

One way ANOVA test shows that drug resistant patterns of *Candida* to different antifungal agents showed highly significant differences ($p<0.01$). Of the total of 92 *Candida* isolates, (71.2%), (7.3%) and (21.5%) *Candida* isolates were susceptible, susceptible dose dependent and resistant to the drug, respectively. High resistant rate was observed for Fluconazole 26/92 (28.3%) and Clotrimazole 22/92 (23.9%). Whereas, 76/92 (82.6%) and 65/92 (70.7%) *Candida* isolates were more susceptible to Amphotericin-B and Clotrimazole, respectively. According to the data in table 7, it was revealed that Amphotericin-B was the most effective antifungal drugs. Whereas, Fluconazole and Ketoconazole were the poorest activity against all species of *Candida* to inhibit the growth of *Candida* isolated in this study.

Drug resistance profile of *Candida* species against fluconazole was (28.3%). The susceptibility of *Candida* species to fluconazole, however, varied from one species to another. (78.4%) of *Candida albicans* was susceptible to the fluconazole. The results of present study revealed that the four antifungal drugs which were tested for susceptibility, Amphotericin-B was the most effective drug. The results showed that most 82.6% (76/92) of the *Candida* isolates were found to be susceptible to Amphotericin-B.

7. RECOMMENDATIONS

Based on the finding of present study the following recommendations are suggested:

- *Candida* species distribution is changing, and antifungal drug resistance is increasing. It is recommended that a national surveillance be conducted to study the epidemiology and susceptibility pattern of *Candida* species isolates to antifungal drugs.
- The emergence of non *albicans Candida* other than *Candida albicans* has increased. Importantly, knowledge of the pathogenic yeast species is a useful guide to the likely pattern of susceptibility and successful patient treatment.
- Epidemiological studies are needed to determine the exact incidence and prevalence of these infections in a country and how they change over time.
- Simple and accessible technologies for identifying and examining yeast susceptibility patterns are recommended to be available in Ethiopia.

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9. APPENDIX

RESEARCH QUESTIONNAIRES

Dear Respondents: This questionnaires is assigned and prepared by the students of Jimma University doing a research for partial fulfillments of requirements for the Degree of Master of Biology. With this questionnaires the researcher intended to **Investigate Diversity, Distribution and Drug Resistant Patterns of Candida Species among Patients Admitted at Tepi General Hospital, Southwestern Ethiopia.** Please answer the questions in from the given alternative box. (Mark ✓ in the box). Thank you for your cooperation, are would like to inform you that all your responses will only be used for academic purpose and be kept confidentially. I appreciate your kind cooperation in completing this questionnaires.

SECTION I: Socio-Demographics Characteristics of Patients

Date of Enumeration: _____

1. Name patient (CODE) …….....

2. Name of village ……

3. Residence

Rural ☐ Urban ☐

4. Gender

Female ☐ Male ☐

5. How old are you?

0-20 ☐ 21-30 ☐ 31-40 ☐ above 41 years ☐

6. Marital status

Single ☐ Married ☐ Divorced ☐ Widowed ☐

7. What is your high level of education achieved?

Illiterate ☐ Basic education ☐ Primary ☐ Secondary ☐

SECTION II: Risk factors of Patients Regarding Candidiasis Infection

1. Your symphonic conditions?

Symptomatic ☐ Asymptomatic ☐

2. Have you taken "broad spectrum" antibiotics or other antibiotics for one month or longer?

Yes ☐ No ☐

3. Are you pregnant? (For female respondents only)

Yes ☐ No ☐

4. Do you know about *Candidia* infection?

Yes ☐ No ☐

5. Have you had athlete's foot, ring worm, "jock itch" or other chronic fungus infections of the skin or nails?

Yes ☐ No ☐

6. Have you taken birth control pills or other contraception?

Yes ☐ No ☐

7. What are the most common signs and symptoms of candidiasis infection? (Mark ✓ in the box)

Itching	☐
Irritation Burning sensation	☐
Vaginal discharge	☐
Skin redness (rash)/ Blisters	☐
Lumpy white patches	☐
Other (specify)	☐
_____	
_____	

8. Risk factors (your current health status) which predisposing to Candida infection? (Mark ✓ in the box)

HIV/AIDS	☐
Diabetic mellitus	☐
Under Antibiotics therapy	☐
Recent Surgery	☐
Intra-abdominal infection	☐
Tuberculosis	☐
____	

Other (specify) ☐

ANOVA FOR CANDIDASPECIES DISTRIBUTION

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	201.8857	4	50.47143	27.89211	1E-09	2.689628
Within Groups	54.28571	30	1.809524			
Total	256.1714	34				

ANNEX III: Amharic Version Assent form for above 18 Years Participants

የተሳታፊ ስምምነት ቅጽ

ይህ ገጽ “Diversity, Distribution and Drug Resistant Patterns of Candida Species Among Patients Admitted at Tepi General Hospital, Southwest Ethiopia” ማለትም “የፈንገሶች ዝርያ አይነት፣ ስርጭትና የጸረ-ፈንገስ መድሃኒቶችን መቋቋም አቅም በቴፒ አጠቃላይ ሆስፒታል ከሚታከሙ ታካሚዎች” በሚል ርዕስ የተሳታፊ ስምምነት ቅጽ ነው፡፡ በመሆኑም እባክዎን ከዝህ በታች የተዘረዘሩትን ነጥቦች ይረዱና፤ ለመሳተፍ ፈቃደኛ ሆነው ከተስማሙ መስማማትዎን የሚያሳይ ፊርማዎን ከታች በተሰጠው ቦታ ላይ እንዲፈርሙ እጠይቃለሁ፡፡

1. “የፈንገሶች ዝርያ አይነት፣ ስርጭትና ከጸረ-ፈንገስ መድሃኒቶች ጋር ያላቸዉ መስተጋብር በቴፒ አጠቃላይ ሆስፒታል ከሚታከሙ ታካሚዎች” የሚለዉን ጥናት አላማ በደንብ ተገንዝቤያለሁ፡፡
2. ከእኔ የሚወሰደው ናሙና ለጥናቱ አላማ ብቻ እንደሚውል ተረድቻለሁ፡፡
3. ሁሉም መረጃዎች እና የናሙና ውጤቱ ምስጢራዊ መሆኑን ተገንዝቤአለሁ፡፡
4. በጥናቱ ላይ በመሳተፍ ምንም የገንዘብ ክፍያ እንደማላገኝ ተረድቻለሁ
5. በጥናቱ ያለመሳተፍ እንዲሁም በማንኛዉም ጊዜ የማቋረጥ መብት እንዳለኝ አውቄአለሁ፡፡
6. ሁሉም መረጃዎች በአስተባባሪዉ/ዎች ተገልጾልኝ በደንብ ተረድቻለሁ።

የተሳታፊ ፊርማ፡----------

የተሳታፊ አድራሻ፡----------

ቀን፡----------

በስምምነቱ ወቅት የነበሩ ምስክሮች

1._____

2._____

ይህንጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋር በተዛመደ መልኩ ስለሚያጋጥመዎት ድንገተኛ ችግር በሚከተለው አድራሻ ይጠቀሙ፡፡

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የባዮሎጂ ትምህርት ክፍል፣ ጅማ ዩኒቨርሲቲ

Annex V: Amharic Version Assent form for Under 18 Years Participants

ይህ ገጽ “Diversity, Distribution and Drug Resistant Patterns of Candida Species Among Patients Admitted at Tepi General Hospital, Southwest Ethiopia” ማለትም “የፈንገሶች ዝርያ አይነት፣ ስርጭትና የጸረ ፈንገስ መድሃኒቶችን የመቋቋም አቅም በቴፒ አጠቃላይ ሆስፒታል ከሚታከሙ ታካሚዎች” በሚል ርዕስ ለሚካሄደው ጥናት ናሙና ለመውሰድ ነው። ከቤተሰብ ወይም ሌላ አዋቂ ሰው እድሜያቸው ከ18 ዓመት በታች ለሆናችሁ ተሳታፊዎች ስምምነት የመጠየቂያ ገጽ ነው::

ለዚህ ጥናት ናሙና እንወስዳለን:: ናሙና የምንወስድበት መሳሪያ ንጽህናው ሙሉ በሙሉ አስተማማኝና ከዚህ በፊት ጥቅም ላይ ያልዋለ ነው:: ናሙና በምንወስድበት ጊዜ የሚደርስ ህመም የሚፈጥር ስሜትም ሆነ አደጋ የሚያስከትል ሂደት የለውም:: ለጥናት የሚወሰደው ናሙና ለጥናት አላማ ብቻ ይውላል።የናሙናው ውጤት ምስጥራዊነት የተጠበቀ ሲሆን በናሙናው ውስጥ የበሽታ አምጭ ተህዋስ ቢገኝ ከጤና ባለሙያው አስፈላጊውን ህክምና ያገኛሉ:: በጥናቱ ላይ በመሳተፍዎ ምንም የገንዘብ ክፍያ አያገኙም። በጥናቱ ለመሳተፍ የመፍቀድም ሆነ ያለመፍቀድ እንዲሁም በማንኛዉም ጊዜ የማቋረጥ መብት አለዎት::

(ስም) በጥናቱ እንዲሳተፍ/እንድትሳተፍ ይፈቅዳሉ? ፈቃደኛ ከሆኑ፡-

የተሳታፊ ፊርማ፣----------

የፈቀደው ግለሰብ ፊርማ፣---------- አድራሻ፣---------- ቀን፣----------

በስምምነቱ ወቅት የነበሩ ምስክሮች

1._____

2._____

ይህን ጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎት ድንገተኛ ችግር በሚከተለው አድራሻ ይጠቀሙ::

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የባዮሎጂ ትምህርት ክፍል፣ ጅማ ዩኒቨርሲቲ

Annex VII Amharic version participant's information sheet

እኔ *መሃመድ* ሰይድ እባላለሁ፡፡ በጅማ ዩኒቨርሲቲ የባዮሎጂ ትምህርት ክፍል የሁለተኛ ድግሪ ተማሪ ስሆን የምርምር ስራየን በመስራት ላይ እገኛለሁ፡፡ እርስዎም በዚህ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል፡፡ በጥናት ለመሳተፍ ፈቃደኛ ሆነዉ ከተስማሙ መስማማትዎን የሚያሳይ ዶክመንት ላይ እንዲፈርሙ እጠይቃለሁ፡፡

መግቢያ

የጥናቱ ርዕስ "የፈንገሶች ዝርያ አይነት፣ ስርጭትና የጸረ ፈንገስ መድሃኒቶችን የመቋቋም አቅም በቱፒ አጠቃላይ ሆስፒታል ከሚታከሙ ታካሚዎች" በሚል ርዕስ የሚካሄድ ጥናት ነው። ይህ ጥናትም በተሳታፊ ሙሉ ፍቃደኝነት ላይ ተመስርቶ የፈንገሶች ዝርያ ስርጭትና ከጸረ-ፈንገስ መድሃኒቶች ጋር ያላቸዉን ተላምዶ ለማወቅ እና አማራጭ መንገዶችን ለመጠቆም ያስችላል፡፡

ከጥናቱ ተሳታፊ የሚጠበቁ

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ የጤና ባለሙያ ከእርሰዎ የሚፈለገውን ናሙና ይሰበስባሉ፡፡ ከተወሰደዉም ናሙና ላይ የሚገኙ መረጃዎች የእርሶን ማንነት የማይገልጹ ማስረጃዎን ማለትም ስም፣ አድራሻና የመሳሰሉት መረጃዎች ሳይጨምርና ለዚህ ጥናት አገልግሎት ብቻ የሚዉል መለያ ቁጥር በመጠቀም ከዚህ ሆስፒታል ዉጭ ለሚገኙ ለሥራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅቦታል፡፡ ናሙና ሰጡ ማለት በሽታው ይገኝብዎታል ማለት አይደለም፡ ፡ በእርሰዎ ናሙና ውስጥ የበሽታ አምጭ ተህዋስ ቢገኝ ከጤና ባለሙያዉ አስፈላጊውን ህክምና ያገኛሉ፡፡

ተሳታፊዉ የሚያጠፋዉ ጊዜ

የተዘጋጀዉን የስምምነት ቅጽ ለመፈረምና ናሙና ለመስጠት 2-5 ደቂቃ ያስፈልጋል፡፡

በጥናቱ በመሳተፍ የሚያስከትለቸዉ ችግሮች

ናሙና የሚወሰደው ፍጹም ንጹህ በሆነ ዕቃና በሰለጠነ ባለሙያ ስለሆነ በሚሰበሰብበት ወቅት ምነም አይነት ችግር አያስከትልብዎትም፡፡

የመረጃዉ ሚስጥራዊነት

ማንኛዉም የሰጡት መረጃ እና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፡፡ ይህን ማህደር ሊያገኙ የሚችሉ የተወሰኑ የጥናቱ ተባባሪ ሠራተኞች ብቻ ናቸዉ፡፡ ከዚህም በላይ ስለ እርስዎ ያለዉን ማንኛዉንም መረጃ የይለፍ-ቃል ባለዉ የኮምፒዉተር የመረጃ ማህደር ዉስጥ እንዲቀመጥ ይደረጋል፡፡

በጥናቱ በመሳተፍ የሚያስከትላቸዉ ጥቅሞች

በዚህ ጥናት በመሳተፍዎ የሚያገኙት የገንዘብ ጥቅማጥቅም የለም፡፡ ለወደፊት በተመሳሳይ ሁኔታ ዉስጥ ሌላ በሽተኞች በመረጃ ላይ የተመሰረተ ህክምና ለመስጠት ያግዛል፡፡ የላቦራቶሪ ዉጤቶችን በነፃ ያገኛሉ፤ እንዲሁም ስለ አስፈላጊዉ ህክምና ከሀኪምዎ ጋር ይነጋገራሉ፡፡

የጥናቱ ተሳታፊዎች መብት

ትብብርዎ ሙሉ በሙሉ በፍቃደኝነት ላይ የተመሠረተና ተሳትፎዎን መተውና በማንኛውም ሰዓት ጥናቱን ማቆም ይችላሉ፡፡ በጥናቱ ዉስጥ ያለዎትን ተሳትፎ በማንኛዉም ጊዜ የማቋረጥ ሙሉ መብትዎ የተጠበቀ ከመሆኑም በላይ ራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም ዓይነት የሆስፒታል አገልግሎት አይኖርም፡፡ ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛዉንም ዓይነት ጥያቄ የመጠየቅና ገለፃ የማግኘት መብት አለዎት፡፡ የላቦራቶሪ ምርመራ ዉጤቱንም በነፃ ማግኘት ይቻላሉ።

ግንኙነትና ጥያቄ

ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎ ድንገተኛ ችግር ወይም ጥያቄ ካለዎት በሚከተለዉን አድራሻ ይጠቀሙ፡፡

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ከዚህ በታች የሚገኘው ፊርማዎ ለእርስዎ የተሰጠውን መረጃ ማንበብዎን፣ መስማትዎን እና መገንዘብዎን የሚያሳይ ነው፡፡ ከመፈረምዎ በፊት እባክዎትን የጥናቱን ዓላማ፣ የተሳትፎ ጉዳትና ጥቅሙን፣ የመተው፣ የማቋረጥ፣ መብትና ነፃነት እንዳለዎት ይረዱ፡፡

ተስማምተዋል? የጥናቱን መግለጫ አንብቤያለሁ/ ሰምቻለሁ እናም ተረድቻለሁ፡፡

መመሪያው ምን እንደሆነና በእኔ ምን ሊከሰት እንደሚችል ተረድቻለሁ፡፡

በጥናቱ ላይ ለመሳተፍ

እስማማለሁ ----------

አልስማማም ----------