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Species Distribution and Antifungal Susceptibility Profile of Yeasts Recovered from Different Clinical Samples at Arsho laboratory, Addis Ababa, Ethiopia.

By: Elias Seyoum

Advisor: Adane Bitew (MSc, PhD, Associate Professor)

Amete mihret (MSC, PHD Candidate)

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Name of the principal Investigator	By: Elias Seyoum Department of Medical Laboratory Sciences School of Allied Health Science, College of Health Sciences, Addis Ababa University
Advisors	Adane Bitew (MSc,PHD,Associate professor) Amete mihret (MSc,PHD candidate) Department of Medical Laboratory Sciences School of Allied Health Science, College of Health Sciences , Addis Ababa University Tel: +251 911039162
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Address of the PI	Email:eliasseyoum2@gmail.com Tel: 251912969651

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Operational Definition

Cross-resistance: Cross- resistance was defined as resistance to two or more antifungals of the same drug class.

Multi drug-resistance (MDR): Multi drug-resistance was defined as resistance to two or more antifungal different drug classes

Abstract

Background: Currently, fungal diseases of humans are among the most problematic illnesses is the result of an increase in opportunistic fungal infections caused by rise in the number of immune-compromised patients. Particularly**,** yeasts are the most common cause of fungal infections leading to a range superficial type to life-threatening invasive infections. The aim of this study is to determine species distribution and antifungal susceptibility profile of yeasts particularly of Candida species recovered from different clinical samples.

Method: A cross sectional study was conducted from January 01, 2018 –September 30, 2018 in Addis Ababa, Arsho laboratory. Oropharengial swabs ,nail scrapings, sputum, corneal scrapings, and vaginal swabs were collected from patients with signs and symptoms of infections. Identification and antifungal susceptibility testing was performed by using YST and AST-YS07 card through VITEK 2 compact system. Data was entered and analyzed using SPSS version 20.

Result: A total of 209 yeasts were recovered of which 49.8% were *Candida albicans*, 43.1% were non-*albicans candida* species and 7.2% were yeasts other than *Candida* species. The current study, eighteen (18) different types of yeast species were identified from different clinical samples. All recognized yeast considered as a causative agent of fungal infection in previously reported data in worldwide. Entire isolates of 85.6 %, 99.4 %, 98.8 %, 98.8 %, 86.2 % were susceptible to fluconazole,Voriconazole,micafungin,Caspofungin , and flucytosine, respectively. *Candida albicans* isolates were 100% susceptible to voriconazole, caspofungin, and micafungin. As of whole isolates were showed 1.6 % cross-resistance and 8.6 % multi drug-resistance.

Conclusion: *C. albicans* remaining the predominant species, but the emergence of non-*albicans Candida* and other than *candida* species have increased .In vitro-resistance to fluconazole, voriconazole and flucytosine was rare among C. *albicans*.However, an increase of non-susceptibility was observed among C. *krusie* and *C.rugosa* for fluconazole and flucytosine. Thus, more studies on *Candida* prevalence and drug susceptibility are needed throughout country.

Key words:*Candida albicans*,Non- *albican candida* species,Candidasis,Antifungal susceptibility

1. Introduction

1.1. Back ground

Currently, fungal diseases of humans are among the most problematic illnesses to manage. Some fungi cause disease in immune-competent persons (fungal true pathogens), but most fungal infections occur in immune-compromised individuals (opportunistic fungal pathogens). Since risk-factors for opportunistic fungal infections continue to increase in frequency, it is likely that the incidence of opportunistic fungal infections will continue to increase in the future. An increase in opportunistic fungal infections are the result of an increase in the number of immune-compromised patients, thereby threaten the achievement of the newest medical advances in cancer care, solid organ and hematopoietic stem cell transplantation, neonatal medicine, autoimmune disease therapies, trauma and intensive care, and sophisticated surgery [1-4].

Fungi can cause infections ranging from easily treatable superficial type to life-threatening invasive infections and it has the capability to infect humans of all age groups [41]. Particularly**,** yeasts are the most common cause of fungal infections, leading to a range of life-threatening invasive diseases such as blood stream candidiasis, pneumonia, and cryptococcal meningitis to non-life-threatening mucocutaneous candidiasis such as genitourinary candidiasis, vulvovaginal candidiasis, and oropharyngeal candidiasis [5]. They are also important cause of superficial mycosis such as onychomycosis [76]. Among fungal infections, blood stream candida infection (candidemia) and cryptococcosis are commonly associated with high morbidity and mortality rate. For example, *Candida* species are among the top ten pathogens causing bloodstream infections resulting in significant increase in the length of patients' hospitalization and in healthcare costs [5].

Currently, there are more than 150 known species of *Candida* [3], and more than 17 different *Candida* spp. are known as etiological agents of human infection [43].Among candida, *Candida albicans* is the most common infectious agent. However, there has been an important shift to non-*albicans Candida* species, particularly those more resistant to antifungal drugs [7, 8]. Although studies demonstrated that antifungal resistance is relatively rare [5,10], antifungal drugs have been used intensively either to control such infections or as prophylactic in long-term treatments, creating serious concerns that might select for drug resistances, thus greatly affecting infection control [10, 11].

Yeasts have various degrees of susceptibility to antifungal drugs. Development of drug resistance to antifungal drugs and frequent isolation of emerging yeasts (i.e., non-*albicans Candida* species) in different clinical samples initiated accurate species identifications and *In vitro* susceptibility testing of medical important yeasts [11]. Antifungal susceptibility profile of yeasts in Ethiopia is poorly known as cultures are rarely performed. As a result of a limited data regarding the antifungal susceptibility profile of yeasts, fungal infections are treated empirically. To this end, early species identification and rapid antifungal susceptibility testing are needed. Although a number of antifungal susceptibility testing systems have been developed and are commercially available, their performance is variable [12]. The VITEK 2compact system (bioMérieux) is a fully automated system that is accurate in the identification of yeasts and evaluating their drug susceptibility [13].The aim of this study is to determine distribution and antifungal susceptibility profile of yeasts particularly of Candida species recovered from different clinical samples collected from patients referred to Arsho Advanced Medical Laboratory for culture and sensitivity testing by using the VITEK 2 compact system.

1.2 .Statement of the Problem

Fungal diseases kill more than 1.5 million, which is more than malaria and similar to the tuberculosis death toll. Nearly a billion people are estimated to have skin, nail and hair fungal infections and many 10's of millions mucosal candidiasis, which have a major impact on their lives. Few realize that over 300 million people suffer from serious fungal-related diseases [44, 45]. However, they are still a neglected topic by public health authorities even though most deaths from fungal diseases are avoidable. Serious fungal infections occur as a consequence of other health problems including asthma, AIDS, cancer, organ transplantation and corticosteroid therapies. Early accurate diagnosis allows prompt antifungal therapy; however this is often delayed or unavailable leading to death, serious chronic illness or blindness [45].

Although firm incidence data are limited due to diagnostic difficulties, invasive candida Infection (ICI) is the third most common cause of infection in Intensive care unit (ICUs) worldwide, accounting for 17% of Infections[46].Different incidence rates and the global emergence of new fungal pathogens with inherent resistance, such as *Candida auris*, have been revealed during the last 20 years. The European data estimate an incidence of Candida blood stream infection (BSI) ranging between 6.7 and 54 per 1,000 ICU admissions, with a mortality of 33.9–61.8 % [47]. While an estimated 46,000 healthcare-associated *Candida* infections occur among hospitalized patients in the United States each year, roughly 30% of patients with blood stream infections (candidemia) with drug-resistant *Candida* die during their hospitalization.

Invasive infection due to *Candida* species is largely a condition associated with medical progress, and is widely recognized as a major cause of morbidity and mortality in the healthcare environment [42]. Particularly in recent years, the infection *Candida albicans* as well as non-*albicans* infection worldwide has risen[48].This thought to be driven largely by the increasing use of prophylactic antifungal agents such as fluconazole. Previously, invasive candidiasis was caused predominantly by *Candida albicans*. As a result of the shift toward non-*albicans Candida* species with various susceptibility patterns, including multidrug-resistant species, fluconazole can no longer be the main stay of empirical antifungal treatment [49].

The global incidence of *Cryptococcus neoformans* varies from 0.04 to 12% and accounts for 13–40% of the annual death rate among HIV-infected patients in areas of sub-Saharan Africa [51]. Moreover, high mortality associated with disseminated fungal infections is of particular concern,

given the limited treatment options and the high adaptive capability of fungal pathogens to stress conditions associated with colonization of different host niches and drug exposure [53].

Therefore, changing epidemiology, increasing resistance rates and a narrow antifungal accessibility may further underline the required attention on resistance fungal infections caused by *Candida* and other yeasts [54]. Whereas, in Ethiopia limited studies of mild fungal or life-threatening invasive fungal infections and lack of surveillance data combined with the unavailability of any diagnostic test for fungi, other than microscopy, reinforces a sense that these infections are rare or non-existent. **In** order that, to find out the profile of yeast species implicated in causing candidiasis and their drug susceptibility pattern is one of the highest priority and timely.

1.3. Significance of the Study

Results obtained highlight the need for national surveillance for examining *Candida* epidemiology and resistance to antifungal drugs and provided current information on local fungal pathogens and their sensitivity patterns as a prime tool in combating such problems. Data can be used to selection of appropriate antifungal treatment for empiric treatment and the study has generated information the different species of yeast causing infection provides supplementary information to plan for infection control and surveillance. Hence, policy makers can use this information for the recommendation and implementation of fungal infection control in hospital acquired infection as well as in the community to prevent treatment failure, to control nosocomial infection in Ethiopian. This information also can be used to create awareness or involvement of practicing clinicians for Ignorance of fungal infections and the government's health capabilities in Ethiopia have consider for any fungal disease and diagnostic test for fungi may support to develop different strategies to deal with this emerging disease.

2. Literature Review.

2.1. Species distribution of yeast

At present , there are over 150 *Candida* species in nature and more than 17 different *Candida* species are known as etiological agents of human infection [3,43] , but greater than ninety percent of invasive disease is caused by the five most common pathogens, *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis,* and *C. krusei*. Each of these organisms has unique virulence potential, antifungal susceptibility, and epidemiology, but taken as a whole, significant infections due to these organisms are generally referred to as invasive candidasis [42].In the last 20 years; a change has been observed in the rates of *Candida* species isolated from patients with candidiasis. The incidence of *Candida albicans* has decreased, while that of the non-*albicans Candida* has increased. This may be new antifungal agents and new therapy strategies such as antifungal prophylaxis, secondary prophylaxis, and preventative therapy have come into use [3].

Oropharyngeal and esophageal candidiasis occur in association with HIV infection and their occurrence is recognized as an indicator of immune dysfunction. In HIV-infected patients, oropharyngeal candidiasis is most often observed in patients with CD4 counts <200 cells/μL [32]. In Brazil in 2010, oral isolates from HIV positive patients and control individuals has been studied. A total of 71 *Candida* isolates from HIV-positive patients were examined with *C. albicans*(59), *C. tropicalis*(9), *C. glabrata*(1), *C. guilliermondii*(1) and *C. krusei*(1). A total of 15 *Candida* isolates were evaluated from control individuals comprised of eleven *C. albicans* and four *C. tropicalis* isolates [20]. Oropharyngeal candidiasis had a high prevalence in hospitalized AIDS patients (83%), and the most prevalent species was *Candida albicans* (56%) [55].

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%) [21]. Likely, *Candida albicans* is by far the most prevalent etiological agent, particularly for the most severe chronic condition known as recurrent vulvovaginal candidiasis[56].

In India one Study published in 2012 illustrated three study groups which are HIV negative ,HIV positive but not start Highly Active Anti-Retroviral Therapy (HAART) and HIV positive started HAART were reported overall culture positivity was 35.33%. *C. albicans* was the most prevalent species (16.67%). This was followed by *C. tropicalis* (8.00%), *C. glabrata* (4.67%), *C. parapsilosis* (1.33%), *C. kefyr* (0.67%), *C. guillermondi* (0.67%), *C. stellatoidea* (0.67%). [33]. In another setting, Candida isolates from patients in the China invasive Candida infection (ICI) in intensive care units (ICUs) were evaluated. Species identified most frequently were *Candida albicans* (40.1%), *Candida parapsilosis* (21.3%), *Candida tropicalis* (17.2%) and *Candida glabrata* (12.9%). Rare species such as *Lodderomyces elongisporus* and *Candida ernobii* were also identified [34]. Another study also in china a three year surveillance result showed of 1072 isolates, 392 (36.6%) were *C. parapsilosis* species complex. *C. tropicalis, C. glabrata* species complex and *C.krusei* comprised 35.4%, 24.3% and 3.7%of the isolates, respectively [35]. Among 760 patients presented with mucocutaneous candidiasis, 307 (40.4%) were infected with N-CA. The majority of N-CA cases were isolated from patients' nails (n = 293, 95.4%) while eight (2.6%) were detected from their skin, and six (2%) from oral mucosa [36].

A study on volvovaginal candidasis conduct by Ali *.et al*. 2016 revealed the cultures were positive for 34(28.3%) samples of vaginal and three Candida species including; *C. albicans* (88.2%), *C. glabrata* (8.8%) and *C. kefyr* (2.9%) [37]. Another study, done in tertiary care hospitals of Peshawar, published in 2018 demonstrated out of 108 *Candida* species isolated from vaginal swabs; there were 45 (41.7%) *Candida albicans,* 18 (16.7%) *Candida tropicalis,* 18 (16.7%) *Candida krusei,* 16 (14.8%) *C. glabrata* and 11 (10.2%) *Candida dubliniensis* [57].

Hamza *et al* .studied species distribution and *in vitro* antifungal susceptibility of oral yeast isolates from Tanzanian HIV-infected patients with primary and recurrent or pharyngeal candidiasis. The result of this study depicted that *Candida albicans* was the most frequently isolated species from 250 (84.5%) patients followed by *C. glabrata* from 20 (6.8%) patients, and *C. krusei* from 10 (3.4%) patients. There was no observed significant difference in species distribution between patients with primary and recurrent oropharyngeal candidiasis[50].As well,

a study with an aim of to determine the prevalence of oral *candida albicans* infection in HIV Sero-positive patients were conduct in Abakaliki, Ghana in 2013. A total of 240 samples were collected from HIV sero-positive males (64) and females (176) in two hospitals. The study demonstrated that the carriage rate of oral candidiasis was 12.5 %(30/240). Among the isolates, *Candida albicans* accounted for 80.00% in HIV sero-postive patients, followed by *Candida pseudotropicalis*(10.0%). More women, 21(8.75) had oral candidiasis than men 9(3.75%). HIV patients were equally affecting by the yeasts regardless of HIV drug therapy. *C.albicans* (76.19%) is the commonest species associated with HIV infected patients on ART (Active Retroviral Therapy) followed by *Candida pseudotropicalis* (14.29%), *Candida tropicalis* (4.76%) and *Candida parapsilosis* (4.76%). Among the patients not on ART *Candida albicans* (88.89%) was most prevalent, followed by *Candida guilliermondii* (11.11%) indicating C. *albicans* still remains the leading cause of oropharyngeal candidiasis in HIV infected persons within the study population [61]. HIV sero-positive people whether or not on ART are predisposed to oral candidiasis. This does not agree with most studies. However, some HIV positive patients with relatively high CD4+ cell counts may develop oral candidiasis.

Study done in Uganda between December 2012 and February 2013 of the 456 high vaginal swabs cultured species distribution illustrated *C. albicans* (78.95%), *C. glabrata* (14.35%), *C. krusei* (3.35%), *C. tropicalis* (1.44%), *C. famata* (0.96%), *C. parapsilosis* (0.48%) and *C. lusitaniae* (0.48%) [58].As well, the HIV epidemic in Uganda has highlighted *Cryptococcus* and *Candida* infections as important opportunistic fungal infections. There are an estimated 4000 *cryptococcal* cases annually in Uganda, Especially, HIV patients developing Cryptococcus infection with total HIV-related *cryptococcal* mortality is approximated at 2412 cases per year [59].Infections caused by emerging *Cryptococcus* non-*neoformans* species are being reported with increasingly frequency [60].

Frequent detection of 'azole' resistant *Candida* species among late presenting AIDS patients in northwest Ethiopia was conducted by Mulu *et al*. The following results were reported from this study. The colonization rate of *Candida* species was found to be 82.3% (177/215). *C. albicans* was the predominant species isolated from 139 (81%) patients but there was a diversity of other species. *C. glabrata* was the most frequent non-*albicans* species isolated in 22.5% (40/177) of the patients followed by *C. tropicalis*14.1% (27/ 177), *C. krusei* 5.6% (10) and other unidentifiable *Candida* species 4% (7/177) [2]. Recurrent episodes of oropharyngeal candidiasis

and previous exposure to antifungal drugs were found to be predisposing factors for colonization by non-albicans species [67]. In Ethiopia study published in 2018 explained of 87 Candida isolates recovered from vaginal swabs, 58.6% were *C. albicans* while 41.4% were non-*albicans Candida* species [16].

2.2. In vitro antifungal susceptibility of yeast species

In *Candida*, susceptibility to azoles varies by species, but [62] Echinocandins are the preferred therapy for invasive candida infections due to *Candida krusei* that is intrinsic resistance to fluconazole [25, 64].

In vitro susceptibility of oral Candida to seven antifungal agents was performed by Kuriyama *et al*.in 2005.Antifungal susceptibility was assessed using a broth microdilution method following the National Committee for Clinical Laboratory Standards (NCCLS) M27-A guidelines. The majority of the test strains were *C. albicans* (n ¼ 521) with few of these being resistant to fluconazole (0.3%). A low incidence of fluconazole resistance (0–6.8%) was similarly evident with all non albicans species (*Candida glabrata*, 5 of 59 resistant; *Candida krusei*, 0 of 7resistant; *Candida tropicalis*, 0 of 13 resistant; *Candida parapsilosis*, 0 of 12 resistant; other *Candida* species, 0 of 6 resistant). voriconazole, ketoconazole, and miconazole also revealed high activity against both *C. albicans* and non albicans isolates, and 23.7% of *C. glabrata* isolates were found to be resistant to itraconazole. There was little difference in the antifungal susceptibilities of *Candid*a isolated from patients who had a history of previous antifungal therapy compared with those who had not received antifungal treatment. This surveillance study of antifungal susceptibility of oral *candida* isolates in the UK, through the collaboration of four dental hospitals, demonstrates that oral *Candida* species have a high level of susceptibilities to a range of antifungal agents [52].

In vitro antifungal susceptibility of *Candida* spp. oral isolates from HIV positive patients and control individuals has been studied in Brazil in 2010. Results demonstrated that the tested antifungal agents showed good activity for most isolates from both groups; however, variability in MIC values among isolates was observed [39].

Antifungal Susceptibility of *Candida* species from mucosal sites in HIV positive patients in Iran by Badieeetalin 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. All species were sensitive to amphotencin B, ketoconazole, nystatin, voriconazole, and caspofungin. In some isolates, resistance touconazole and itraconazole was noted [21].*Candida* isolates from patients in the China invasive Candida infection (ICI) in intensive care units (ICUs) were evaluated. Fluconazole susceptibility was evident in 85.9% (134/156) of *C. albicans*, 62.7% (42/67) of *C. tropicalis* and 48.2% (40/83) of *C. parapsilosis* isolates. Susceptibility to voriconazole was ≥90% among all species. All isolates were susceptible to amphotericin B and caspofungin except *C. glabrata* [86.0% (43/50) susceptible to caspofungin. Cross-resistance between fluconazole and voriconazole was observed for *C. parapsilosis* and *C. glabrata* [34]. In china a three year surveillance result showed of 1072 isolates over 99.3% of the isolates were of Wild Type (WT) phenotype to amphotericin B and 5-flucytosine. Susceptibility/WT rates to azoles among *C. parapsilosis* species complex were ≥97.5%. However, 11.6% and 9.5% of C. tropicalis isolates were non-susceptible to fluconazole and voriconazole, respectively (7.1% were resistant to both). Approximately 14.3% of *C. glabrata* sensu stricto isolates (n¼258) were fluconazole resistant, and 11.6% of *C. glabrata* sensu stricto isolates were cross-resistant to fluconazole and voriconazole. All *C. krusei* isolates were susceptible/ WT to voriconazole, posaconazole and itraconazole. Overall, 97.7%–100% of isolates were susceptible to caspofungin, micafungin and anidulafungin, but 2.3% of *C. glabrata* were non-susceptible to anidulafungin. There was no azole/echinocandin co-resistance [35].

A retrospective study in Thaiwan demonstrated Of 709 *Candida* isolates which are found from candidmia patients. The fluconazole-susceptible rate was 96.5% in *Candida albicans,* 85.8% in *Candida tropicalis* and 92.1% in *Candida parapsilosis* by the revised CBPs. The susceptible rates to fluconazole were 96.5% (333/345) for *C. albicans,* 85.8% (127/148) for *C. tropicalis* and 92.1% (93/101) for *C. parapsilosis* isolates. More than 97% of the isolates of *C. albicans*, *C. parapsilosis* and *C. krusei* were susceptible to voriconazole. Among 21 fluconazole non-susceptible *C. tropicalis* isolates, 18 (85.7%) were also voriconazole non-susceptible. These results showed high susceptibility rates of all the three echinocandins tested against *C. parapsilosis*. In terms of susceptibility to 5-flucytosine, 5.5% (19/345) of *C. albicans,* 2% (3/148) of *C. tropicalis* and 1% (1/101) *C. parapsilosis* isolates were non-wild type to 5-flucytosine [36]. All the *Candida krusei* and *Candida famata* isolates and two of *Candida glabrata* were resistant to fluconazole [55].

A study on volvovagina candidasis conducted by Ali *.etal*, 2016 in Iran showed that only one isolate of C. *albicans* was resistant to caspofungin at the concentration of 2 µg/ml after 24h incubation that increased to 2 isolates after 48h incubation. All isolates were sensitive to fluconazole at the MIC ranges of 1-0.25 µg/ml, while 88.2% of them were inhibited at 0.25 µg/mL of clotrimazole. Candida albicans remains the most common agent of fungal vaginitis [37]. Another study in antenatal clinic in Uganda result demonstrated *C. krusei* showed a high resistance of 71.43% to fluconazole. *C. glabrata*, *C. krusei*, *C. famata* and *C. lusitaniae* exhibited 100% resistance to itraconazole [58].

Antifungal susceptibility pattern of clinical isolates of *Candida albicans* isolated from urine and vaginal swab were investigated by Doughari Peter in Nigeria in 2009. Among the isolates 3(37.5%) susceptible while the remaining 11(78.57%) were resistant fluconazole, ketoconazole and nystatin [38]. The same way in Burkinafaso study showed relatively high resistance to commonly and widely used azoles (fluconazole, ketoconazole) for In vitro *C. albicans* antifungal susceptibility test [48]. A cross sectional study was conducted from November 2015 to December 2016 at the Family Guidance Association of Ethiopia demonstrated all Candida isolates were 100% susceptible to voriconazole, caspofungin, and micafungin. *C. albicans*, was 100% susceptible to all drugs tested except fluconazole and flycytosine with a resistance rate of 2% each drug. *C. krusei*, was 100 and 33.3% resistant to fluconazole and flu cytosine, respectively [16].

In vitro antifungal susceptibility of *Candida albicans* isolates from oral cavities of patients infected with human immunodeficiency virus in Ethiopia was conducted by Wubie et al out of 42 isolates, forty one (97.7%) of all isolates were fully susceptible to amphotericin B, 40 (95.3%) to nystatin, and 39 (92.9%) to ketoconazole and miconazole. On the other hand, the isolates showed highest rates of resistance against fluconazole (11.9%). They also reported that there was little difference in the antifungal susceptibilities of *C.albicans* isolated from patients who had a history of previous antifungal therapy compared with those who had not received antifungal treatment. The study did not report other *candida* species [28]. Another study in northwest Ethiopia was conducted by Mulu *et al*. title of frequent detection of 'azole' resistant *Candida* species among late presenting AIDS patients result showed irrespective of the *Candida* species

identified 12.2% (11/90), 7.7% (7/90) and 4.7% (4) of the isolates were resistant to fluconazole, ketoconazole and itraconazole, respectively. In contrast, resistance to micafungin, amphotericin B and 5-Fluorocytosine was infrequent [2].

3. Objective

3.1. General objective

- To determine species distribution and antifungal susceptibility profile of yeasts recovered from different clinical samples**.**

3.2 .Specific objectives

- To determine species distribution of yeast species in different clinical samples
- To determine the antifungal susceptibility patterns of the isolates

4. Materials and Methods

4.1. Study area

The study was conducted at Arsho Medical laboratory, Addis Ababa, Ethiopia. Arsho is a brand of private diagnostic Laboratory practice in Ethiopia. It originated in 1972 in a small individual practice and grows up to internationally accredited big service and name. Their personnel specializing in a wide variety of disciplines including microbiology work to ensure best quality and quick delivery of medical result. Daily patient flow for microbiology diagnosis approximately ten patients per day

4.2 Study design and Period: A cross sectional study was conducted from January 01, 2018 –September 30, 2018.

4.3. Population

4.3.1. Source Population: The source population was all patients referred to bacteriological culture to the study site.

4.3.2. Study Population: Patients who have with sign and symptoms of infection of onychomycosis, vaginal, keratits, Oropharengeal and lung.

4.4. Inclusion and exclusion Criteria

4.4.1. Inclusion Criteria: All patients clinically suspected of onychomycosis, Vaginal, keratits, Oropharengeal and lung infection were included at the study site and in the specified period of study.

4.4.2. Exclusion Criteria: Persons who did not want to participate in the indicated study**.**

4.5. Study variables

4.5.1. Dependent variables

- ✓ Distribution of yeast species in different clinical samples
- ✓ Yeast species drug susceptibility profile.

4.5.2. Independent variables

- ✓ Age
- ✓ Sex

4.6. Sample size and Sampling method

4.6.1: Sample size: - To increase the finding of positive result all consecutive patients was recruited at the Arsho medical laboratory during the study period.

4.6.2. Sampling Method:-Non probability of purposive sampling method was used because study subject was recruited based on characteristics of infection.

4.7: Data Collection Procedures

4.7.1. Sample collection: All samples were collected according to standardized operating procedures (Annex IV). Clinical samples including oropharengial swab, nail scrapings, sputum, corneal scrapings, and vaginal swabs were collected from patients with signs and symptoms of infections and referred to the study site for culture and susceptibility testing .A portion of each clinical specimen was inoculated onto bacteriological culture media for routine activity and incubated at appropriate temperature and period according to standard protocols related to each sample. The other portion of each clinical samples were inoculated on to Sabouraud dextrose agar (Oxoid, Basingstoke,UK) to which 50µg/1ml gentamicin is incorporated. Inoculated sample were kept at least 72 hours a temperature of 37^0 C. Yeast isolates were transferred to tryptic soya broth with 20% glycerol and transport to the Ethiopian public health institute national reference laboratory of clinical bacteriology and mycology case team and stored at minus 80^o Refrigerator until used.

4.7.2: Identification and Antifungal susceptibility testing: Identification testing was performed by YST card through VITEK 2 system. Antifungal susceptibility testing of these isolates were performed for Caspofungin, Fluconazole, Flucytosine, Micafungin, Voriconazole by using VITEK card AST-YS07**.** The VITEK 2 cards containing serial two fold dilutions of Caspofungin, Fluconazole, Flucytosine, Micafungin, Voriconazole were provided by the manufacturer.

Compared the performance of the VITEK 2 YST identification card to the RapID Yeast Plus system using 750 clinical yeast isolates, using 16S rRNA sequence analysis was used as the reference method ,98.2% of isolates were correctly identified to the species level by the VITEK 2 system [84]. The VITEK 2 was found also to have excellent qualitative and quantitative agreement relative to reference method of broth micro dilution (BMD) for the generation of

amphotericin B, flucytosine, and voriconazole susceptibility data with Candida spp. It produces highly reproducible, rapid results that reliably produce MICs that are comparable to BMD while ensuring that each test is performed in a highly standardized fashion [85].

The purity and viability of yeast original cultures were checked by plating yeast colonies on Sabouraud dextrose agar (Oxoid, Basingstoke,UK). Yeast identification and *in vitro* antimicrobial susceptibility testing of yeasts were determined by the automated VITEK 2 compact system (bioMérieux, France) using YST-21343 and AST-YS01 cards. The inoculums suspensions for the VITEK 2 were prepared in sterile saline at a turbidity equal to a 2.0 McFarland standard, as measured using a Densichek instrument (bioMérieux).The YST-21343 and AST-YS01 cards were automatically filled with the prepared culture suspension, sealed, and incubated by the VITEK 2 instrument. The cards were incubated at 35.5 °C for 18 h, and data were collected at 15-min intervals during the entire incubation period and final identification and antimicrobial testing (MIC and interpretation) results were obtained in approximately 18 h or less. The final profile and MIC results were compared with the database, and the identification of the unknown organism was obtained. A final identification of excellent, very good, good, acceptable, or low- discrimination was considered to be correct. A set of clinical isolates (24 totals) were used as challenge strains to evaluate the ability of the VITEK 2 system to determine the antifungal susceptibility of yeasts.

4.8. Data Management and Quality Assurance

4.8.1. Pre-analytical

Expiry date of media, VITEK card of YST and AST-YS07, sterility and performance of media and VITEK card checked and a standard protocol was followed. Specimens were collected following SOPs and processed in Arsho microbiology laboratory. Isolates were transported to the laboratory of Ethiopian public health institution national reference laboratory of clinical bacteriology and mycology as soon as possible after identify isolates for further identification and antifungal susceptibility test. Pre-test was done before regular data collection was started.

4.8.2. Analytical:

For performance check VITEK 2 Compact system tested by using Standard strains of *Candida albicans* ATCC 10231 before any isolate tested. Reference strains for quality control were passed.

4.8.3. Post-analytical:

Quality of the data were maintained by coding the isolates with unique number and finally all clinical isolates were preserved in deep freeze using 20% glycerol with trypticase soya broth (TSY) in case needed or for future further investigation. Data were checked for completeness before analysis.

4.9. Data Processing and Analysis

Results were compiled and entered in to SPSS version 20 Software and were analyzed to determine frequency, percentage and compare distribution of each etiologic agent by anatomic site of infection involved.

4.10. Ethical Consideration

All ethical considerations and obligations were duly addressed, and the study was conducted after the approval of the department of research and ethical review committee (DRERC) of the department of Medical Laboratory Sciences. Written informed consent was obtained from the participants before data collection. Each respondent was given the right to refuse to take part in the study and to withdraw at any time during the study period. All the information obtained from the study subjects were coded to maintain confidentially. When the participants are found to be positive for fungal pathogen, they were informed to the hospital clinician and received proper treatment. An assent form was completed and was signed by a family member and/or adult guardian for participants under the age of less than 18 years.

4.11. Dissemination of Results: The findings of the study was forwarded to the department of medical laboratory science, college of health science, school of allied health science, Addis Ababa University and Ethiopian public health institute and thesis defense then an attempt will be made to present the findings in different conferences and will be sent to publication on peer reviewed scientific journals.

5. Results

5.1. Species distribution of yeast isolates

A study participant socio-demographic data demonstrated a minimum and a maximum age was 21 and 83, respectively. A mean of age was 40 with standard deviation 13. Around 70% participant was female.

As shown in Table 1, a total of 209 yeasts were recovered of which 49.8% (104/209) were *Candida albicans*, 43.1% (90/209) were non-*albicans candida* species and 7.2% (15/209) were yeasts other than *Candida* species. The two hundred nine isolated from different body sites which consisted of *24% (50/209)* oropharengial, 41 % (87/209) vaginal, 12% (25/209) nail 5.7% (12/209) eye discharges and 17 % (35/209) sputum were collected and evaluated for yeast species distributions and their corresponding susceptibility patterns. Yeast species with major *candida* species identified in different quantity of distribution. The most commonly isolated species were: *Candida albicans* 49.8% (104/209) followed by *Candida krusie 6.7 % (14/209)*, *Candida famata* 6.2% (13/209), each of *Creptococcus laurenti and Candida rugosa* 4.8 %(10/209) ,*Candida lusitaniae* 4.3% (9/209), each of *Candida parapsilosis, Candida kefyr* ,*Candida lipolytica* and *Candida ciferrii* 2.9% (6/209), *Candida guilliermondii 2.4% (5/209),Candida dubliniensis* 3.3 % (7/209), each of *Cryptococcus neoformans* and *Candida pelliculosa* 1.9% (4/209) and other yeast species 2.4% (5/209).

The proportion of *C. albicans* to non- *albicans candida* species was 1.2 to 1 .*C. krusei* 15.6% (14/90), *C. famata* 14.4 %(13/90), *C. rugosa* 11.1 % (10/90), and *C. lusitaniae* 10.0% (9/90) were the commonest isolates among non-*albican candidia* species. *Candida krusi* the main isolated in vaginal isolate while *Candida rugosa* and *Candida famata* from sputum and nail specimen, respectively. Whereas, yeast fungal infections of the eye showing highest variability of non-*albican candida* species with equal frequency (Table 1). Among yeasts other than *Candida* species *Cryptococcus laurenti* represented 66.7% (10/15*)* this group of yeasts.

Table 1. Species distribution of yeasts isolated from different clinical samples from January 2018 to September 2018 in Addis Ababa, Ethiopia.

	Clinical samples					
Species	Vaginal Discharge	Oropharyngeal	Nail	Eye Discharge	Sputum	Total
Candida albicans	52	28	4	5	15	104
Sub-total						**104**
Non-albicans candida						
C. krusie	11	0	0	2	1	14
C .famata	2	4	5	1	1	13
C. guilliermondii	0	3	1	1	0	5
C. lipolytica	2	4	0	0	0	6
C .pelliculosa	1	0	3	0	0	4
C. intermedia	0	0	1	0	0	1
C. utilis	0	1	0	0	0	1
C .rugosa	0	2	1	1	6	10
C. glabrata	2	0	0	0	0	2
C. lusitaniae	3	2	3	1	0	9
C. kefyr	1	2	1	1	1	6
C. dubliniensis	5	0	0	0	2	7
C. parapsilosi	2	4	0	0	0	6
C. ciferrii	2	0	0	0	4	6
Sub total						**90**
Other yeast						
Cryptoccocus laurenti	3	0	4	0	3	10
C. neoformans	1	0	1	0	2	4
Trichosporn mucoides	0	0	1	0	0	1
Sub-total						15
Grand Total	87	50	25	12	35	**209**

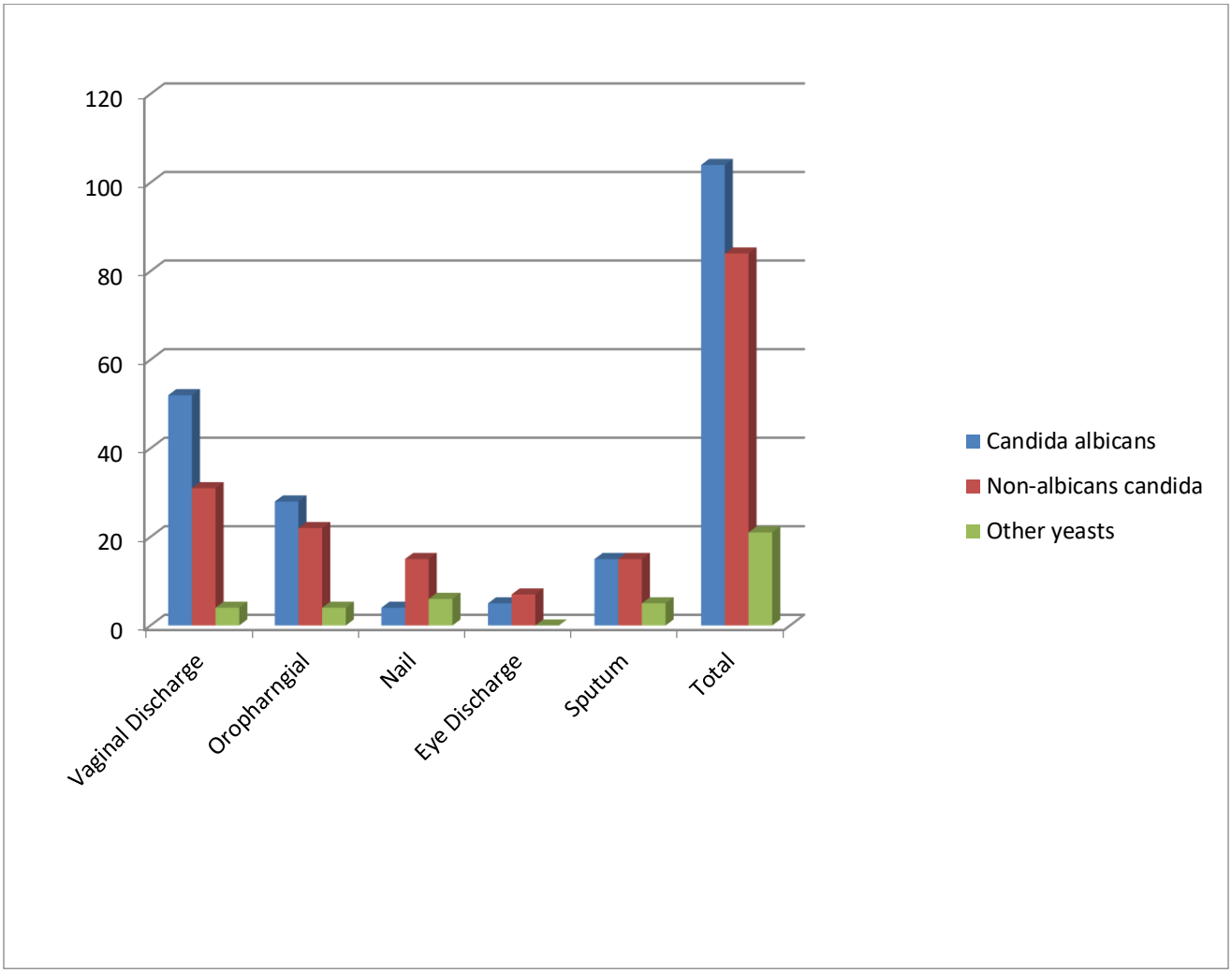

Figure I: Frequency of *Candida albican*, non-*albicans Candida* and other yeasts species from different clinical samples at Arsho medical laboratory in Addis Ababa, Ethiopia from January, 2018 to September, 2

5.2. Antifungal susceptibility profile of yeast isolates

The *in vitro* antifungal susceptibility profile of *Candida* species to the five antifungal drugs expressed as MICs in µg/m list shown in Table 2 (I- V).

Out of 194 Candida species tested against fluconazole the MIC value was obtained for only 181 isolates as the MIC value for *C. famata* was not proved to all drugs tested by the VITEK. Of the 181 isolates 85.6%, 10.5% and 3.9% isolates were susceptible, resistant, and intermediate to the drug, respectively. *C. krusie*, which was the 2nd predominant isolate, was 100% resistant to fluconazole followed by *C. pelliculosa* (25%) and *C. rugosa* (20%). Around 98 %(102/104) of *Candida albicans* were susceptible to fluconazole .There is no *Candida albican* resistance for fluconazole in the present study and only 2 % (2/104) of isolated showed intermediate Table 2(I). Out of 181 *Candida* isolates 99.4% of the isolates were susceptible to voriconazole. All *Candida* species except 1 species of *C. ciferrii* were susceptible to the drug. Voriconazole confirmed highly susceptible to *Candida* species. It has a good activity against fluconazole-resistant *C. krusie and c.rugosa* strains Table 2(II). Among 194 *Candida* species evaluated for their *in vitro* susceptibility profile to echinocandins (caspofungin and micafungin) MIC values were obtained to 175 isolates, because MIC values to *C. famata* and *C. ciferri* were not provided by the VITEK. Among the 175 isolates 98.8% of the isolates were susceptible to caspofungin and micafungin . *C. rugosa* (10%) and *C. lipolytica* (16.7%), were the isolates where resistance strains to both drugs were recorded Table 2 (III, IV). Out of 181 *Candida* isolates were tested for their in vitro drug susceptibility profile against flucytosine, 86.2%, 6.6% and 7.1% were found out to be susceptible, resistant, and intermediate, respectively. The most resistant species was C. *krusei* accounting for 78.6% followed by *C. albicans* with 1% resistance Table 2(V). Regarding drug resistance profile of yeast other than Candida species no MIC values were obtained for both *C. laurenti* and *T. mucoid*. *C. neoformans* was 100% susceptible to fluconazole and flycytosine, but MIC values were not obtained against the remaining drugs tested (Table 3).

Table 2. In vitro antifungal susceptibility pattern of *Candida* species to five antifungal drugs (I-V) samples from January 2018 to September 2018 in Addis Ababa, Ethiopia.

I. Fluconazole

***Candida* species**	**S (%)**	**R (%)**	**I (%)**	**MIC(µg/ml)-Range**	**Total**
C .albicans	102 (98)	0	2(2)	≤1-32	104
C.guilliermondii	5 (100)	0	0	≤1-32	5
C. famata (13)	*	*	*	*	*
C. lipolytica	4 (66)	1 (17)	1 (17)	≤ 1 -64	6
C. ciferrii	4(66)	1(17)	1(17)		6
C. pelliculosa	3 (75)	1 (25)	0	-2 ≥ 64	4
C. intermedia	1(100)	0	0	≤ 1	1
C. utilis	1 (100)	0	0	-2	1
C. rugosa	7 (70)	2 (20)	1 (10)	≤ 1-64	10
C. glabrata	2 (100)	0	0	≤ 4	2
C. lusitaniae	9 (100)	0	0	≤ 1-2	9
C. kefyr	6 (100)	0	0	≤ 1	6
C. krusi	0	14(100)	0	-≥16 -≥ 64	14
C. dubliniensis	5 (71.4)	0	2 (28.6)	≤ 1-32	7
C. parapsilosi	6 (100)	0	0	≤ 1	6
Total	155 (85.6)	19(10.5)	7 (3.9)		181

MIC=Minimum inhibitory concentration, S=Susceptible=Resistance, I=Intermediate.

*=The VITEK 2 Compact system did not provide antifungal susceptibility test result.

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II. Voriconazole

Candida species	S (%)	R (%)	I (%)	MIC(µg/ml)-Range	Total
C. albicans	104 (100)	0	0	≤ 0.12 -1	104
C.guilliermondii	5 (100)	0	0	≤ 0.12	5
C. famata(130)	*	*	*	*	*
C. lipolytica	6 (100)	0	0	≤ 012-32	6
C. ciferrii(6)	5(83)	1(17)	0	<0.12	6
C. pelliculosa	4 (100)	0	0	≤ 012-0.5	4
C. intermedia	1 (100)	0	0	≤ 012	1
C .utilis	1 (100)	0	0	≤ 012	1
C. rugosa	10 (100)	0	0	≤ 012	10
C. glabrata	2 (100)	0	0	≤ 012	2
C. lusitaniae	9 (100)	0	0	≤ 012-0.5	9
C. kefyr	6 (100)	0	0	≤ 0.12	6
C. krusi	14 (100)	0	0	≤ 0.12-0.25	14
C. dubliniensis	7 (100)	0	0	≤ 0.12	7
C. parapsilosi	6 (100)	0	0	≤ 0.12	6
Total	180 (99.4)	1(0.6)	0	-	181

MIC=Minimum inhibitory concentration, S=Susceptible=Resistance, I=Intermediate.

*=The VITEK 2 Compact system did not provide antifungal susceptibility test result.

III. Caspofungin

Candida species	S (%)	R (%)	I (%)	MIC (µg/ml) -Range	Total
C. albicans	1004(100)	0	0	≤ 0.06 -4	104
C.guilliermondii	5(100)	0	0	≤0.25-1	5
C. famata	*	*	*	*	*
C. lipolytica	5(83.3)	1(16.7)	0	≤ 012-4	6
C. ciferrii (6)	*	*	*	*	*
C. pelliculosa	4(100)	0	0	≤0.25	4
C. intermedia	1(100)	0	0	≤0.25	1
C. utilis	1(100)	0	0	≤0.25	1
C. rugosa	9(90)	1(10)	0	≤ 0.25-4	10
C. glabrata	2(100)	0	0	≤ 0.25	2
C.lusitaniae	9(100)	0	0	≤ 0.25-1	9
C. kefyr	6(100)	0	0	≤ 012-0.25	6
C. krusi	14(100)	0	0	≤ 0.12-0.5	14
C. dubliniensis	7(100)	0	0	≤ 0.12-0.25	7
C. parapsilosi	6(100)	0	0	≤ 0.12-1	6
Total	173(98.8)	2(1.2)	0	-	175

MIC=Minimum inhibitory concentration, S=Susceptible=Resistance, I=Intermediate.

*=The VITEK 2 Compact system did not provide antifungal susceptibility test result.

IV. Micafungin

Candida species	S (%)	R (%)	I(%)	MIC (µg/ml) - Range	Total
C.albicans	1004(100)	0	0	≤0.06- 4	104
C.guilliermondii	5(100)	0	0	≤0.06-0.5	5
C. Famata	*	*	*	*	*
C.lipolytica	5(83.3)	1(16.7)	0	≤ 0.06- 4	6
C. ciferrii(6)	*	*	*	*	*
C. pelliculosa	4(100)	0	0	≤0.06-0.12	4
C. intermedia	1(100)	0	0	≤ 012	1
C. utilis	1(100)	0	0	≤ 012	1
C. rugosa	9(90)	1(10)	0	≤0.06-≥4	10
C. glabrata	2(100)	0	0	≤ 0.06	2
C. lusitaniae	9(100)	0	0	≤ 0-06-1	9
C. kefyr	6(100)	0	0	≤0.06-0.12	6
C. krusi	14(100)	0	0	≤0.06-0.25	14
C. dubliniensis	7(100)	0	0	≤0.06-0.25	7
C. parapsilosi	6(100)	0	0	≤ 0.25-2	6
Total	173(98.8)	2(1.2)	0		175

MIC=Minimum inhibitory concentration, S=Susceptible=Resistance, I=Intermediate.

*=The VITEK 2 Compact system did not provide antifungal susceptibility test result.

V. Flucytosine

Candida species	S (%)	R (%)	I (%)	MIC (µg/ml) Range	Total
C. albicans	100 (96)	1(1)	3(3)	≤1-64	104
C.guilliermondii	5(100)	0	0	≤ 1	5
C. Famata(13)	*	*	*	*	*
C. lipolytica	4(66.7)	0	2(33.3)	≤ 1-16	6
C. ciferrii	5(83.3)	-	1(16.7)	≤ 1-16	6
C. pelliculosa	4(100)	0	0	≤ 1-4	4
C. intermedia	1(100)	0	0	≤ 1	1
C. utilis	1(100)	0	0	≤ 1	1
C. rugosa	5(50)	0	5(50)	≤ 1-16	10
C. glabrata	2(100)	0	0	≤ 1	2
C. lusitaniae	9(100)	0	0	≤ 1	9
C. kefyr	6(100)	0	0	≤ 0.5 -1	6
C.krusi	3(21.40	11(78.6)	0	≤ 1-64	14
C. dubliniensis	5(71.4)	0	2(28.6)	≤ 1-16	7
C. parapsilosi	6	0	0	≤ 1	6
Total	156(86.2)	12(6.6)	13(7.1)		181

MIC=Minimum inhibitory concentration, S=Susceptible=Resistance, I=Intermediate.

*=The VITEK 2 Compact system did not provide antifungal susceptibility test result.

Table 3: Antifungal susceptibility profile of yeasts other than Candida species in Addis Ababa, Ethiopia January to September 2018

Species	Fluconazole				Voriconazole				Caspofungin				Micafungin				Flucytosine			
	S(%)	R(%)	I(%)	MIC	S(%)	R(%)	I(%)	MIC	S(%)	R(%)	I(%)	MIC	S(%)	R(%)	I(%)	MIC	S(%)	R(%)	I(%)	MIC
C. Laurenti(10)	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
T. mucoid (1)	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
C. neoformans (4)	4(100)	0	0	≤1-4	*	*	*	*	*	*	*	*	*	*	*	*	4(100)	0	0	≤1-8

MIC=Minimum inhibitory concentration, S=Susceptible=Resistance, I=Intermediate.

*=The VITEK 2 Compact system did not provide antifungal susceptibility test result.

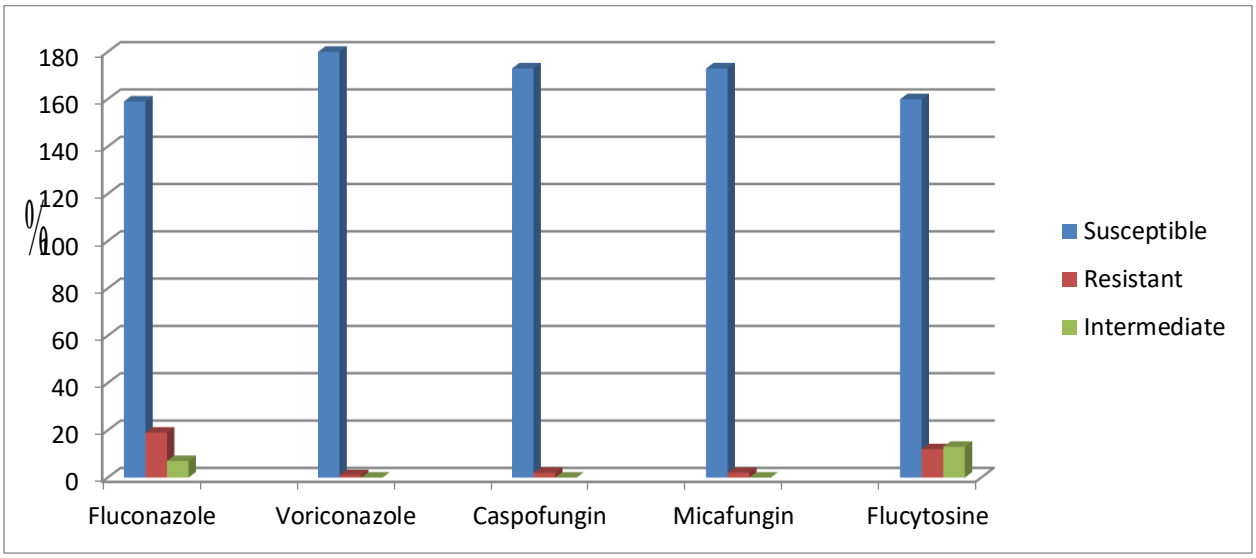

Figure II: Antifungal susceptibility pattern of *Candida* species of yeast to different of antifungal in Addis Ababa, Ethiopia January to September 2018.

5.3. Cross-resistance and multi-drug résistance

Out of 185 yeast species three (3) isolates demonstrated cross-resistance with *Candida rugosa* (1/3) and *candida lipolytica* (1/3) and *Candida ciferrii (1/3).* Generally, 1.6 %(3/185) yeast species were showed cross-resistance. All yeast cross-resistance explained in multi drug resistance and every one cross-resistance yeast were within antifungal drugs of Echnocandin group between Caspofungin and micafungin except *Candida ciferrii* that was cross resistance occurred between fluconazole and voriconazole. All caspofugin resistance isolates were micafungin cross-resistance (Table 4).

Of the 16 yeast isolates which were resistant to two or more antifungal agents, the main isolates were *candida krusie* which reflect the intrinsic less susceptibility of non albicans. There was no multi drug resistance (MDR) in *candida albicans*. The higher frequency isolate with MDR were *Candida krusie* (11/16) followed *Candida rugosa* (3/16),*Candida lipolytica* (1/16), *Candida ciferrii(1/16).*All isolate showed higher multi drug resistance with azole and flucytocine antifungal drug .There was no echnocandin group plus other antifungal group of drugs were demonstrated with multi drug resistance (Table 4).

Table.4: Multi-drug resistance (MDR) and Cross-resistance distribution in different yeast isolates in, Addis Ababa, Ethiopia January to September 2018.

Yeast species	MDR n (%)		Cross-resistance n (%)		Total
	Yes	**No**	**Yes**	**No**	
Candida albicans	0	104 (100)	0	104	104
Candida guilliermondii	0	5 (100)	0	5	5
Candida ciferrii	1(16.7)	5 (83.3)	1(16.7)	5 (83.3)	6
Candida lipolytica	1(16.7)	5 (83.3)	1(16.7)	5 (83.3)	6
Candida pelliculosa	0	4 (100)	0	4 (100)	4
Creptococcus neoformans	0	4 (100)	0	4 (100)	4
Candida intermedia	0	1(100)	0	1(100)	1
Candida utilis	0	1(100)	0	1(100)	1
Candida rugosa	3 (30)	7 (70)	1(10)	9 (90)	10
Candida glabrata	0	2 (100)	0	2 (100)	2
Candida lusitaniae	0	9 (100)	0	9 (100)	9
Candida kefyr	0	6 (100)	0	6 (100)	6
Candida krusie	11(78.6)	3 (21.4)	0	14 (100)	14
Candida dubliniensis	0	7 (100)	0	7 (100)	7
Candida parapsilosi	0	6 (100)	0	6 (100)	6
Total	16 (8.6)	169 (91.4)	3 (1.6)	182 (98.4)	185

MDR=Multi drug resistance

6. Discussion

6.1. Species distribution of isolates

The incidence of opportunistic fungal infections such as *Candida* infections has considerably increased recently. Given the fact that many clinical microbiology laboratories in Ethiopia do not perform identification of yeasts beyond direct microscopy, accurate identification of *Candida* down to the species level is crucial. This is because different species have different antifungal susceptibility profile and the incidence of non-*albicans candida* is increasing. In the present study, identification of yeasts down to the species level and their drug susceptibility profile were determined in an automated manner by the VITEK 2 compact system that delivers quantitative MIC results that are reproducible and precise when compared with other reference methods [12, 14, and 15].

Although the relative prevalence of the yeast species depends on the geographical location, patient population, and clinical settings [65], with a rise in the immunosuppressive patients, increased number of fungal infections has been reported worldwide. Simultaneously, the profile of human yeast pathogens has also been increasing [66].Whereas, In Ethiopia little is known regarding the distribution and the *in vitro* antifungal susceptibility profile of yeasts isolated from patients. The different types of yeast species were identified from clinical suspect volvovaginal, oropharngial, sputum, nail and eye fungal infection patient with major *Candida* species of yeast. These are *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, *Candida guilliermondii*, *Candida lusitaniae*, *Candida dubliniensis*, *Candida pelliculosa*, *Candida kefyr*, *Candida intermedia*, *Candida lipolytica*, *Candida famata*, *Candida rugosa*, and *Candida pelliculosa*, *Creptococus laurentii* , *Creptococus neoformans*, *Trichosporn mucoides*, *Candida ciferrii*. This result demonstrated similar finding with previously published data, which reported considered as a causative agent of fungal infection, in worldwide [3,43]. There is no recognized information regarding the profile of yeast species from Ethiopian patient s similar result with this data.

The present study, 49.8% were *C. albicans*, 43.1% were non-*albicans candida* species, and 7.1% were yeasts other than *Candida* species; the ratio between *C. albicans* to non-*albicans Candida* species was being1.2 to 1. Among non- *albicans Candida* spp. *C. krusei* (14), *C. famata* (13), *C. rugosa* (10) and *C. lusitanitae* (9) were the predominant species. C. *albicans* and C. *krusei* as the 1st and the 2nd predominate species were similar with a study conducted in Ethiopia

[16] but, *C. famata* and *C. rugosa* two of the dominant species in the current study were not recovered at all by previous Ethiopian study [16], as well as in the world consider as rare finding of human pathogen [3]. Occurrence rate of *Candida rugosa 4.8* % (10/209) isolated from sputum isolate followed by oropharengial, coneal discharge and nail. These data result compare to Ghana isolates reported 0.7% of total *Candida* isolates (4/600), which is similar to the reported incidence of 0.6% worldwide. The variation arise may be due to regional and patient population of in this study [68] .Clinically, species of the *C. rugosa* complex have been isolated from a range of sources including blood, urine, sputum, and swabs from different anatomical sites [69]. Members of the *Candida rugosa* species complex have been described as emerging fungal pathogens and are responsible for a growing number of *Candida* infections [70].Studies are needed to better clarify the frequency of *Candida rugosa* infections in Ethiopian patients.

A total of 50 *yeast* isolates from oropharngial specimen with predominant *Candida albican* 56 %(28/ 58) isolated. It has great variation previously reported data in Ethiopia 81% [67] and Ghana 76.19% from HIV patients[61].However, one study in China from patients in intensive care units (ICU) result comparable *Candida albican* (40.1%) to this finding [34]. This deviation possibly arises from the study population difference and low number of sample size. In oropharengial, the incidence rate of non-*albican candida* yeasts were around 44 %(22/50) and other yeast was not found which is similar with previously study [67, 20].

5.7% (12/209) from total of isolate recovered from eye discharge with main isolate of Candida albican and high variability of non-albican candida species with equal frequency of each 0.5% (1/209) that were *C. lusitaniae,C. kefyr,C. rugosa,C. famata,C. krusi* and *C. guilliermondii.* Fungal keratitis is one of the most challenging types of infectious keratitis which has been gradually increasing during the past few decades. It now accounts for approximately 50% of infectious corneal diseases **[71]**.

In the current study, the occurrence rate of non *candida* yeast isolates were 7.1% with highest frequency of *Creptococcus laurenti* 4.8 % (10/209) followed by *Creptococcus neoformans* 2% (4/209) and *Trichosporn mucoides 0.5%(1/209). Cryptococcus laurenti* and *Trichosporn mucoides* are a rare human pathogen, but *Creptococcus neoformans* as typical fungal pathogen to immunocompromised [59]. When comparing these data of *Creptococcus laurenti* 4.8 % (10/209) to previously one study showed 0.6%(1/155) recovered rate in Ethiopia from orophrengial

[72].Other than *Cryptococcus neoformans* species have classically been considered to be non-pathogenic. However, *Cryptococcus albidus* , *Cryptococcus laurentii* , *Cryptococcus luteolus* ,*Cryptococcus uniguttulatus* , *Cryptococcus curvatus,* have emerged as opportunistic pathogens over the last few years[73]. They have been described as opportunistic pathogens in HIV positive individuals, as well as in patients with other predisposing factors [74]. *Creptococcus laurentii* has been implicated in 18 cases of opportunistic infection, predominantly of the skin, bloodstream, and central nervous system. Within the non-*neoformans Cryptococcal* species, *Cryptococcal laurentii* and *Cryptococcal albidus* account for 80% of pathogenic infections. Although *C. laurentii* is found worldwide and its natural habitat remains largely unknown [66, 75], the occurrence rate (4.8%) of its current finding is higher.

Trichosporon spp are yeast-like fungi found in soil and water. Furthermore, they belong to the normal flora of the human skin and gastrointestinal tract. The first case of onychomycosis caused by *Trichosporon mucoides* was published in 2011 by Malini A et al [76]. An additional cases were published in 2015 and 2016 by Capoor et al and Rizzitelli et al [77].The published data showed *Trichosporon mucoides* a clinical significant isolate that is consider as human pathogen even if the occurrence of this fungi rare. This fungus is only one (1/25) *Trichosporon mucoides* recovered from nail that is agreeing from previously finding. However, clinical history of this patient was not evaluated. All epidemiological papers of fungal diseases in Ethiopia were reviewed. Where there was no Ethiopia data align with this finding .Perhaps, by reason of these diseases are often understudied.

Generally, this is the first report including other than *candida* species in Ethiopia demonstrated as species level by this amount and variance of yeast species. This explains incidence of *C. albicans* has decreased, while that of the non-*albicans Candida* and other yeast species has increased. Although the reason for the emergence of non-*albicans Candida* and other than *candida* species in large proportion in this study is not clear, the precision of the identification method (VITEK 2 compact system) and use of antifungal drugs for prophylaxis and treatment empirically in Ethiopia could be possible explanations.

6.2. Antifungal susceptibility profile of the isolates

In the present study, percentage drug resistance profile of *Candida* species against fluconazole was 10%. Similar findings of fluconazole resistant rate illustrate 11.9% previously reported data in Ethiopia [28]. The susceptibility of *Candida* species to fluconazole, however, varied from one species to another. Ninety- eight percent of *C. albicans* was susceptible to the fluconazole. However, yeast isolates such as *C. krusei*, *C.pelliculosa* and *C. rugosa and C.lipolytica* were 100%, 25 %, ,20% and 17% resistant to the drug, respectively. The azole antifungal drugs such as fluconazole are the most frequently used drugs to treat *Candida* infection due to the fact that they are less toxic, inexpensive and can be administered orally. It has been suggested that prolonged or repeated exposure to low-dose fluconazole is associated with emergence of fluconazole resistance among strains of *Candida albicans* [17] and the potential selection of non-*albicans Candida* species such as *C. kruse* [18].

Fluconazole is a first line (choice) drug in the treatment of candidiasis in the current guideline of the Ethiopian Ministry of health [19] and this fact may be cited as a possible explanation for a higher isolation rate of *C. krusei* than other non-*albicans candida* species in the present study. Among yeast isolates other than non-albican *candida* species only 66% of *Candida ciferrii* was susceptible to fluconazole. *Candida ciferrii*, as new fluconazole-resistant yeast causing systemic mycosis in immunocompromised patients has been reported by Gunsilius *et al* [20]. As result, the needs for a revision of antifungal therapy guidelines in Ethiopia. Echinocandins have been recommended for the treatment of *Candida* infections showing resistance to fluconazole [25].

In the current study, 99.4% (180/181) of the *Candida* isolates were found to be susceptible to voriconazole which is similar result previously study in Ethiopia and other countries [16,52] Voriconazole is a second generation triazole antifungal agent derived from fluconazole, but with better antifungal activity than its mother compound (i.e., fluconazole). The antifungal agent showed a high activity against a number pathogenic yeasts including *Candida krusei*[[21].Our finding was in line with earlier findings as almost all candida isolates including *C. krusei* ,which is reported to be intrinsically resistant to fluconazole [64] ,were susceptible to the voriconazole. We do not have an immediate explanation on the susceptibility difference between fluconazole and voriconazole against *C. krusei* as all azole antifungal drugs have a common mechanism of

action, i.e., inhibition of ergosterol synthesis. [23]. According to literature, low resistance rate of Voriconazole for *Candida* species was reported [35, 34] . Voriconazole showed both superior potency and a wider spectrum of activity than fluconazole .Only one of *Candida ciferrii* isolate was resistant to voriconazole (Table 2 II). In general, these results and other study point upped the best choices for treatment and have no intrinsically or acquired resistance yeast species for voriconazole.

The current study, the in vitro resistance rates of all yeast isolates was 1.2 % to both caspofungin and micafungin which block fungal cell wall synthesis by inhibiting the enzyme that synthesizes β-glucan. Resistance occurred in only in *Candida rugosa* and *Candida lipolytica* . This finding align with earlier studies reported that resistance rate *C. albicans* and most Candida species to echinocandinss was <3% [24]. Echinocandin resistance is even more rare .The reason of this resistance is not clear. Although it is difficult to explain, it could be due to associated with Echinocandin group of antifungal resistance in yeasts is mediated via point mutations resulting in target modification or resistance can also occur when environmental factors lead to colonization or replacement of a susceptible species with a resistant one [79]. The study of Pham et al [26] demonstrated also regional difference may contribute to variation in drug resistance.

In the present study, among 181 yeast isolates tested against flucytosine, about 6.6% was resistant to the drug, the exception being *C. krusei* with a resistance rate of 78.6%. A 33.3% resistant rate of *C. krusei* to the agent was reported by earlier study conducted in Ethiopia [16]. Possibly, resistance of yeasts to flucytosine is mediated by enzymatic modifications that either interferes with drug uptake into the cell or the conversion of flucytosine to 5-fluorouracil or 5-fluorouracil to 5-fluorouridine monophosphate [80]. Indeed, it has been documented that, Flucytosine is currently used as an antifungal drug in combination therapy, but fungal pathogens are rapidly able to develop resistance against this drug, compromising its therapeutic action [81]. Although, the prevalence of flucytosine resistance in most yeast remains low, rapid emergence of resistant yeasts during flucytosine mono therapy limited the use of the drug to combination therapy mainly with amphotericin B [29].

6.3. Cross-resistance and multi-drug resistance

Cross-resistance is a resistance to the two or more antifungals of the same drug class [78]**.** These study data illustrated 1.6 %(3/185) yeast species were showed cross-resistance. For *Candida rugosa* and *candida lipolytica* cross-resistance occurred within antifungal drugs of Echnocandin group between Caspofungin and micafungin and *candida ciferrii* that was cros-resistance occurred between fluconazole and voriconazole (table 4). Compare this finding to previously study published in 2008 demonstrated that *in vitro* cross-resistance between micafungin and caspofungin does exist among clinical isolates of *Candida [6]* .In addition, using CLSI methods and interpretive criteria another study illustrated micafungin may serve as an acceptable surrogate marker for the prediction of susceptibility and resistance of Candida to caspofungin [9]. So that, current data support the above mentioned scientific evidence and also one study done by Kołaczkowska et al. published in 2016 explained resistance to a single drug may be accompanied by cross-resistance to the entire group of antifungal of the same class, as well as to representatives of multiple structurally unrelated groups[53].Micafungin and caspofungin have an identical microbial spectrum of activity.Micafungin shares with caspofungin an identical spectrum of in vitro activity against candida albicans ,non-albicans species of candida and Aspergillus species ,as well as several but not all pathogenic molds[40].

Multi drug-resistance was defined as resistance to two or more antifungal drug classes [63]. Of the 8.6 % (16/185) yeast isolates which were resistant to two or more antifungal agents. The present study illustrated increasing appearance of species of *Candida* resistant to azoles and flucytocine confirms the importance of monitoring possible changes in the distribution of pathogenic species and susceptibility patterns.

Changes in species distribution and a shift to more resistant isolates are increasingly described in global antifungal surveillance study [62]. Likewise, this study identifies several less-common species of *Candida* with decreased susceptibility to azoles and flucytosine. Although a limited number of antifungal agents against isolated were tested, these data confirm have higher frequency of multidrug-resistance within the yeast isolates. It is mainly occurring due to Intrinsic

or primary resistance is inherent (not acquired) resistance and it is predictive of clinical failure. Examples of intrinsic resistance are the resistance of *C. krusei* to fluconazole [64].

Most MDR *Candida* infections involve isolates belonging to species with intrinsic resistance, for example candida auris which is intrinsically multidrug resistant and currently emerging yeast. Multidrug resistance in species that possess no intrinsic resistance is rare, as in general it requires acquisition of several resistance mechanisms and these often come at a fitness cost [67]. Moreover, multi-drug resistance infections are associated with high morbidity and mortality and can be associated with healthcare-associated transmission. Outcomes are poor with these resistant infections; thus, an accurate mycological diagnosis and therapy guided by susceptibility testing should be used to optimize management [63].

8. Strength and limitation of the study

8.1 .Strength

The strengths of this study are its prospective and multi clinical sample design with collection of different clinical sign and symptom of patients with fungal infection reflecting broad wide yeast species kind.

8.2. Limitation of this study

- There are several limitations to this study; most prominent is not incorporating patient clinical history or the extent of prior exposure to antifungal therapy is not known.
- Even though the isolates were collected from different anatomical sites, each specimen has not equal representative in the overall data, so that distribution of yeast isolates from different specimen could not compare result of each other.
- The clinical significance of the increased multi drug resistance with *C. krusei* and also cross resistance of *Candida* species could not be analyzed due to the lack of clinical data.

7. Conclusion and Recommendation

7.1. Conclusion

A total of eightheen18 different species of yeast were isolated with C. albicans remaining the predominant species in all specimen type. The emergence of non-*albicans Candida* and other than *candida* species yeast have increased. Importantly, knowledge of the species of pathogenic yeast is a useful guide to the probable pattern of susceptibility and for successful treatment of patients. Regarding antifungal susceptibility; Voriconazole was highly potent in vitro against a wide range of yeast species. It was notably more potent against *C. krusei and C.rugosa* than fluconazole. It is also notably more active than fluconazole in terms of both potency and spectrum. In vitro-resistance to fluconazole, voriconazole and flucytosine was rare among C. albicans, but an increase of non-susceptibility was observed among C. *krusie* and *C.rugosa* for fluconazole and flucytosine. These results point upped the best choices for treatments have no intrinsically or acquired resistance yeast species for voriconazole.

7.2. Recommendation

- This information clearly indicates the growing importance of non-albican *candida* caused infections. Therefore, epidemiological studies are required to determine the exact incidence and prevalence of these infections in a country trends over time. With the emergence of inherently drug resistant nona-lbicans *candida* species, more studies on *Candida* prevalence and drug susceptibility are needed throughout country.
- Regular monitoring of *candida at* a regional level could therefore be an important tool to aid in the prescription of antifungals based on the prevalent species and their susceptibilities to antifungal drugs in areas where routine microbiological laboratory testing is not available.
- Clinicians and other health care provider need to be trained and to be made aware of these infections, in order to improve the diagnosis and treatment.
- These data showed yeast species especially non-albican *candida* is resistant to fluconazole which is widely used in public health settings in Ethiopia and is used empirically in the treatment of systemic or localized *Candida* infections, needs to be revised guideline of treatment .

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10. Annex

Annex I

Information sheet (English and Amharic)

- **Title :**The **s**pectrum of yeast isolate in different clinical samples and their antifungal susceptibility profile at Arsho medical laboratory , Addis Ababa Ethiopia
- **Purpose:** To determine spectrum of candida species in different clinical samples and determine their antifungal susceptibility profile

Procedure: First, we will ask you about demographic information then we need only small amount of samples. The samples will be tested at Arsho laboratory and Ethiopian Public Health Institute using a code so that no one will know about your results except your clinician for your management. All information collected during this study will be kept private and will only be known by the investigators.

Benefits: This project will help you and other people living with fungal infection and took different antibiotic for treatment. We will use these results to decide whether there is resistance for the commonly used antibiotics fungal infection treatment. If we find that the resistance in drugs you use at the time of investigation the clinician will change the drug prescription.

Risks: There is no risk to you from answering the questions however; there will be a discomfort while we took samples for culture test.

Privacy: We will keep information about you private. We will not collect your name. Only the investigators will have access to the data and only for study purpose. We will not use any information that might identify you when we present or publish the study's results.

Payment: There is no cost to you for being part of the project. The approximate time that this study will take is 15 minutes. There will be no involvement past today.

Consent Form I (English)

Participant Agreement: The project has been explained for me. I have been given a chance to ask questions. I feel that all my questions have been answered. Being in this study is my choice. I may change my mind and leave the study any time during the interview.

Participant Signature_____

Date_____

Name of persons obtaining consent_____

Signature of persons obtaining consent_____

Date_____

Supervisor Name _____

Supervisor signature_____

Date_____

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Annex II. Information sheet (English version)

- **Title :The s**pectrum of yeast isolate in different clinical samples and their antifungal susceptibility profile at Arsho medical laboratory , Addis Ababa Ethiopia

Purpose: To determine spectrum of candida species in different clinical samples and determine their antifungal susceptibility profile.

Procedure: First, we will ask your child about demographic information then we need only small amount of samples. The samples will be tested at Arsho laboratory and Ethiopian Public Health Institute using a code so that no one will know about your child results except your clinician for your

management. All information collected during this study will be kept private and will only be known by the investigators.

Benefits: This project will help your child and other people living with fungal infection and took different antibiotic for treatment. We will use these results to decide whether there is resistance for the commonly used antibiotics fungal infection treatment. If we find that the resistance in drugs your child use at the time of investigation the clinician will change the drug prescription.

Risks: There is no risk to you from answering the questions however; there will be a discomfort while we took samples for culture test.

Privacy: We will keep information about you private. We will not collect your name. Only the investigators will have access to the data and only for study purpose. We will not use any information that might identify you when we present or publish the study's results.

Payment: There is no cost to you for being part of the project. The approximate time that this study will take is 15 minutes. There will be no involvement past today.

Annex II. Ascent form (English version)

- **Participant Agreement:** The project has been explained for me on behalf of my child. I have been given a chance to ask questions. I feel that all my questions about my child have been answered. Allowing my child being part of in this study is my choice. I may change my mind and leave the study any time during the interview.
- Participant Signature_____
- Date_____
- Name of persons obtaining consent_____
- Signature of persons obtaining consent_____

- Date_____
- Supervisor Name _____
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Annex-IV: Standard Operating Procedures

1. Nail Scraping

i. Patient preparation

Preferably, before administration of antibiotics

ii. Type of Specimen

Specimen type	Container	Volume	Transport	Storage and Stability
Nail Scrapings	sterile container with	Scrape lesion with glass slide or scalpel;	< 72 hour at RT	72 hrs at RT

	lid	Clip nail near skin.		

iii. Nail Scraping Specimen Collection

a). Materials and Reagents

- Disposable glove
- Surgical blade knife / scalpel
- sterile Petri dish
- Marker
- cotton swab
- 70% alcohol/other antiseptic

b). Collection Procedure

Step	Action
1	Clean nail with 70% alcohol or other antiseptic
2	Dorsal plate scrapes outer surface and discard; scrape the deeper portion.
3	Remove a portion of debris from under the nail with a scalpel
4	Collect whole nail or nail clippings
5	Place all material in a clean sterile Petri-dish
6	Label the Patient's Name, Collection Time, Date, Collectors name or initial, specific collection site
7	Transport to the laboratory in a dry container within two hours

2. Tongue Swab

i. Patient preparation

Preferably, before administration of antibiotics

ii. Type of Specimen

Specimen type	Container	Volume	Transport	Storage & Stability
Tongue swab	Sterile test tube lid	The whole wet cotton swab.	< 2 hour RT	24 hrs. refrigerator

iii. Tongue Swab Specimen Collection

a). Materials and Reagents

- Disposable glove
- sterile Petri dish
- Marker

- cotton swab
- physiological saline

b). Collection Procedure

Step	Action
1	Using cotton swabs take materials from the affected area of tongue
2	Insert swabs into sterile test tube
3	Add physiological saline
4	Label the patent's name or ID, Collection Time and date, collector's initial or name, specific site for collected specimen
5	Transport to laboratory within one hr

3. Laryngeal Swab

i. Patient preparation

Specimen should be taken, if possible, before antimicrobial therapy. Otherwise, drugs administered and duration of treatment should be given in the request form. Collect early morning before food and drinks are taken**.**

ii. Type of Specimen

Container	Volume	Transport and Packaging	Storage	Stability
Sterile test tube that has little saline solution (0.9%)	Wetted Cotton Swabs (2Ml Normal Saline)	Should be transported within one hour. If transportation cannot be done within 8 hours, refrigerate until transported	2-8℃	8hrs if saline added to sample

Laryngeal **Swab** Specimen Collection

a). Materials

Disposable glove

- Wooden cotton swabs
- Sterile test tube
- Waste disposal container
- Tongue suppresser
- N-95 Respirator
- Physiological saline (0.9%)

b). Collection Procedure

Step	Action
1	Fix tongue root with wooden tongue depressor
2	Insert the cotton swab into the affected parts of the Laryngeal and rotate the swab several times until wet
3	Insert swab into sterile test tube
4	Label tube with patient identifier

4. **Eye Swab**

i. Patient preparation

Preferably, before administration of antibiotics and preferably before application of local anesthetic.

ii. Type of Specimen

Specimen type	Container	Volume	Transport	Storage and Stability
Eye Swab	Sterile container with lid or transport media	Two well wetted cotton swabs rolled on the affected area	< 2 hour RT	< 24 RT

iii. Eye Swab Specimen Collection

a). Materials and Reagents

- Disposable glove
- Sterile test tube
- Marker
- Wooden cotton swabs
- Physiological saline

b). Collection Procedure

Step	Actions
1	If there is make up and ointment gently remove with sterile swab and normal saline
2	Collect specimen using the cotton swab from the affected parts of the eye and rotate the swab several times until wet (Take care of contamination of skin flora from skin outside the eye)
3	Put /Insert swab into sterile test tube or into the transport media. Push the cap to bring the swab into contact with the transport medium
4	Medium may be inoculated at time of collection
5	Label the container with Patient's name, ID, Date, Collector name or initial, Time of collection and the culture site on the specimen tube (even left or right eye)
6	Store at room temperature
7	Send specimen to the laboratory

Procedure Notes

- Do not touch external surface of eye
- Small volume aspirates may be transported in the syringe (take care of contamination, leakage and needle stick injury)

- Sample each eye with separate swabs (never ever use the same swab to collect sample from both eyes

5. Throat Swab

i. Patient preparation

Preferably, before administration of antibiotics

ii. Type of Specimen

Specimen type	Container	Volume	Transport	Storage and stability
Throat swab	Sterile container with lid or transport media	Two well wetted cotton swabs rolled on the affected part of tonsil/pharynx	< 2hour RT	24 our RT

iii. Throat Swab Specimen Collection

a). Materials and Reagents

- Disposable glove
- Tongue depressor /spatula
- Wooden cotton swabs
- Waste disposal container
- Sterile test tube
- Marker
- Physiological saline
- Transport media

b). Collection Procedure

Step	Action
1	Let the patient tilt the head back and open the mouth wide
2	Hold the tongue down with the depressor. Use a strong light source wipe area of inflammation and exudates in the posterior pharynx and the tonsillar region of the throat behind the uvula
4	The swab is applied to any area that appears either very red or discharging pus.
5	Label the Patient's name, ID, Date, Collection name or initials, Time of collection and the culture Site on the specimen tube.
6	Send specimen to the laboratory immediately unless we use transport media

Procedure Notes

- Do not use antiseptic mouthwashes before sample collection
- Do not touch the teeth, cheeks, gums, or tongue during collection

- If processing is delayed, refrigeration is preferable to storage

6. Genital Specimen

i. **Patient preparation**

Preferably, before administration of antibiotics and female patients should not wash their genital area.

ii. Type of Specimen

Specimen type	Container	Volume	Transport	Storage and stability
Urethral, Anal, Cervical and Vaginal swab	Sterile container with lid or Transport media	Two well wetted cotton swabs rolled on the affected part of Genital	2 hours RT or 24 hrs. at transport media	24 hours at room temperature

iii. Genital Specimen Collection

a). Materials and Reagents

- Disposable glove
- Speculum
- Sterile test tube
- Marker
- Wooden cotton swabs
- Physiological saline
- Transport media

b). Collection Procedure (Cervical Secretion)

Step	Action
1	No, cleaning of vagina for overnight.
2	Insert the speculum into the vagina canal to open the cervix
3	Remove mucus and secretions from the cervical area with swab, and discard the swab.
4	Firmly yet gently sample the endo-cervical canal with a new sterile swab.
5	Insert swabs into sterile test tube
6	Add physiological saline
7	Label the Patient's Name, Time of collection, Collectors name or initial and the culture Site on the specimen tube.
8	Transport specimen to the laboratory immediately unless we used transport media

c). Collection Procedure (Vaginal Secretion) (High Vaginal Swab)

Step	Action
1	Instruct the patient to open her genital area fully
2	Wipe away old secretion and discharge.
3	Obtain secretion from the mucosal membrane of the vaginal wall with a sterile swabs
4	Insert the swab in to sterile container such as tube
5	Label the Patient's Name, Time of collection, Collectors name or initial and the culture Site on the specimen tube.
6	Transport to laboratory immediately

Procedural Notes;

- Do not refrigerate the specimen
- Better to send the specimen immediately for observing motile pathogens such as Trichomonas vaginalis

d). Collection procedure (Urethral Discharge) (Both male and female)

Step	Action
1	Instruct the patient not to wash their urethra prior to sample collection
2	Wipe away old secretion and discharge
3	Obtain secretion from the tip of urethra
4	Insert the swab in to sterile container such as tube (preferably to transport media)
5	Label the Patient's Name, Time of collection, Collectors name or initial and the culture Site on the specimen tube.
6	Add sterile normal saline and transport to laboratory immediately unless we used transport media

Procedural Notes:

- Don’t try to massage the penis for production of secretion, it will contamination with normal skin flora

- Take care for not touch of urethra by labia of female (will cause contamination with normal skin flora)

e). Collection Procedure (Anal Swab)

Step	Action
1	Insert swab past anal sphincter, move swab from side to side
2	Allow the swab 10-30 seconds for better absorption, and withdraw
3	If contaminated with feces, recollect until swab free from any fecal contamination is obtained
5	Label the Patient's Name, Time of collection, Collectors name or initial and the culture Site on the specimen tube.
6	Add sterile normal saline and transport to laboratory immediately unless we used transport media (using transport media is preferable)

7. Sputum

i. Patient preparation

Preferably, before administration of antibiotics and must be collected before food (morning sputum).

ii. Type of Specimen

Specimen type	Container	Volume	Transport	Storage and Stability
Sputum	Sterile container with lid or use transport media	>1ml	2hrs at RT	< 24 hrs at RT

iii. Sputum Specimen Collection

a). Materials

- Gloves
- Marker
- Sterile container
- Waste disposal container

b). Collection Procedure

Step	**Action**
1	Have the patient rinse mouth with water to avoid excess oral flora
2	Instruct patient to cough deeply to produce a lower respiratory specimen (not postnasal

	fluid) on to a wide-mouth sterile container
3	Collect in to sterile container
4	Check specimens for quality: Volume (at least 3–5 ml) and describe sputum consistency (mucoids, purulent, bloody, or watery)
5	Label the Patient's Name, Time of collection, Collectors name or initial and the culture Site on the specimen tube.
6	Transport the specimen to laboratory

Procedural Notes:

- Collect the specimen outside the laboratory in a designated and open space away from other people.
- A hot drink or breathing deeply over a steam vessel (i.e. pan of boiling water) may help raise sputum.
- Avoid 24hr urine sputum collection
- Avoid swab specimen

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Elias Seyoum (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Adane Bitew (MSc, PhD, Associate Professor)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Amete mihret

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Elias Seyoum Deribe entitled: Species distribution and antifungal susceptibility profile of yeasts recovered from different clinical samples at Arsho laboratory, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

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