Isolation, identification, and antifungal resistance of *Candida* species from various samples

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Abstract

This study in Aden, Yemen, collected 67 samples from patients attending hospitals and laboratories. Samples were taken from different body areas, covering various age groups and genders. Fungal infections, particularly *Candida* species, are a significant cause of death worldwide. The emergence of azole-resistant *Candida* has raised concerns and highlighted the need for better antifungal agents.

In the study, 68.66% of the collected samples contained pathogenic *Candida* fungi. Some samples also showed bacterial growth or both bacteria and fungi. Suspected *Candida* growth on corn meal agar exhibited chlamydospore formation and germ tubes on human serum. Carbohydrate fermentation tests were positive for Glucose, Maltose, Galactose, and Xylose, while Sucrose and Lactose were negative. All *Candida* isolates (100%) were accurately identified to the species level using CHROM agar *Candida*. Candida's pathogenicity was linked to various virulence factors, including hydrolytic enzyme secretion. Nystatin was the most effective antifungal agent (100% sensitivity), while Fluconazole (73.92%) and Amphotericin B (52.17%) showed varying levels of sensitivity. However, Voriconazole, ketoconazole, and Miconazole displayed high resistance rates (89.13%, 84.78%, and 80.44%, respectively) against the isolated *Candida*.

This study concluded that *Candida* spp., particularly *Candida albicans*, were frequently found in the samples from patients, with vaginal swaps being a common site of infection. The identification of *Candida* isolates to the species level using CHROM agar *Candida* was accurate. Nystatin, Fluconazole, and Amphotericin B were the most effective antifungal agents against *Candida* spp.

Keywords: *Candida* species, Candidiasis, Antifungal resistance.

Introduction

Fungal infections caused by *Candida* species is one of the hospitals acquired infections in immunocompetent and immunocompromised patients(**49**). Etiology for the emergence of *Candida* spp., as important nosocomial pathogens include - prolonged use of antimicrobial agents, steroid therapy, malignancy, indwelling catheters, total parenteral nutrition etc.(**21**). The genus *Candida* comprises over 200 species, with 15 isolated from infections in humans and animals (**39**). Fungi are free-living, eukaryotic organisms that exist as yeasts, and as molds, or a combination of these two dimorphic fungi. *Candida* has emerged as a major group of opportunistic pathogens that cause superficial and invasive infections in humans(**18**). *Candida albicans* (*C. albicans*), is commonly inhabits in oral, vaginal mucosa and gastrointestinal tract of human, the most prevalent species in human superficial and invasive *Candida* infections, although there is concern over the increasing rates of non-*C. albicans* (NCA), infections worldwide(**2**). It causes opportunistic infections in immunocompromised patients ,produces allergic reactions and rarely causes morbidity and mortality(**8**).

It also causes a variety of infections that range from mucosal candidiasis to life-threatening disseminated candidiasis (**42**). *C. albicans* is still the most commonly isolated organism from bloodstream cultures, other Candida species, have emerged as clinically important pathogens in their own right (**51**). Different *Candida* isolates can play a role in invasive infections and colonization. Multiple characteristics of the *Candida* spp., have been proposed to be virulence factors that enable the organism to cause disseminated infections in a susceptible host. *Candida* virulence are caused by the switch between yeasts and hyphae and the organism's adhesion to surfaces (**41**). *Candida* species, can inhibit the host complement activation system, inactivate antimicrobial peptides and inhibit other immune cell functions, thus weakening host defenses .

Antifungal drugs targeting cell-wall (echinocandins) or the ergosterol biosynthesis pathway (azoles) are used as first options to treat infection, but *C. albicans* can develop resistance via the up regulation of azole drugs, the acquisition of mutations affecting the structure or expression of the azole target or the induction of compensatory changes in cell wall in response to echinocandins (**18**). The resistance of *Candida* spp., to antifungal drugs is becoming an increasingly difficult problem for treating its infections. The complicated structures of such as capsule and contribute to resistance to antifungals drugs (**45**).

The study aims to iidentify the prevalence of *Candida* species and analyse their susceptibility to locally available antifungal agents. Improve treatment strategies for fungal infections in the local population based on the findings obtained.

Materials and Methods

Study area and Study population

The current study was conducted in Aden Governorate, which was chosen due to its environmental conditions, including high temperature and strong moisture, that create a suitable environment for fungal growth and potential infection in individuals. The study was carried out in various healthcare facilities, including private and government hospitals, and private laboratories located in Aden, Yemen. The participating healthcare facilities were German Aden Hospital, Alreyada International Hospital, Algomhoria Hospital - Aden, Al-Awlaki Laboratories, Almadinah Medical Centre, Al-Razi Diagnostic Centre - Aden, Modern Medicine Laboratories, Al-Usra Laboratory, Aden Diagnostic Clinic, and Al-Bashaer Laboratory, Aden, Yemen.

Study population and *Candida* spp., isolates

The study was conducted on a diverse group of 67 clinical samples suspected to be infected with fungi, which were transferred to the Microbiology Laboratory at the University of Science and Technology in Aden. All pure isolates of *Candida* species from these clinical samples were selected during the period from May to August 2022, while other non-fungal samples such as bacteria were **excluded**. All these pure isolates were **included** in the study for various analyses and investigations.

Ethical considerations

Official approval has been obtained from both the department chair and the dean of the College of Sciences, allowing access solely to public hospitals. To ensure confidentiality, patient records/information have been anonymized. Consequently, all information was acquired directly from the hospital administration.

Sample collection

Vaginal swabs were obtained from women with a confirmed clinical diagnosis of vaginal yeast infection after examination by Gynaecologists in Aden City. For other samples, including oral, mucosa, skin, sputum and anal swab, both men and women with confirmed clinical diagnosis of yeast

infection were examined by Internal Medicine Doctors, Dermatologists, and Ear, Nose, and Throat doctors. The average age of the patients ranged from 2 to 55 years. All samples were stored under cooled conditions before being sent to the Microbiology Laboratory at the University of Science & Technology, Aden, Yemen.

Upon arrival at the laboratory, samples were subjected to direct examination and inoculated onto the surface of Sabouraud Dextrose Agar (SDA; HI Media, INDIA) supplemented with 250mg/l of chloramphenicol to suppress bacterial growth and ensure purity of the cultures. Petri dishes were then incubated at 37^{o} C until the appearance of colonies. The isolated yeasts were identified using classical methods (**52**), and the phenotypic identification was further confirmed using CHROM agar Candida (HI Media, India).

Direct microscopic examination

Gram Staining: The gram staining technique was employed to separate most bacteria into two groups based on cell wall composition. In this study, all fungi, including yeast cells of *C. albicans*, were observed as gram-positive single and budding yeast cells under the 100X objective (**48**).

Lactophenol Cotton Blue (LPCB) Staining: LPCB stain, both wet and dry smears, was used as a mounting medium and staining agent for preparing slides for microscopic examination of fungi. Positive results were indicated by the appearance of budding cells with or without pseudo-mycelium under the microscope (**48**).

Potassium Hydroxide (KOH) Dye: KOH (10%, 20%), was used as a reagent for qualitative procedures to detect fungal elements in clinical specimens by microscopic examination (https://www.cdc.gov/labtraining).

Methylene Blue Stains (MB): MB stains (0.06%), were used in one study for the diagnosis of fungal candidiasis, and they were found to be a fast and effective method for the early diagnosis of fungi (**23**). One drop of MB (0.06%) was added to the slide, covered with a cover slip, and then examined by direct microscopy.

Growth at 45°C: Fungal colonies were inoculated into Sabouraud Dextrose Broth (SDB) and incubated at 45°C. Turbidity was monitored at different time points over a period of 7 to 10 days. The ability of *C. dubliniensis* to grow at 45°C served as a distinguishing factor from *C. albicans* (**14**).

Test for Chlamydospores Formation: The suspected Candida cultures were inoculated on corn meal agar medium (CMAM) with a pH of 7, containing 4% glucose, 1% Tween 80, and 1.5% agar in double-distilled water. The plates were incubated at 25°C until colonies appeared (**19**).

Test for Germ Tube Formation: The suspected Candida cultures were inoculated into 0.5 ml of human serum in a small tube and incubated at 37°C until colonies appeared. After the desired incubation period, a loop-full of culture was placed on a glass slide and covered with a cover-slip (**13**).

Homolysis Activity: Homolysis activity was determined using a blood plate assay (**50**). Media were prepared by adding 7% fresh sheep blood to Sabouraud Glucose Agar (SGA), supplemented with glucose at a final concentration of 3% (w/v). Standard inoculums of *Candida* isolates (6.10^3 - 7.10^3 cells/ml) were deposited onto the medium. An additional 10 ml of saline without yeast cells was overlaid onto the same plate. Cultures were then incubated at 37°C for 24-48 hours, after which blood haemolysis was visually detected.

Carbohydrate Fermentation Tests: Fermentative yeasts recovered from clinical specimens produce carbon dioxide and alcohol during fermentation. Maltose, Lactose, Galactose, Xylose, Glucose, and Sucrose were used in the test. A mixture of 5 ml of carbohydrate medium (pH 7.4) containing 1% peptone, 1% sugar, 0.3% beef extract, 0.5% NaCl, and 0.2% Bromothymol blue in distilled water was dispensed into sterilized Durham tubes. Then, 0.2 ml of a saline suspension of the test organism was added, and tubes were incubated at 37°C for 10 days (**48**).

Phenotypic identification of yeasts

Chrome agar *Candida* medium kindly supplied by the chrome agar Company, (HI Media, India), has been recommended for rapid presumptive identification of many common *Candida* species. Yeast cultures were streaked on the medium surface and incubated at 37˚C until the appearance of colonies. Chemical colorimetric reaction on agar allows distinction between *C. albicans* (green colonies), *C. tropicalis* (metallic blue colonies), *C. krusei* (pink-fuzzy colonies), *C. glabrata* (mauve-dark pink colonies), and *C. parapsilosis* white-pale pink colonies (**33**).

Antifungal susceptibility test:

The disk diffusion method adopted by the National Committee for Clinical Laboratory Standards **(31)** was used to evaluate the in-vitro sensitivity of yeasts to 6 antifungal agents. Interpretative breakpoints of antifungal agents against *Candida* isolates shown in (table 1).

Table 1: Resistance of *Candida* species against several antifungal drugs

Antifungal agents	**Disc content**	**Zone diameter in mm**		
		R	**I**	**S**
Nystatin (NS)	100 Units/disk	< 10	10-14	≥ 15
Fluconazole (FLU)	25mg/disk	≤ 14	15-18	≥ 19
Amphotericin B (AmB)	100 Units/disk	< 10	10-14	≥ 15
Ketoconazole (KT)	10 mg/disk	≤ 20	21-27	≥ 28
Miconazole (MC)	10 mg/disk	≤ 11	12-19	≥ 20
Voriconazole (VRC)	1mg/disk	≤ 13	14-18	≥ 17

S= Susceptible; **I=** Intermediately susceptible; **R=** Resistant

Disk diffusion technique

The disk diffusion technique of Antibiotic Susceptibility Tests (AST) involves the assignment of antibiotic disks onto Muller Hinton Agar (MHA) supplemented with 2% glucose and 0.5µg of methylene blue/ml plates (**31**). Mueller Hinton Agar is recommended by Bauer, Kirby and Tuck for performing (AST) using a single disk of high concentration **(3).**

Select four-five well-grown colonies of the same morphology from SDA plate culture. By touching the top of each colony with a wire loop and the growth is transferred into MHA plate. Now spread the culture by streaking the sterile cotton swab three to four times over the entire surface of the agar, performing the streaking each time rotate the plate about 55-60 °C to ensure an even distribution of the inoculums. Allow the plates 3-5 minutes, to dry the surface of agar before applying the antimycotic disks. Moreover, through forceps place the proper antimicrobial-impregnated disks on the surface of the MHA agar culture plate and inverted for 24-72h at 37 ^{0}C. After 24-72hours of incubation hold the petri dish a few inches above on a black, nonreflecting background and lightened with reflected light, zones of complete growth inhibition around each of the disks are carefully examined with the scale on every petri plate.

Results

Patient characteristics and distribution of *Candida* species Sixty-seven samples with suspected fungi infection were identified over a 4-mounth period.

Total of fungus infections

A total of 67 various clinical samples were cultured on blood agar, and selective media (SDA) **fig. 1**. Forty-six samples 46/67 (68.66%) were positive for growth of pathogenic fungi and bacteria 16 (23.88%) while 5 (7.46%) mixed microbes (fungi and bacteria) **table 2.**

Figure 1. Photograph of Sabouraud dextrose agar (SDA) showing the isolates of *Candida albicans*

Table 2: Fungal clinical isolates distribution

Total samples	Fungus	bacteria	Mix
67	46 (68.66%)	16 (23.88%)	5 (7.46%)

Frequency of *Candida* species:

Patients suspected with Candidiasis 46 different samples from gave positive evidence for yeast infections in consecutive patients. All 46 isolates of yeast which gave positive results included: *Candida albicans* was the most prevalent fungal species (54%), followed by *C. tropicalis* (17%), *Candida glabrata* (15%), *C. krusei* (9%), *Candida dubliniensis* (2%), and *C. parapsilosis* (2%) **table3.**

Table 3: List of fungal clinical isolates and their frequency in percentage

Fungi	No. of isolates (n=46)	% Frequency
C. albicans	25	54.35%
C. tropicalis	8	17.39%
C. glabrata	7	15.22%
C. krusei	4	8.70%
C. dubliniensis	1	2.17%
C. parapsilosis	1	2.17%

Samples were collected from 67 patients, 15 (22.38%) of them were males and 52 (77.61%) were females (**table 4**). An overall rate of 68.66% (46/67) for Candidiasis was found in this study with female accounting for **(***n*=67, 40 59.70%) as against (*n*=67, 6 8.96%) for the male counterparts.

Table 4: The *Candida* spp. distribution between male and female patients

Six	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. krusei*	*C. dubliniensis*	*C. parapsilosis*	Total
Male	1	4	0	1	0	0	6/15 (8.96%)
Female	24	4	7	3	1	1	40/52 (59.70%)
total	25	8	7	4	1	1	46/67 (68.66%)

The incidence of *Candida* spp., infection by age group is summarized in **table 5**. The median age of the patients was 21 years (range, 2 years to 55 years), The results showed that 2.99% of Candidiasis

cases were in the age group (2-10), while (65.67%) of Candidiasis cases were in the age group (11-55). It is obvious that *C. albicans* was the predominant species isolated. Moreover, age groups of 11 to 55 years were the most infected individuals.

Table 5: The Prevalence of *Candida* spp. among age groups

Ages	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. krusei*	*C. dubliniensis*	*C. parapsilosis*	Total
2-10	1	1	0	0	0	0	2/5(2.99%)
11-55	24	7	7	4	1	1	44/62(65.67%)
Total	25	8	7	4	1	1	46/67(68.66%)

Direct microscopic examination

In direct microscopic examination of yeast cells on normal saline (**fig. 2**), budding cells were the most prevalent fungal structures found in the various clinical samples, present in 46 *Candida* spp., (46/46; 100 %). Our results showed pseudohyphae and true hyphae were observed in most of the vaginal samples (25/46; 54.35 %).

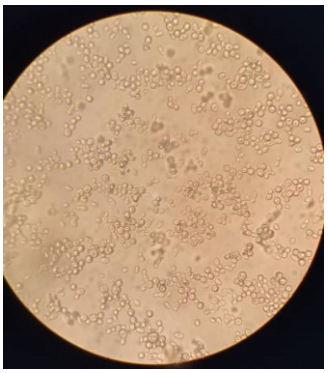

Figure 2. Yeast cells on Normal saline

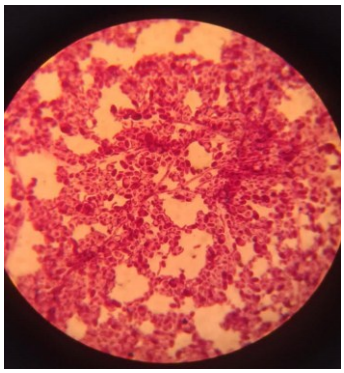

Figure 3. Yeast cells in Gram stain

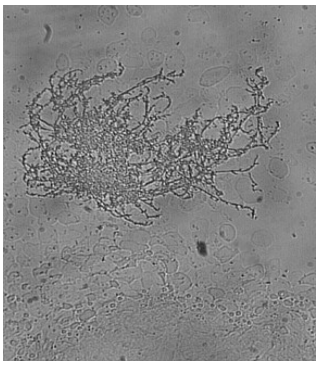

Figure 4. Yeast cells in potassium hydroxide 10X

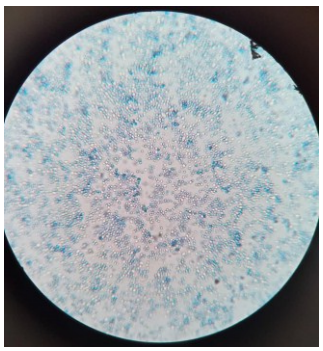

Figure 5. Yeast cells in Lactophenol blue cotton stain

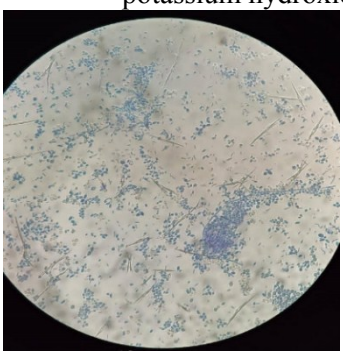

Figure 6. Yeast cells in Methylene blue 10X

Results of direct microscopy using Gram stain:

Candida yeast cells (fig. 3) can be detected in gram staining preparation. In Gram-stained smears, *Candida* appears as gram-positive budding yeast cells 44/67(65.67%). Large numbers of budding yeast forms are seen, many of which are intracellular, with some pseudohyphae.

Direct microscopy using KOH:

Direct microscopic examination of the 67 specimens was done using 10% KOH. Forty-one samples 41/67(61.19%) were KOH positive and 5 patients (7.5%) were negative (fig.4).

Results of direct microscopy using Lactophenol cotton blue (LPCB) stain of *Candida* spp.,:

Out of 67 cases tested, 17 (29.16%) were confirmed to be fungal pathogens using direct microscopy and Lactophenol cotton blue (LPCB) stain (see fig. 5).

Direct microscopy using Methylene blue stain:

Candida yeast cells can be detected in Methylene blue stain preparation (fig. 6). *Candida* appears as gram-positive budding yeast cells 36/67(67%).

Fungal Isolates

The distribution of fungal isolates with the associated specimens is illustrated in **table 6.** In this study, a total of 46 clinical specimens were *Candida* spp. The most common specimens processed in the different laboratories were V. swap (*n*=19, 41.30%), followed by throat swap (*n* = 9, 19.57%) and sputum (*n* = 7, 15.22%). The majority of the fungal isolates were recovered from the vaginal swap specimens. The most common fungal isolated were *C. albicans* 10(21.74%), followed by throat swap *C. albicans* 5(10.87%), *C. albicans* 3(6.52%), sputum. *C. albicans* 2(4.35%) isolated from Mouth, Ear and anal. *C. albicans* with 1(2.17%) isolated from Skin. *C. tropicalis* isolated from 2(4.35%) from each V. swap, Throat swap and Skin. *C. tropicalis* isolated from 1(2.17%) from each Sputum and anal. *C. glabrata* isolated from V. swap 3(6.53%), Throat swap 2(4.35%), Sputum and Mouth each from 1(2.17%).

Table 6: Distribution of fungal isolates with the specimens

Specimen	Fungi	*n* (%)	Total
Vaginal swap	***C. albicans***	10(21.74%)	19/46(41.30%)
	C. tropicalis	2(4.35%)	
	C. glabrata	3(6.53%)	
	C. krusei	2(4.35%)	
	C. dubliniensis	1(2.17%)	
	C. parapsilosis	1(2.17%)	
Throat swap	***C. albicans***	5(10.87%)	9(19.57%)
	C. tropicalis	2(4.35%)	
	C. glabrata	2(4.35%)	
Sputum	***C. albicans***	3(6.53%%)	7(15.22%)
	C. tropicalis	1(2.17%)	
	C. glabrata	1(2.17%)	
	C. krusei	2(4.35%)	
Mouth swap	***C. albicans***	2(4.35%)	3(6.52%)
	C. glabrata	1(2.17%)	
Ear swap	***C. albicans***	2(4.35%)	2(4.33%)
Skin scraping	***C. albicans***	1(2.17%)	3(6.52%)
	C. tropicalis	2(4.35%)	
Anal swap	***C. albicans***	2(4.35%)	3(6.52%)
	C. tropicalis	1(2.17%)	
Total			**46(100%)**

Carbohydrate fermentation tests for *Candida* spp.,

The carbohydrate fermentation tests for *Candida spp.,* are presented in **table 7.** All *Candida* spp., can be fermented all carbohydrate of glucose used in this study, but can't fermented lactose sugar. *Candida albicans* fermented of glucose, maltose, galactose and xylose were positive. *C. tropicalis* fermented sucrose, maltose, galactose and xylose. *C. glabrata* fermented of Maltose and Galactose but negative for fermentation of Sucrose and Xylose. *C. krusei* fermented of Xylose, but negative for Sucrose, Maltose and Galactose. *C. dubliniensis* fermented of Maltose and Galactose, and varies with

Xylose, but negative for Sucrose. *C. parapsilosis* fermented of Glucose, Sucrose, Maltose, Galactose and Xylose.

Table 7: Carbohydrate fermentation tests for *Candida* spp.,

Candida Spp.,	Glucose	Maltose	Galactose	Xylose	Sucrose	Lactose
C. albicans	+	+	+	+	+	-
C. tropicalis	+	+	+	+	+	-
C. glabrata	+	+	+	-	-	-
C. krusei	+	-	-	+	-	-
C. dubliniensis	+	+	+	+/-	-	-
C. parapsilosis	+	+	+	+	+	-

Profile Phenotypic of *Candida* spp.

The confirmatory test for *C. albicans* were carried by using two different medias i.e., corn meal agar for production of chlamydospores. The other confirmatory test for *C. albicans* were carried by using germ tube test (**fig.7**). Our results showed that only *C. albicans* had ability to produce chlamydospores and germ tube formation (**table 8**).

Table 8: Microscopic characters, for *Candida* spp. isolated from various samples

Candida spp.	Chlamydospores	Germ tube
C. albicans	+	+
C. tropicalis	-	-
C. glabrata	-	-
C. krusei	-	-
C. dubliniensis	-	-
C. parapsilosis	-	-

Figure 7. Micrograph of Germ tube formation in *Candida species*; two *C. albicans* (short, slender tube structure without constrictions-Germ tube positive

Hemolytic activity

Hemolytic activity of *C. albicans*, C. *tropicalis,* C. *glabrata, C. krusei, C. parapsilosis* and *C. dubliniensis* strains were streaked onto SGA (**table 9**). The resulting cultures showed the presence of a translucent halo around the inoculum site indicated 13/25 (52%) positive hemolytic activity (**fig.8**).

Figure 8. *Candida albicans* β hemolysis in Sabouraud dextrose agar with (7% human blood agar; 250 mg/L chloramphenicol)

Table 9: Enzymatic hemolytic activity of *Candida* spp.,

Candida spp.	***C. albicans***	***C. tropicalis***	***C. glabrata***	***C. krusei***	***C. dubliniensis***	***C. parapsilosis***	**Total**
Hemolysis	9	1	2	0	1	0	13

Antifungal Susceptibility

Sensitivity test of various clinical specimens to 6 different antifungal therapeutic agents which included Amphotericin-B (AmB), Ketoconazole (KT), Fluconazole (MC), Fluconazole (FLU), Voriconazole (VRC) and Nystatin (NS), (**table 10**). Nystatin exhibited a strong activity towards all *Candida* strains 46/46. Fluconazole was effective against most of the tested strains 35/46. Amphotericin-B was effective against most of the tested strains 24/46. *C. dubliniensis* and *C. parapsilosis* were sensitive to the different antifungal agents used in our study.

Data of the present research showed also that several isolates of *Candida* spp., were resistant to one or more of the frequently prescribed azole antifungal agents.

Table 10: General patterns of Antifungal susceptibility test of *Candida* species

Species		**Amphotericin B**	**Voriconazole**	**Ketoconazole**	**Fluconazole**	**Miconazole**	**Nystatin**
C. albicans	**S**	14/25 56%	0/25	3/25	19/25 76%	3/25	25/25 100%
	I	7	2	1	2	2	0
	R	4	23	21	4	20	0
C. tropicalis	**S**	2/8 25%	0/8	0/8	4/8 50%	0/8	8/8 100%
	I	3	1	0	3	2	0
	R	3	7	8	1	6	0
C. glabrata	**S**	3/7 42.86%	0/7	0/7	5/7 71.43	0/7	7/7 100%
	I	1	0	0	2	0	0
	R	3	7	7	0	7	0
C. krusei	**S**	3/4	0/4	0/4	4/4	0/4	4/4(100%)
	I	0	0	1	0	0	0
	R	1	4	3	0	4	0
C. dubliniensis	**S**	1/1	1/1	1/1	1/1	1/1	1/1
	I	0	0	0	0	0	0
	R	0	0	0	0	0	0
C. parapsilosis	**S**	1/1	1/1	1/1	1/1	1/1	1/1
	I	0	0	0	0	0	0
	R	0	0	0	0	0	0
Total of Candida spp.,	**S**	24/46 52.17%	2	5	34/46 73.91%	5	46/46 100%
	I	11/	3	2	7	4	0
	R	11	41/46 89.13%	39/46 84.78%	5	37/46 80.43%	0

I= Intermediately susceptible; **R=** Resistant; **S=** Susceptible.

Discussion

In this study, the isolated strains of *Candida* spp. were sub-cultured onto Sabouraud Dextrose Agar (SDA), a commonly used medium for the isolation of *Candida* spp. The colonies typically appear as cream-colored to yellow and can exhibit varying textures, such as smooth, glistening, or dry, depending on the species. Under optimal nutrient conditions, yeast cells grow in the log phase as budding cells, which are spherical to oval in shape. (**43;16**).

The mean age of patients in the study was 21 years (range: 2 to 55 years). *Candida* spp. was the predominant species isolated from clinical samples, accounting for 68.66% of the cases. This finding aligns with a study in Hajjah Governorate, Yemen, where *Candida* spp. also predominated 70.45% (**11**). Our study's prevalence was higher than the overall prevalence reported in Lagos, Southwest Nigeria, which was 21.5% (**4**), but slightly lower than a study in Poland where the prevalence was 75.47% (**22**). Differences in prevalence rates may be attributed to variations in personal hygiene practices and social awareness in different societies.

Gram staining is a widely used diagnostic method for bacterial infections, but it has also been employed for identifying yeast cells like *Candida albicans*. *C. albicans* is described as a dimorphic fungus, existing in blastospore and hyphal forms (**53**). Gram staining shows Gram-positive yeast cells (dark blue/purple color). Direct microscopic examination using KOH effectively identified fungal elements in 61.19% of the samples, while negative results (7.5%) may indicate the absence of fungal elements in those samples. Lactophenol cotton blue (LPCB) stain detected fungal pathogens in 29.16% of the samples, suggesting its effectiveness in identifying *Candida* species. Methylene blue stain successfully identified *Candida* yeast cells in 67% of the samples, indicating a significant presence of *Candida*. Further tests and species identification are recommended for comprehensive diagnosis.

Candida albicans (54.35%), was reported to be the most virulent among the *Candida* spp., and can cause several forms of candidiasis, this finding is partially consistent with the several works reported by **(10)** in Yemen (61.2%), and **(32)** in Nigeria (60%), and higher than finding by **(6)** in Ethiopia (41.4%), which mean that *C. albicans* is highly adapted to the human mucosal surfaces and possesses virulence factors formation (**1**).

Female patients had a higher prevalence rate compared to male patients (40/52 (59.70%) vs 6/15 8.96%). The factors that influence the severity of this Candidiasis, including continuous, general health problems like diabetes or hypertension, and the age of the patients. Our result not in line with the study of (**35),** Sir Sunderlal Hospital, Banaras Hindu University ,Varanasi where, India, were they isolated Candida spp. And they found 51 (64.56) in male, and 28 (35.44) in female. Regardless of these problems, *Candida* spp., are the most frequently diagnosed (**11**). According to the study by (**11**), our results showed higher rates of *Candida* spp. infection among female patients compared to males, indicating that females are more vulnerable to *Candida* infections.

Our results showed a significantly higher percentage of females infected by *Candida* spp., with greater intensity of growth in the female group compared to males, presenting microbiologically as intermediate, intense, and abundant growths of yeast. Moreover, *Candida* spp. exhibited higher rates among female patients than male patients, indicating the greater vulnerability of females to candidiasis, suggesting a higher susceptibility of females to candidiasis.

Moreover, age groups of 11 to 55 years were the most infected individuals. Our result in line with the study of (**30).** When they found Candidemia was found more commonly in the age group of 11-90 years. One of reasons for more urethral infection among young adults compared to children and other ages, may due to sexual activities.

C. albicans was the most common species (54.35%) followed by the other (NCA), remaining unspecified. Our results supported a work done in Iran, (**26**). *C. albicans* was the most common species among the isolates (93.9%) followed by (NCA). In agreement with our results, a study by (**20**),

showed that *C. albicans* (32.7%) were the most frequent fungal isolates. There are several other studies from other countries which have reported this shift from *C. albicans* to *C. tropicalis* as the major cause of candidemia (**28**). *C. tropicalis* has been found to be the most common *Candida* spp., (19/66) causing candidemia. Other studies on candidemia from western India by Rajni *et al.,* have also found *C. tropicalis* (36/95; 38%), to the commonest cause of candidemia (**37**).

Hemolysin is a virulence factor believed to contribute to *Candida* pathogenesis. Its secretion, followed by iron acquisition, facilitates hyphal invasion in disseminated candidiasis (**9**). Research on Hemolysin activity in *C. albicans* is limited, and no studies have been conducted on its activity in oral *C. albicans* isolates from type 2 diabetes mellitus patients. Hemolytic factor production varies among *Candida* spp., with *C. parapsilosis* being the least hemolytic species. No interspecies differences in beta-hemolytic activities are found among isolates belonging to *C. parapsilosis*, *C. albicans, C. glabrata, C. krusei, and C. tropicalis*, which aligns with our findings (**15**). Interestingly, *C. albicans* isolates from female diabetes mellitus patients showed significantly higher hemolytic activity than those from males, and female patients had higher fasting plasma glucose values than males. Further investigations are needed to clarify this issue.

The germ tube test is a rapid and highly reliable test for the presumptive identification of *C. albicans* and it has been widely used for many years. This technique is a simple and cheap alternative to other rapid test methods and may, therefore, be favored by laboratories trying to work economically (**27**). In fact, *C. albicans* is found in the environment and is usually a part of the natural flora of human skin and mucous membranes. Many other fungi have the ability to produce chlamydospores and germ tube formation as well. For example, some other strains of *Candida* (such as *Candida tropicalis*) can produce chlamydospores, and some species of *Aspergillus* are among the other fungi that produce chlamydospores (**24**). Additionally, some other fungi can also form germ tubes, such as other species of *Candida* (like *Candida dubliniensis*), some *Cryptococcus* species, and certain strains of filamentous fungi.

Culture of the clinical samples was the most sensitive method as compared to gram stain as noted in other study (**34**). In the present study, similar results were obtained regarding speciation of *Candida* by CHROM agar methods but, CHROM agar has the advantage of being rapid, simple and cost effective as compared to conventional methods which are slow, technically demanding and expensive (**44**). CHROM agar *Candida* took 24-48 hours for the speciation of *Candida* isolates. Phenotypic assay based on the CHROM agar *Candida* was preliminary presumptive test, but are not accurate and precise for *Candida* identification, earlier studies noticing the same results (**17**). CHROM agar *Candida* was found to accurately identify over 95% of stock and clinical isolates of *C. albicans*, *C. tropicalis*, *C. krusei* and *C. glabrata* (**47**). CHROM agar *Candida* has also been used for identification of *Candida spp.,* directly from various clinical specimens (**29**), but are not accurate and precise for *Candida* identification, earlier studies noticing the same results (**12**).

This has important threatening infections like candidemia where rapid identification and antifungal susceptibility results are extremely important in determining the correct therapeutic measures to be taken (**40**). We found that (NS) was more active than (FLU) and (AmB) against all the *Candida* isolates tested. Data of the present work showed also that several isolates of *Candida* were resistant to one or more of the frequently prescribed azole antifungal agents.

Nystatin is a polyene antifungal that is frequently used as a topical agent in the treatment of oral candidiasis **(46)**. The antifungal susceptibility results, showed that, the highest sensitivity of antifungals against was (NS) than (FLU) than (AmB). This finding is **(10)** (Nystatin 127 (94.8%); Fluconazole 122 (91.04%); Amphotericin B 119(88.8%), and not in line with the work of **(6)**, (were all *Candida* spp.,100% susceptible to voriconazole, caspofungin, and micafungin). Nystatin pastilles at a dose of 400,000 IU resulted in a higher mycological cure rate than the dose of 200,000 IU. With regard to treatment duration, administration of (NS) pastilles for 4 weeks showed better clinical

efficacy (76.9%) than its administration for 2 weeks (**25**). Poor taste and gastrointestinal adverse reaction were the most common adverse effects of (NS).

Amphotericin B is generally regarded to have the broadest spectrum of antifungal activity and is used in cases of serious and invasive *Candida* infections, such as in the treatment of systemic infection in hospitalized patients. Resistance to (AmB) remains uncommon during treatment. Resistance to (AmB) remains uncommon during treatment, but reports of isolates exhibiting elevated minimum inhibitory concentration (MC) have become more frequent (**36**).

Fluconazole is active against *Candida* species; the low sensitivity of some antifungal under interest previously study (**20**) may be due to development of gene resistance against some fungal isolates when compared with (VRC), (KT) and Miconazole. Prolonged or repeated exposure to low-dose (FLU) was associated with increased frequency of (FLU) resistance in *C. albicans* isolates (**5**). In our study, 64.5% of total *Candida* species were sensitive to (FLU), and dose-dependent susceptibility was seen in 6 isolates. Swinn et al. reported 55% and 65% of the Candida and (NCA) isolates were susceptible to (FLU) (**38)**. Resistance of *Candida* spp., to the antifungal agents (VRC), (KT) and (MC) were not in line with previously study such as (**20**).

Recommendation

We recommended the increasing the examination must be implemented genital system, digestive system and skin, which may be cause the disease, of fungi in the Aden city, Yemen. We recommended Doctors should encourage patients to make cultural tests to detect the cause of the disease and give the best treatment. Antimycotic such as nystatin, fluconazole and amphotericin B needs further examination to check their activity.

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فصل وتشخيص ومقاومة الفطريات من نوع الكانديدا من عينات مختلفة

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الملخص

هذه الدراسة التي أجريت في عدن، اليمن، جمعت 67 عينة من المرضى الذين يراجعون المستشفيات الحكومية والخاصة والمختبرات الخاصة. جُمعت العينات من مناطق مختلفة في الجسم، تغطي مجموعات عمرية وجنسيات متنوعة. العدوى الفطرية، وخاصة نوع *Candida*، هي سبب رئيسي للوفيات في جميع أنحاء العالم. ظهور سلالات *Candida* مقاومة للأزول أثار مخاوف وأبرز حاجة إلى وجود عوامل مضادة فطرية أكثر فعالية.

في الدراسة، احتوت 68.66% من العينات المجمعة على فطريات *Candida* الممرضة. بعض العينات أظهرت أيضًا نمو بكتيري أو نمو لكل من البكتيريا والفطريات. وقد أظهر نمو *Candida* المشتبه فيه على محيط الذرة وجود تشكيلات جراثيم على مصل الإنسان. أظهرت اختبارات تخمر الكربوهيدرات نتائج إيجابية للغلوكوز والمالتوز والجالاكتوز والزيلوز، بينما كانت السكروز واللاكتوز سلبية. تم تحديد جميع العزلات من الفطر *Candida* (100%) بدقة إلى مستوى النوع باستخدام محيط CHROM agar *Candida*. كانت الفطريات *Candida* مسبباته مرتبطة بعوامل الضراوة المختلفة، بما في ذلك إفراز الإنزيمات المحللة لكريات الدم الحمراء. كان النيستاتين أكثر وكيل مضاد للفطريات فعالية (100% حساسية)، بينما أظهر الفلوكونازول (73.92%) وأمفوتريسين ب (52.17%) مستويات حساسية متفاوتة. ومع ذلك، أظهر الفوريكونازول وكيتوكونازول الميكونازول معدلات مقاومة عالية (89.13%، 84.78%، و80.44% على التوالي) ضد فطريات *Candida* المعزولة.

خلصت الدراسة إلى أن نوع *Candida*، وبخاصة *Candida albicans*، يوجد بشكل متكرر في العينات المأخوذة من المرضى، وأن الفحص والتشخيص بناءً على محيط CHROM agar *Candida* كان دقيقاً. وكان النيستاتين والفلوكونازول وأمفوتريسين ب العوامل المضادة للفطريات الأكثر فعالية ضد نوع *Candida*.

الكلمات المفتاحية: أنواع المبيضات، داء المبيضات، مقاومة الفطريات.