BIODIVERSITAS
Volume 24, Number 7, July 2023
Pages: 4166-4174

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240761

Phenotypic and genotypic diversity of yeast isolated from cancer patients and their environment and their antifungal sensitivity pattern

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Manuscript received: 31 May 2023. Revision accepted: 30 July 2023.

Abstract. *Al-Darraji ZDG, Mohammed-Ameen MKM. 2023. Phenotypic and genotypic diversity of yeast isolated from cancer patients and their environment and their antifungal sensitivity pattern. Biodiversitas 24: 4166-4174.* This study aimed to investigate yeast diversity and their antifungal susceptibility patterns in 853 samples collected from cancer patients, their apparently healthy companions, their beds, their tables, and indoor hospital air. The samples were cultured and identified using classical phenotypic characteristics and a molecular method utilizing amplification of ITS conservative regions of rRNA. The sum of phenotyping and genotyping identification revealed that 102 yeast isolates included 6 genera and 14 species. *Candida* sp. was the most dominant genera (84.31%), followed by *Naganishia* sp. (10.78%). *Candida albicans* (24.50%) and *C. krusei* (16.66%) were the most prevalent yeast species. Fluconazole, caspofungin, and voriconazole exhibited potent antifungal activities against the most yeast species with low minimum inhibitory concentration (MIC) values compared with high MIC (64 µg/mL) values against *Meyerozyma guilliermondi*, *Naganishia difflunes*, and *C. tropicalis*. To conclude, the antifungal activity was isolate- and species-specific and the higher antifungal concentrations led to a more-rapid expression of activity. The high isolation rate of yeasts from beds and tables, advocated the use of various monitoring systems to ensure thorough cleaning and consistent disinfection of surfaces around patients and health care providers. The study findings showed that the molecular method was superior to the phenotyping technique in identifying yeast isolates. While similar research has been performed in different locations, the current project represents a unique milestone for this particular institution, and offers an opportunity to advance research in this area.

Keywords: Antifungal, cancer patients, environment, genotyping, phenotyping, yeasts

INTRODUCTION

A diverse variety of biological contaminants, including bacteria, fungus, viruses, algae, insects, and their byproducts, including endotoxins, mycotoxins, volatile organic compounds, etc., can be found in the indoor air environment (Moldoveanu 2015; EPA 2020). Biological pollutants have been divided into groups based on their potential to cause allergic, infectious, poisonous, or inflammatory reactions in people. Biological contamination in the interior environment and its possible origins in the development of various illnesses are currently not well known (Kumar et al. 2021). Bioaerosols are biologically produced aerosols such as metabolites, poisons, or microbe fragments that are widespread in the environment. International interest in bioaerosols is increasing quickly in order to further develop the comprehension of their identification, quantification, distribution, and health implications (e.g., infectious and respiratory disorders, allergies, and cancer) (Kim et al. 2018). Bioaerosols may contain pathogenic and/or non-pathogenic dead or alive microorganisms (e.g., viruses, bacteria, and fungi) (Karimpour Roshan et al. 2019). Bioaerosols have a tiny size and light weight; therefore, they are easily transported from one habitat to another (Van Leuken et al. 2016).

Microorganisms are commonly found everywhere in the environment, including hospital wards, and they are critical for human health. As a result, it is critical to analyze microorganisms in hospital air both quantitatively and qualitatively (Montazeri et al. 2020). The exposure to a high inoculum of fungi in their natural habitat is another key socioeconomic and geoecological factor that affects the prevalence and incidence of fungal infection globally (Bongomin 2017). The incidence of fungal infections has increased in the last decade, owing in part to recent medical advances such as increased survival of immuno-compromised patients, widespread use of antibiotics, immunosuppressors, or medical devices such as catheters (Gabaldón and Carreté 2016; Rayens and Norris 2022).

Yeasts are becoming more prevalent, especially for those suffering from cancer, immune deficiency syndrome, renal failure, or who take immunosuppressive medications (Calderone and Fronzi 2001). For instance, there are over 30 different species among the etiological causes of invasive Candidiasis (Gabaldón et al. 2016), while more than half are regarded as extremely uncommon (i.e., 0.1% of cases). As a consequence, the concept of emerging fungal pathogens is now prevalent (Papon et al. 2013). Clinical diagnosis of nosocomial *Candida* infections is challenging, and treatment is ineffective. As a result, it's critical to obtain a quick and correct laboratory diagnosis in order to administer the proper antifungal therapy (Sharma and Aggarwal 2013). Opportunistic yeasts are highly similar related species, and due to the numerous limitations of traditional yeast identification methods, they are frequently misidentified or reported as unknown yeast

species. Some of these species, on the other hand, have intrinsic or acquired resistance to current antifungal drugs (Walsh et al. 2004).

The genetic mechanisms of resistance that fungi possess, in addition to any alterations in the permeability barrier or target location, lead to an increase in resistance to antifungal drugs (Sobel 2016). As a result, identifying and determining their susceptibility profiles to antifungal medicines is critical for optimal patient treatment and infection control in both nosocomial and community-acquired settings. To overcome the limits of phenotypic methods of yeast recognition, improved molecular techniques, such as molecular identification approaches based on polymerase chain reaction (PCR), such as PCR-RFLP, real-time PCR, or other techniques, have been introduced in recent decades (Mirhendi et al. 2005). These techniques are definitely more sensitive and faster than conventional techniques, although they do not effectively distinguish all yeast species (Mirhendi et al. 2006). The DNA sequencing technique is recognized as one of the most effective quick standard methods for fungus diagnosis (Alkhuwailidy and Alrufae 2022).

Therefore, this work aimed to study the phenotyping and genotyping diversity of yeasts isolated from cancer patients and their environments, in addition to the antifungal susceptibility patterns. While phenotypic and genotypic diversity of yeast isolated from cancer patients, has previously been executed in Iraq and Basra, this is the first time such an endeavor is being undertaken in our hospital setting. While similar undertakings have taken place in different locations, the current project represents a unique milestone for this particular institution, and presents an opportunity for advancements within this area of research.

MATERIALS AND METHODS

Clinical specimen collection

A total of 191 skin swabs were collected from patients (males and females) (17-70 years old) undergoing chemotherapy treatment for different types of cancer at The Center of Oncology and Hematology, Al-Sader Teaching Hospital, and from children (1 month-14 years old) at Basrah Children Specialist Hospital, Basrah governorate, Iraq. The specimens were collected during the period from November 2021 (rainy season) to April 2022 (dry season). In addition, 150 swabs were collected from apparently healthy companions. The samples were collected under complete aseptic precaution using sterile cotton swabs, labeled, and transported immediately to the microbiology laboratory. The swabs were inoculated directly onto potato dextrose agar (PDA; Himedia, India) and Sabouraud Dextrose Agar medium (SDA; Himedia, India) supplemented with chloramphenicol (250 mg/L) to prevent the growth of bacteria. The inoculated plates were incubated at 37°C for (1-14) days.

Environmental samples

A total of 512 environmental samples were collected from different sources, including 130 air samples from inside the hospital (the hall corridor and the cancer patients' ward). The air samples were collected by the open plate method according to Nageen et al. (2021), in which the opened PDA plates were exposed to air for 10-15 minu, then tightly closed and submitted to laboratory as quickly as possible. Also, 382 samples were collected from patients' beds and the tables of a patient lying in the hall using sterile cotton swabs (191 samples each). The swabs were inoculated onto PDA and SDA media supplemented with chloramphenicol. All environmental samples were incubated at 25°C for (1-14) days under aerobic conditions (Al-akeel et al. 2013). After incubation, the cultured media were macroscopically investigated to determine the shape, color and texture of the developed colonies and microscopically by using lactophenol cotton blue.

Phenotypic identification of yeasts

Growth on chromogenic agar medium

As a basic technique to identify yeast species in 48 h, the chromogenic media's ability to identify the species of yeast based on the color of the colony was used. Chromogenic media (Thermo Fisher Scientific, USA) was sterilized and prepared according to manufacturer instructions. Each isolate was subcultured onto CHROM *Candida* agar and incubated at 37°C for 48 h. Daily inspection of the inoculated plates was carried out, and the growth morphology was recorded (Scharmann et al. 2020).

Germ tube test

Yeast isolates were activated by inoculation onto SDA and then incubated at 37°C for 1-3 days. To evaluate the ability of yeast for germ tube formation, a small portion of the activated colony was added to a tube containing 0.5 mL of human serum and incubated at 37°C for 3 h. After incubation, the samples were inspected microscopically at 10X and 40X to detect the creation of an extended tube without constriction from mother cells, which indicated germ tube formation (Matare et al. 2017).

Chlamydospores forming test

After the yeast samples were activated on SDA medium for 24-48 hrs at 37°C, a small portion of this activated sample was taken by a sterile loop and inoculated onto corn meal agar- tween 80 medium and incubated for 3-7 days at 25°C. Then a part of the yeast colonies was taken and placed on a clean glass slide, covered gently with coverslip, and stained with lactophenol cotton blue. The sequamous spore production was examined microscopically at 40X (Böttcher et al. 2016).

Growth onto tobacco agar medium

The identification of both *C. albicans* and *C. dubliniensis* isolates was confirmed by subculture on tobacco agar medium, which discriminates between them. The medium was prepared as described by Tendolkar et al. (2003). Simply, 50 g of tobacco from a commercial cigarette currently available (tar, 6 mg; nicotine, 0.5 mg)

was mixed with 1000 mL of distilled water, boiled for 30 min and filtered by a clean and dry gauze. Then 20 g of agar was added to the filtrate, bringing the volume to 1 liter. Tobacco agar plates were streaked with a small amount of fresh subcultured colonies, incubated at 30°C, and monitored daily for up to 4 days for colony characteristics, especially the color and formation of fringes.

Molecular identification of yeasts

DNA extraction

Yeasts were subcultured onto Sabouraud dextrose agar plates and incubated at 30°C for 2 days, and DNA was extracted and purified according to the manufacturer`s protocol (Gene Aid, Taiwan). The isolated DNA was examined using 0.8% Agarose gel electrophoresis as described by Sambrook et al. (1989).

Polymerase chain reaction (PCR) amplification

PCR was used for amplification of the ITS conservative regions of rRNA using the universal primers ITS1-F 5′-TCC GTA GGT GAA CCT GCG G-3′ and ITS4-R 5′-TCC TCC GCT TAT TGA TAT GC-3′ according to Mirhendi et al. (2006) with some modifications. PCR was carried out in a final volume of 25 µL. The PCR reaction mix consisted of 12 µL of master mix, 1.0 µL of template DNA, 1.0 µL of each forward and reverse primer, and 10.0 µL ddH2O. Amplification was performed in a Thermocycler (Thermo Scientific, USA) with the following conditions: 94°C for 1 min (1 cycle), 94°C for 1 min, 55.5°C for 45 s, 72°C for 1 min (35 cycles), and 72°C for 10 min (1 cycle). The amplified PCR products were visualized by 2.0% (w/v) agarose gel electrophoresis in 1XTBE buffer, stained with ethidium bromide, and a 100-bp DNA ladder (BIONEER) was used for determining the size of the PCR products. For Sequencing, The PCR products were sent for analysis by Microgen Company and identified in "BLAST" provided by the NCBI.

Antifungal susceptibility test for yeast

The minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) of fluconazole (FLC), voriconazole (VRC), and caspofungin (CAS), against yeast isolates were tested using the broth microdilution method in 96-well microtiter plates as recommended by the Clinical and Laboratory Standards Institute (CLSI 2017). Two-fold serial dilutions of fluconazole, voriconazole, and caspofungin at concentrations (64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, and 0.125 µg/mL) were prepared. Hundred microliters of each antifungal concentration were delivered to 100 µL of SDA broth in each microtiter plate well. From each yeast isolate, 100 µL inoculums at a concentration of 1.5×10^6 CFU/mL (corresponding to 0.5 McFarland turbidity standards) were added to each microtiter plate well. Positive control experiments were carried out using the yeast isolate in the SDA broth medium, while negative control wells just contained SDA broth. The microtiter plates were incubated for 24 hrs at 37°C. The MIC was determined as the lowest concentration that inhibited yeast growth. A 100 µL aliquots from each well that did not show any yeast growth after incubation were transferred to SDA plates and incubated at 37°C for 24 h. The lowest concentration without any yeast growth or less than three CFUs was taken as MFC. The antifungal susceptibility testing was performed in duplicate.

RESULTS AND DISCUSSION

Isolation, distribution, and phenotypic identification of yeasts

Isolations of yeasts from the clinical and environmental samples were done on PDA and SDA media supplemented with chloramphenicol, and the yeast colonies were first selected based on colony morphology, aiming at select colonies of varying morphology. Yeast isolation from different samples proved that a total of 102 yeasts were isolated and distributed as follows: beds (29), patients (28), tables (22), apparently healthy companions (18), and indoor air (5) (Table 1). That means beds, patients, and tables were the most likely sources of yeast compared to air samples. A variety of monitoring systems should be used to ensure complete cleaning and consistent disinfection of surfaces near patients or potentially touched by patients and healthcare professionals (Centers for Disease Control and Prevention 2003).

The high prevalence of yeast among patients is attributed to immunocompromised situations and prolonged use of antibiotics (Martins et al. 2014). Also, the incidence of yeasts among apparently healthy cancer patient companions was high (18) because the yeasts are commensal to healthy humans (Sardi et al. 2013). At present, there is a shortage of awareness of biological contamination in the indoor environment and its possible sources of illness transmission (Kumar et al. 2021). All the pure yeast cultures were identified by phenotypic characters using the chromogenic agar medium, which identifies yeasts to species level based on the color of the colony (Figure 1.A). Chromogenic medium is frequently carried out to detect and identify the yeasts to the species level in a cost-efficient and more rapid way (Scharmann et al. 2020). The germ tube test detects the ability of yeast to form long tube like projections extending from the yeast cells (Figure 1.B). The germ tube formation in human serum is a rapid method for identification of *Candida* sp., these morphological changes often represent a response of the fungus to changing environmental conditions and may permit the fungus to adapt to different biological niches (Deorukhkar et al. 2012). Chlamydospores forming test was carried out for determination the ability of yeast for the production of sequamous spores (Figure 1.C). Chlamydospores are produced by a variety of fungi, including the closely related human-pathogenic dimorphic yeasts *Candida albicans* and *C. dubliniensis* (Böttcher et al. 2016). *C. albicans and C. dubliniensis* are the only known producers of chlamydospores in addition to yeasts, pseudohyphae, and genuine hyphae (Moran et al. 2012). The growth on tobacco agar medium was performed for discrimination between yeasts depending on the colony

characteristics, especially the color and formation of fringes (Figures 1D and 1E). Khan et al. (2004) utilized tobacco agar to differentiate between *C. albicans* and *C. dubliniensis*; the results suggested that this method is a straightforward tool for screening *C. albicans* and non-*albicans* yeast. The ability of tobacco agar to identify *C. dubliniensis* from *C. albicans* was tested. This medium provides an easy way to distinguish *C. dubliniensis* from *C. albicans* (Al Mosaid et al. 2001).

The phenotyping examination of 102 clinical and environmental yeast isolates revealed that 77 isolates were identified to species level, while 25 isolates were untyped. 77 out of 102 (75.49%) yeast isolates belonged to *Candida* sp. These results coincided with the previous findings by Tang et al. (2014) and Xia and Wang (2019). The most prevalent *Candida* spp. were *C. albicans* 22 (21.56%), *C. krusei* 17 (16.66%), *C. dubliniensis* 15 (14.70%), and *C. glabrata* 14 (13.72%). *C. albicans* is a common opportunistic pathogen and a commensal microbe in human systems. It takes on several morphogenic forms in order to thrive in diverse host niches with varying environmental conditions (Prasad and Tippana 2023). Our findings about the dominant species were nearly consistent with the results previously mentioned by Bitew and Abebaw (2018) and Xiao et al. (2022). Aslani et al. (2018) shed light on the prevalence of *Candida* species isolated from the oral cavity of Iranian cancer patients, including new *Candida* species and uncommon yeasts. They recorded that oral Candidiasis was more common in patients with hematological malignancies. The obtained findings showed that the isolated yeasts belonged to groups of ascomycetes and basidiomycetes, where ascomycetes yeasts appeared in their sexual and asexual phases, and the appearance of the anamorphic was the most frequent 86 (84.31%), while the teleomorphic was for one species only 1 (0.98%), while the basidia fungi appeared in the anamorphic phase only 15 (14.70%) (Figure 2).

These results go hand in hand with those recorded in a previous study by Minati and Ameen (2020). Rostami et al. (2016) assessed indoor and outdoor airborne fungi in an educational, research and treatment center. They reported that 62.03% of indoor samples and 96.42% of outdoor samples were positive for fungi, and the most isolated fungi were yeast species (17.12%). The prevalent fungal group in all examined sources was the anamorphic Ascomycota (85.34%), followed by Zygomycota (5.46%). Teleomorphic Ascomycota was only at (3.64%) and Basidiomycota accounted for less than 1%.

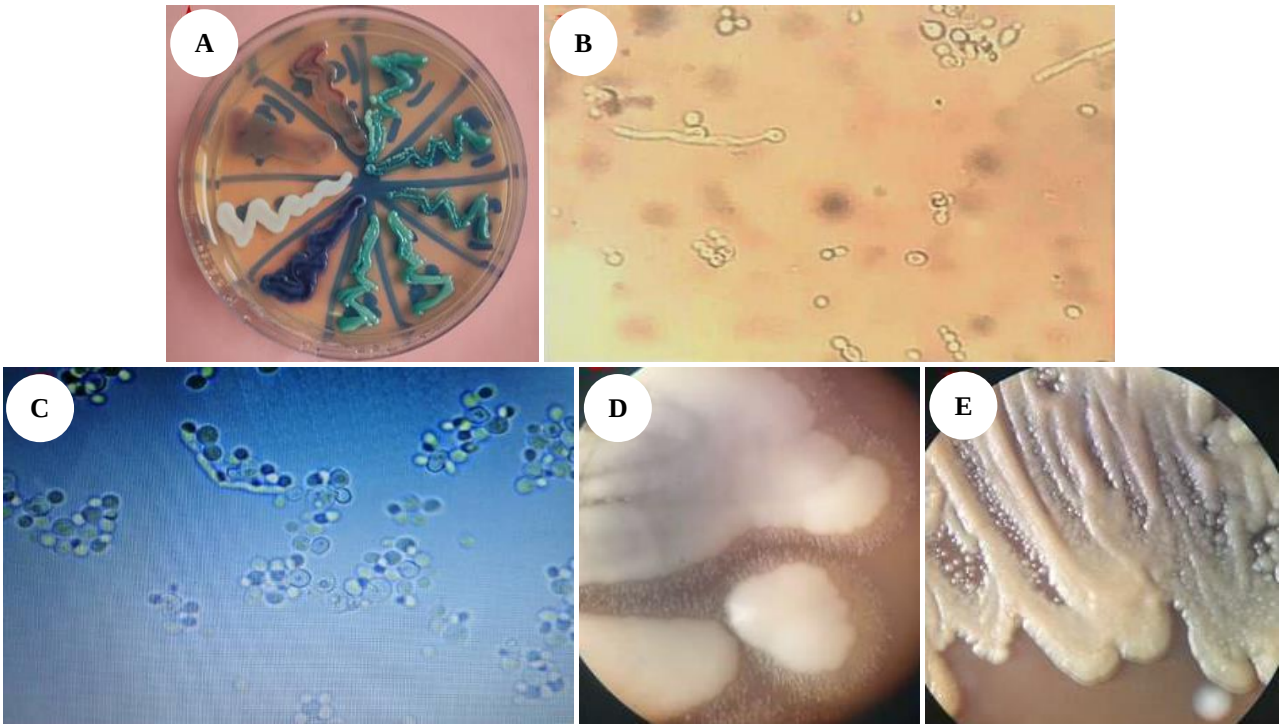

Figure 1. A. Growth of yeasts on CHROMagar Medium, (*C. albicans*) Pale green, (*C. dubliniensis*) Dark green color, (*C. parapsilosis*) White color, and (*C. tropicalis*) Blue color, B. Germ tube of *C. albicans* grown on human serum 40X, C. Single chlamydospores of *C. albicans* (100X), D. Peripheral hyphal fringes extending from colonies of *C. dubliniensis* on tobacco agar medium, E. White to cream colored colonies of *C. albicans* on tobacco agar medium

Table 1. Phenotypic identification and distribution of yeasts in the clinical and environmental samples

Yeast isolates	No &% of isolates	Indoor air	Beds	Tables	Apparently healthy	Cancer patients
Candida albicans	22 (21.56%)	2	8	4	3	5
Candida krusei	17 (16.66%)	-	1	5	5	6
Candida dubliniensis	15 (14.70%)	2	6	3	1	3
Candida glabrata	14 (13.72%)	-	7	3	-	4
Candida kefyr	5 (4.90%)	-	1	2	1	1
Candida tropicalis	4 (3.92%)	-	3	-	-	1
Untypable strains	25 (24.50%)	1	3	5	8	8
Total	102	5	29	22	**18**	2**8**

Molecular identification

Twenty-five untypable yeast isolates were subjected to molecular identification utilizing amplification of ITS1-5.8S-ITS2 rRNA region by means of universal primers ITS1 and ITS4. The utilized protocol (Gene Aid, Taiwan) for DNA extraction yielded clear, highly purified DNA (Figure 3). The pure extracted DNA was successfully used in the PCR amplification of the ITS conservative regions of rRNA using a set of universal primers. The PCR products were seen on an agarose gel electrophoresis in comparison to the DNA ladder, as shown in (Figure 4). The yeast's internal transcribed spacer 1 (ITS1) and ITS2 sections, as well as their 5.8S ribosomal DNA (rRNA), were amplified using the universal primers ITS1 and ITS4. The ITS2 region was amplified simultaneously using the universal primers ITS3 and ITS4. Erami et al. (2022) identified *Candida* sp. by PCR sequencing using the ITS1 and ITS4 primers. PCR amplification followed by electrophoresis appears to be a promising approach for rapid identification of common and uncommon yeast strains from culture colonies (Bayraktar et al. 2017). Twenty-five yeast isolates yielded successful sequences after being identified by PCR. The DNA sequencing findings of the isolates were analyzed and compared with their reference strains in the GenBank database using the BLAST analysis provided by the NCBI site. By sequence analysis, all the tested yeast strains were correctly identified at the species level. PCR amplification using ITSI and ITS4 primers was a rapid, easy, reliable, and applicable technique in clinical laboratories for the identification of medically important *Candida* sp. (Shokohi et al. 2010).

The genotypic identification showed that *Naganishia* sp. was the most prevalent genus, followed by *Candida* sp., while Trichosporon sp., *Cutaneotrichosporon* sp., and *Meyerozyma* sp. were the least prevalent yeast species. The obtained findings revealed that yeast isolates belonged to six genera and nine levels of species (Table 2). Multiple strains of a novel yeast belonging to the genus *Naganishia* were isolated from environmental surfaces aboard the International Space Station (Bijlani et al. 2022). Obeed et al. (2022) reported that *Naganishia globose* was first recorded as a nosocomial agent in Iraq. Abu-Mejdad et al. (2019) found that the most frequently isolated species from soil in Iraq belong to the genus *Naganishia*, with five species. They added that all the described species were recorded for the first time in Iraq. *C. parapsilosis* was the dominant *Candida* sp., this finding was consistent with the fact that it is an exogenous pathogen of the skin rather than the mucosal surface (Shokohi et al. 2010). Amplification of tested yeasts with ITS1 and ITS4 primers yielded fragments ranging from 350 to 569 bp long. The tested organisms showed mainly species-specific differences in the sizes of the PCR products. Meanwhile, the bp length of the PCR products was nearly equal for some species. The BLAST comparisons revealed 99-100% identity among the tested yeast isolates (Table 3). Oliveira et al. (2021) reported similar results. The identification of 25 untypable yeast isolates by molecular method proved that the genotyping identification was more accurate, reliable, and sensitive than the phenotyping technique. Molecular biology techniques are much more accurate, rapid, and sensitive at identifying fungal isolates than conventional phenotyping techniques (Shokohi et al. 2011).

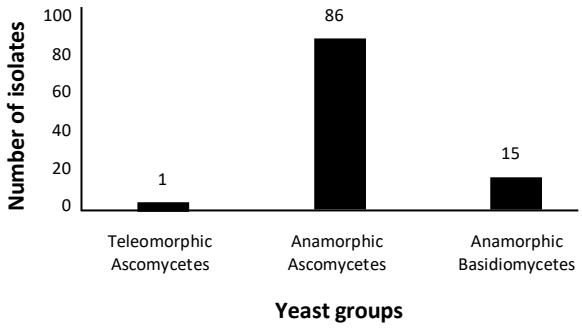

Figure 2. Yeast group distribution among 102 yeast isolates

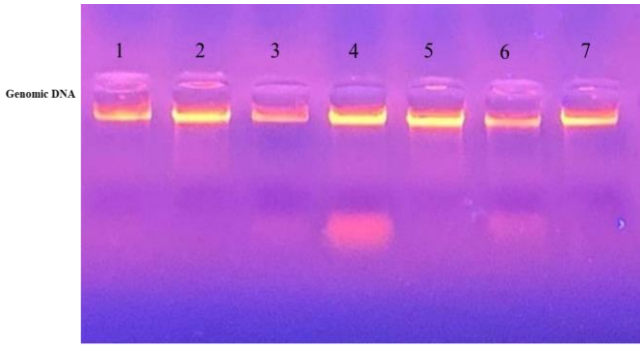

Figure 3. Agarose gel electrophoresis of GDNA

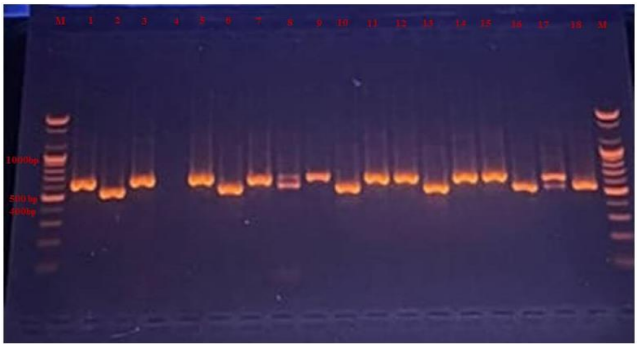

Figure 4. Agarose gel electrophoresis of PCR products of yeast ITS regions: Lane M(100bp DNA ladder), Lane 4 (negative control), Lanes 1-3, and Lanes

Table 2. Genotyping identification and distribution of yeasts in the clinical and environmental samples

Yeast isolates	No. of isolates	Indoor air	Beds	Tables	Apparently healthy	Cancer patients
Candida orthopsilosis	1	-	-	-	-	1
Candida parapsilosis	5	-	-	2	3	-
Candida albicans	3	1	1	-	1	-
Naganishia albida	5	-	-	-	3	2
Naganishia difflunes	6	-	-	1	1	4
Trichosporon asahii	1	-	1	-	-	-
Cutaneotrichosporon. jirovicii	1	-	1	-	-	-
Rhodotorula mucilaginosa	2	-	-	2	-	-
Meyerozyma guillieroii	1	-	-	-	-	1
Total	25	1	3	5	8	8

Table 3. The genotyped yeast strains deposited at the GenBank

Yeast species	Length bp	Identity	Accession no. of closet species
Naganishia diffluens ZDK1	400	100%	OQ851756
Candida orthopsilosis ZDK2	446	100%	OQ860781
Naganishia diffluens ZDK3	350	99%	OQ888796
Candida parapsilosis ZDK4	464	100%	OQ876043
Naganishia albida ZDK5	569	100%	OQ888839
Meyerozyma guilliermondii ZDK6	548	100%	OQ888795
Naganishia albida ZDK7	564	100%	OQ888837
Naganishia albida ZDK8	562	100%	OQ888838
Naganishia diffluens ZDK10	560	100%	OQ888797
Candida parapsilosis ZDK11	463	100%	OQ876044
Naganishia diffluens ZDK12	558	100%	OQ888801
Naganishia diffluens ZDK13	564	100%	OQ888805
Candida parapsilosis ZDK14	460	100%	OQ873592
Candida albicans ZDK15	476	99%	OQ866240
Naganishia albida ZDK16	562	100%	OQ888840
Naganishia albida ZDK17	565	100%	OQ888841
Candida parapsilosis ZDK18	461	100%	OQ873593
Naganishia diffluens ZDK19	550	100%	OQ889159
Candida albicans ZDK20	472	100%	OQ866241
Rhodotorula mucilaginosa ZDK111	541	99%	OQ890327
Rhodotorula mucilaginosa ZDK128	539	99%	OQ890744
Cutaneotrichosporon jirovecii ZDK113	437	100%	OQ913170
Trichosporon asahii ZDK114	480	99%	OQ890746
Candida parapsilosis ZDK124	446	100%	OQ890745
Candida albicans ZDK121	464	100%	OQ913169

Table 4. The MIC values of fluconazole, caspofungin, and voriconazole against yeast isolates

Yeast isolates	No. of isolates	Fluconazole MIC(µg/mL)	Caspofungin MIC(µg/mL)	Voriconazole MIC(µg/mL)
Candida albicans	25	16-32	8-16	8-16
Candida krusei	17	2-4	0.25-1	0.25-1
Candida dubliniensis	15	0.25-1	0.5-1	0.25-1
Candida glabrata	14	4-8	0.125-0.5	0.5-1
Naganishia diffluens	5	8-16	4-8	4-8
Naganishia albida	5	0.25-1	2-4	4-8
Candida kefyr	5	0.125-0.5	0.125-0.5	0.5-1
Candida parapsilosis	5	4-8	0.25-0.5	0.25-0.5
Candida tropicalis	4	32-64	8-16	4-8
Rhodotorula mucilaginosa	2	0.5-1	0.5-1	0.5-2
Naganishia diffluens	1	64	32-64	32-64
Trichosporon asahii	1	4-8	4-8	8-16
Cutaneotrichosporon jirovecii	1	4-8	4-8	8-16
Meyerozyma guilliermondi	1	64	32-64	64
Candida orthopsilosis	1	4-8	0.25-0.5	0.25-0.5

Table 5. The MFC values of fluconazole, caspofungin, and voriconazole against yeast isolates

Yeast isolates	No. of isolates	Fluconazole MFC µg/mL	Caspofungin MFC µg/mL	Voriconazole MFC µg/mL
Candida albicans	25	64	32	32
Candida krusei	17	8	2	2
Candida dubliniensis	15	2	2	2
Candida glabrata	14	2	2	2
Naganishia diffluens	5	32	16	16
Naganishia albida	5	2	8	16
Candida kefyr	5	1	1	2
Candida parapsilosis	5	16-32	1	1
Candida orthopsilosis	1	16	1	1
Candida tropicalis	4	64	32	16
Rhodotourla mucilaginosa	2	2	2	4
Naganishia diffluens	1	>64	>64	>64
Trichosporon asahii	1	16	16	32
Cutaneotrichosporon jirovecii	1	16	16	32
Meyerozyma guilliermondi	1	>64	>64	>64

The genotyped strains were registered and deposited at the Gene bank (Table 3). The sum of phenotyping and genotyping identification revealed that *Candida* sp. was the most dominant genus (84.31%), followed by *Naganishia* sp. (10.78%). *C. albicans* (24.50%), *C. krusei* (16.66%), *C. dubliniensis* (14.70%), and *C. glabrata* (13.72%) were the most prevalent yeast species. The results of the *Candida* sp. isolation rate were consistent with those reported by Reda et al. (2023). The detected isolates of *Candida* non-*albicans* were identified as *C. dubliniensis*, *C. glabrata*, and *C. Tropical*, as well as *Rhodotorula mucilaginosa* (Alrubayae et al. 2020). *C. albicans* are still the most frequent isolates from patients with esophageal Candidiasis (Jafarian et al. 2021). The most common species was *C. albicans*, with lower percentages of *C. dubliniensis*, *C. parapsilosis*, and *Meyerozyma guilliermondii*, respectively (Al-laaeiby et al. 2020). Obeed et al. (2022) demonstrated that the most yeast isolates were from the genus *Candida*, which included *C. albicans* (47.5%), *C. glabrata* (16.4%), *C. tropicalis* (11.48%), *C. parapsilosis* (9.84), *C. krusei* (4.91%), and *C. dubliniensis* (4.91%).

Antifungal susceptibility testing (AFST) for yeast isolates

AFST is useful in the development of new antifungal medications. Antifungal susceptibility testing can be used to establish the degree of activity of a new possible antifungal against a panel of isolates of various species from a number of laboratories (Pfaller et al. 1994). For a variety of reasons, the amount of antifungal susceptibility testing undertaken has increased in recent years, because the number of patients with risk factors for invasive fungal infection has increased (Berkow et al. 2020). Antifungal medications are frequently used as a regular preventive precaution or for at-risk individuals who have a fever (Jørgensen et al. 2014). Different MIC values were recorded for fluconazole, caspofungin, and voriconazole against the tested yeast isolates (Table 4).

The present investigation showed that FLC, VRC, and CAS exhibited potent antifungal activity against the majority yeast species with low MIC values compared with high MIC (64 µg/mL) values reported against *M. guilliermondi*, *N. diffluens*, and *C. tropicalis*. These results agreed with the findings reported by Borman et al. (2020). *Candida kefyr*, and *C. dubliniensis* were highly sensitive to

FLC, VRC, and CAS with MIC/ MFC range values (0.125 - 1.0/ 1.0-2.0 µg/mL), (0.25- 1.0/ 2.0 µg/mL) respectively. These results were consistent with those stated by Lone et al. (2014). Badiee and Alborzi (2011) reported that resistance varies depending on the species and the respective antifungal agents, and the lowest MIC was observed for caspofungin (0.5 µg/mL), the MIC for all *Candida* sp. were 64 µg/mL for fluconazole and 2 µg/mL for voriconazole. They added that 45.5% of *C. kefyr* were resistant to fluconazole but sensitive to the other antifungals. Yesudhason and Mohanram (2015) reported that 37.7% of C. *tropicalis* isolates were fluconazole sensitive. Similar findings were recorded by Abeed and Alrubayae (2022), who found that the yeast isolates exhibited sensitivity to the antifungals tested, with the exception of one *Candida glabrata* isolate that showed resistance to voriconazole. Mishra et al. (2014) mentioned that all *C. glabrata*, 50% *C. tropicalis*, and 12% *C. albicans* isolates were found to be resistant to fluconazole. This study proved that low MFC values of FLC, VRC, and CAS were shown against tested species, except *M. guilliermondi* and *N. diffluens*, where MFC values were >64 µg/mL as shown in Table 5. *M. guilliermondii* can be considered intrinsically resistant to fluconazole (Pfaller et al. 2019; Xiao et. 2020). Caspofungin showed superior activity against all *Candida* spp. (Erami et al. 2022). Higher antifungal concentrations result in a more rapid expression of action, and the fungicidal activity is isolate- and species-specific. (Espinel-Ingroff et al. 2004; Favel et al. 2004; Maheronnaghsh et al. 2020).

ACKNOWLEDGEMENTS

We want to thank Prof. Dr. Wijdan Al-Timimi and Muntathar Aziz Al-Sawafi for their assistance and guidance with this work.

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