



Evaluation of *Candida auris* Colonization using Clinical Skin Swabs: A Single-Center Study in Isfahan, Iran

Fatemeh Safari ¹, Mahboobeh Madani ^{1,*}, Hossein Mirhendi ^{2,3} and Majid Kheirollahi ⁴

¹Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran

²Department of Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

³Research Core Facilities Laboratory, Mycology Reference Laboratory, Isfahan University of Medical Sciences, Isfahan, Iran

⁴Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

*Corresponding author: Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran. Email: mmadani66@gmail.com

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Abstract

The multidrug-resistant fungal pathogen *Candida auris* has been associated with healthcare. We need to address COVID-19 pandemic as well as the ongoing global fungal epidemic caused by *C. auris*, a multi-drug-resistant fungus spreading rapidly throughout the world. This study was conducted on patients admitted to an ICU in Isfahan, Iran, from November 2020 to February 2021 to determine the spectrum of *C. auris* colonization in immunocompromised patients hospitalized in an intensive care unit (ICU). Therefore, clinical swabs were collected from 32 immunocompromised patients for *C. auris* detection upon ICU admission after 7 to 14 days. A rich culture medium was used to evaluate *C. auris* growth at a higher temperature (40°C) and salinity (10% wt/vol) in Sabouraud dextrose agar, which can be used in combination with a *C. auris*-specific polymerase chain reaction method. *C. auris* was not isolated in the clinical samples of patients. The most common colonizer was *Candida albicans*, followed by *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*. *Candida glabrata* was the only species with noticeable growth in the Salt SDA, with D-Mannitol as a carbon source. Currently, *C. auris* is not a common cause of systemic or superficial fungal infections in Iran. The screening of patients admitted to the ICU for *C. auris* could aid in the identification of colonized patients and could simplify the application of infection control measures.

Keywords: *Candida auris*, Colonization, Culture, *C. auris*-specific PCR

1. Background

The recent emergence of multidrug-resistant *Candida auris* has become an international health problem during the past decade (1). *C. auris* is a recently discovered *Candida*/Clavispora clade member, which was first isolated from a female patients' ear discharge in 2009 in Japan (2). Subsequently, *C. auris* has quickly gained a reputation as a remarkable nosocomial pathogen causing many infections throughout the world (3), including three confirmed cases from Iran, which were genetically distinct from the other known *C. auris* clades (4-6). *C. auris* is a global health issue because of its rapid global expansion, multidrug-resistance features, skin colonization capacity, nosocomial epidemic production with high mortality rates (7). Most infections were reported in immunocompromised and/or ill subjects suffering severe underlying diseases (8).

Even though the risk factors for *Candida* infections seem to be the same regardless of species, a recent study found that prolonged hospitalization and prolonged antifungal drug exposure are the most common risk factors

for *C. auris* infection (9, 10). The ICUs have proven a fertile ground for establishing and spreading *C. auris* during the ongoing COVID-19 epidemic (11-16). Since most *Candida* species inhabit the gastrointestinal tract, *C. auris*' ability to remain on the skin surface is an unusual trait that may contribute to its efficient nosocomial transmission (3). As a result, limiting the further spread of *C. auris* requires early and accurate detection (17).

There are no known causes for the recent emergence of this yeast. According to whole-genome sequencing, *C. auris* isolates have undergone the geographical classification of five distinct clades, which have emerged independently (18). The inadequacy of conventional and commercially available biochemical tests to identify *C. auris* infections is a severe barrier to timely diagnosis and appropriate treatment (19).

There is the possibility of misinterpreting *C. auris* as other *Candida* species or non-*Candida* yeast species, even when utilizing commercial biochemical techniques allowing for species-level identification (20). Therefore, several DNA-based approaches were developed to detect

C. auris in environmental and clinical specimens, some of which have been validated (21). Matrix-assisted laser desorption/ionization-time of flight mass spectrometry and polymerase chain reaction (PCR) are the most practical diagnostic techniques to accurately identify *C. auris* (17). In most underfunded mycology laboratories, the high cost of their procurement and operation remains a barrier (22). Several *C. auris* strains are reported with resistance to numerous antifungal categories (23).

2. Objectives

This study aimed to determine the spectrum of *C. auris* colonization from clinical skin swabs in immunocompromised patients hospitalized in ICU using the salinity-rich culture and *C. auris*-specific PCR.

3. Methods

This descriptive study was conducted to evaluate *C. auris* colonization from the skin swabs of patients with a weak immune system or immunodeficiency in the ICUs, in Omid hospital, Isfahan, Iran, after obtaining ethical clearance by the Falavarjan Branch, Islamic Azad University, Isfahan, Iran. An informed consent was obtained from the patient or the next of kin (in the case of the unconscious patient). The active surveillance of 32 admitted patients was conducted from November 2020 to February 2021 (IR.IAU.FALA.REC.1398.046). The ICUs were the host of a mixed population of patients with immunodeficiency, including breast, intestinal, gastric, and lung tumors, leukemia, and neuroblastoma. The information, including age, gender, length of hospitalization, medical specialty, diagnosis on admission, and bathing habits of patient, were recorded upon admission (Table 1). All patients were evaluated for skin surface colonization with an expected duration of > 7 days without bathing.

3.1. Clinical Samples

The skin swab samples (ie, 31 axillaries, 31 groins, two nares, one tongue, and one ear) were collected individually using the sterile swab and a transport system. Each swab was solved in 1 mL Sabouraud Dextrose Broth medium [Merck, Germany] in sterile round bottom tubes from the patients and immediately transferred to the laboratory for analysis. The swab samples were obtained by cleaning the swab in a circular motion while rotating the swab 360 degrees and applying moderate pressure to the surface. All the specimens (round bottom tubes) were put in a shaker incubator for 24 h at 35°C after receiving. Then, each tube was vortexed for 30 sec to release the specimen from the swab tip after 24 h.

Table 1. Distribution of 32 Patients Admitted to ICUs Based on Different Characteristics

Characteristics	No. of Patients
Gender	
Male	10
Female	11
Child	11
Age groups	
< 10	10
11 - 19	2
20 - 29	3
30 - 39	2
40 - 49	5
50 - 59	7
60 - 69	2
≥ 70	1
Underlying diseases	
Acute lymphocytic leukemia (ALL)	8
Acute myeloid leukemia (AML)	16
Lung, breast, colon, stomach and brain cancer	7
Neuroblastoma	1

3.2. Culture Conditions

About 100 µL of suspension was cultured on Sabouraud dextrose agar (SDA; Merck, Germany) supplemented with chloramphenicol and incubated at 35°C for 24 to 48 h. Then, 100 µL was cultured at the same time on Salt SDA (S.N.M) containing antibacterial chloramphenicol (0.5 g/L), NaCl (10% wt/vol), and D-Mannitol (as carbon sources) (SIGMA, Germany) and put in an incubator for two weeks at 40°C. In the next procedure, the colonies were studied, cultured on CHROMagar Candida (BioMerieux, France), and incubated at 35°C for 24 to 48 h. Finally, the plates were investigated for the color of colonies (24).

In this study, the clinical strain of *C. auris* (access number: MZ389242) was used as a positive control (Figure 1) (5).

3.3. DNA Extraction

The DNA of the yeast isolates was extracted by the boiling technique. Then, several procedures, including the suspension of a new yeast colony in 50 µL distilled water, boiling at 95°C for 20 min, and centrifugation at 5000 rpm for 5 min, were conducted. The supernatant was stored at -20°C and used as the DNA template (25).

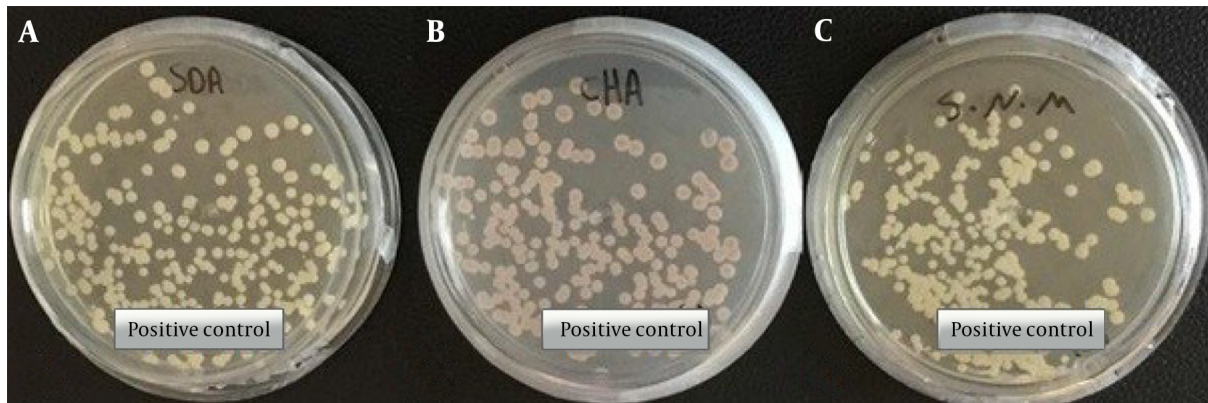


Figure 1. Morphological characteristics of pure colonies of *C. auris* (MZ389242) white to cream on Sabouraud dextrose agar (A), pink on CHROMagar upon 2 days of incubation at 35°C (B), white to cream on Salt Sabouraud dextrose agar upon 7 days of incubation at 40°C (C) (5).

3.4. *C. auris*-specific PCR

All extracted DNAs from yeast isolates were subjected to amplification by direct *C. auris*-specific PCR by the primer pairs, namely F250 (5'-ATTTGCATACACTGATTGG-3') and R250 (5'-AATCTTCGCGTGGCGTT-3'). Regarding the PCR temperature program, the initial denaturation was performed at 95°C for 5 min. Then, 35 cycles of 94°C for 15 sec and 60°C for 30 sec was done. In the next stage, elongation were carried out at 72°C for 30 sec, as well as a final extension step at 72°C for 5 min. All the products of PCR were electrophoresed on 1% agarose gel stained with 0.5 µg/mL ethidium bromide (5). Then, the internal transcribed spacer PCR-RFLP was used to identify the yeasts isolated on CHROM agarTM *Candida* medium with the restriction enzymes *MspI* (Figure 2) (26).

4. Results

In this study, 32 immunocompromised patients were screened for skin surface colonization, who were evaluated by culture and *C. auris*-specific PCR. The average age of the samples was eight months to 71 years. The majority of the patients were under the age of 10 years. There were 10 male cases, 11 female cases, and 11 children. Only cases with underlying disease, including (patients with weak immune systems or immunodeficiency, were studied. In addition, 15 of 32 patients had valid results by culture, and patients with negative culture were excluded. The skin swabs of patients in the groin (13 swabs), axilla (4 swabs), nare (1 swab), and tongue (1 swab) were positive based on culture assay (SDA). Fungal species present were determined on skin surfaces. The results showed that the most common fungal types contaminated on skin surfaces were *Candida albicans*

(n = 11), *Candida glabrata* (n = 4), mix (*C. albicans* and *C. glabrata*: n = 2), mix (*C. albicans* and *C. parapsilosis*: n = 1), and mix (*C. albicans* and *C. glabrata* and *C. tropicalis* and *C. parapsilosis*: n = 1), but *C. auris* was not isolated (Table 2).

Table 2. Fungal Species Isolated from Immunocompromised Patients to Evaluate the Colonization of *C. auris* Using DNA-based Methods Listed by Frequency

Source / No. of Samples	No. of Positive Culture (yeast)	Causative Organism / Frequency
Groin swabs (n = 31)	13 swabs	<i>Candida albicans</i> (n = 8)
		<i>Candida glabrata</i> (n = 2)
		Mix (<i>C. albicans</i> & <i>C. glabrata</i>) (n = 2)
		Mix (<i>C. albicans</i> & <i>C. glabrata</i> & <i>C. tropicalis</i> & <i>C. parapsilosis</i>) (n = 1)
Axillary swabs (n = 31)	4 swabs	<i>Candida glabrata</i> (n = 2)
		<i>Candida albicans</i> (n = 1)
		Mix (<i>C. albicans</i> & <i>C. parapsilosis</i>) (n = 1)
Nare swabs (n = 2)	1 swabs	<i>Candida albicans</i> (n = 1)
Tongue swab (n = 1)	1 swabs	<i>Candida albicans</i> (n = 1)
Ear swab (n = 1)	0	0

The results indicated that the colonized patients had many live *C. albicans* on their skin (axilla/groin). The dominant pathogen in inpatient clinical specimens was markedly *C. albicans* with *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*. *C. auris* is not a common cause of systemic or superficial fungal infections in Iran. Most *C. albicans* isolated from the skin swabs were obtained through recovery from the groin, axillary, and other body. In 13 out of 15 subjects, gynecological colonization occurred more frequently. The

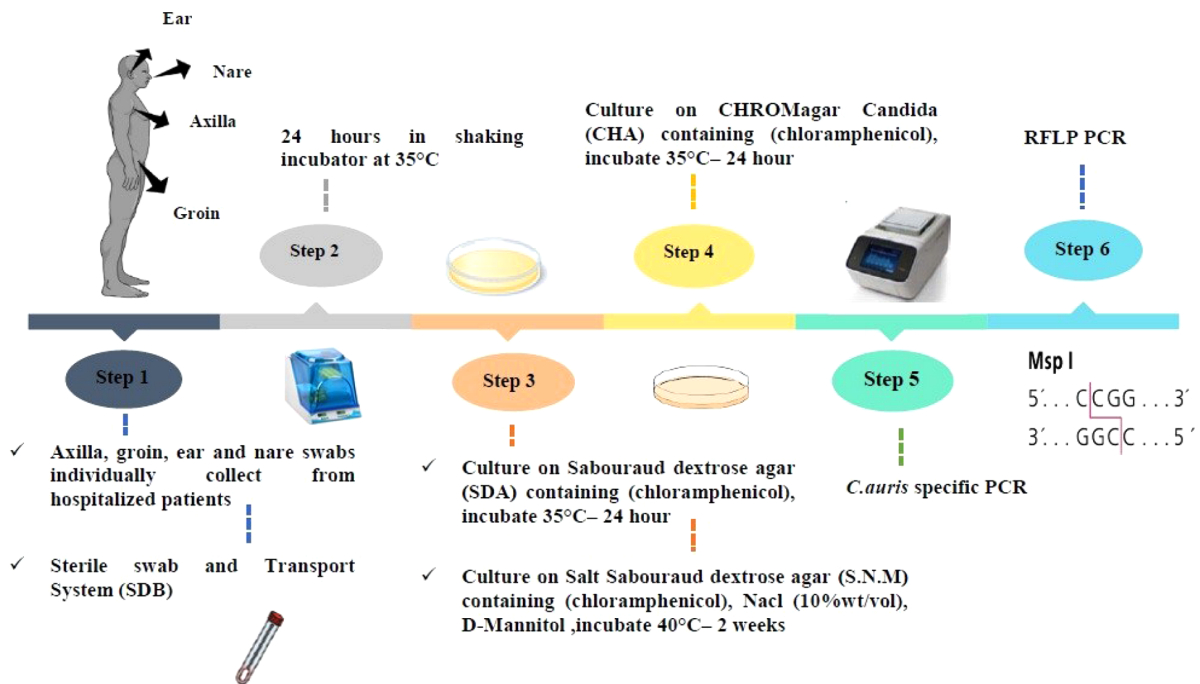


Figure 2. Schematic of the rich culture medium method for *C. auris*

findings showed the groin as the preferred site of *C. albicans* colonization compared to the axilla in the colonized subjects (representing moist areas).

Interestingly, only one patient had a positive result for at least one site (one groin) of the 66 swab samples cultured in Salt SDA (S.N.M) (at 40°C). A positive result was identified for *C. glabrata* when the RFLP-PCR assay was repeated using the stored DNA specimen for this patient. This feature indicates the high ability of this species to tolerate salinity and high temperatures compared to *C. auris*, which grows significantly in Salt Sabouraud with D-Mannitol as a carbon source.

5. Discussion

C. auris is recognized as a colonizing organism and a source of infection in people in healthcare systems worldwide (2). These infections were linked to invasive medical devices, mechanical ventilation, extended stays in ICUs, and prior exposure to broad-spectrum antibiotics (27). Furthermore, the COVID-19 pandemic has provided excellent conditions for *C. auris* to propagate (8). Given the limited capacity of ICUs for the number of COVID-19 patients in need of critical care and the difficulty in implementing conventional infection prevention and control measures, it is possible to unintentionally facilitate the silent spread

of *C. auris* through the lengthy utilization of personal protective equipment by healthcare staff (13).

In this study, a *C. auris*-specific PCR assay and salinity-rich culture were used to effectively suppress other microorganisms and isolate *C. auris*, using a combination of high temperature and salinity. Using the culture-based method and specific-species PCR, no *C. auris* strains were isolated from immunocompromised patients admitted to the ICU. Although nobody can guaranty the *C. auris* as a non-fungal infectious agent forever, it is not a common cause among patients with systemic or superficial fungal infections in Iran.

As mentioned, *C. glabrata* was the only species growing poorly in Salt SDA with D-Mannitol as the carbon source at high temperatures and in the salinity medium. These findings are consistent with those of Welsh et al. (24) confirming salinity-rich culture processing of skin swabs does not eliminate the development of species other than *C. auris*.

The results revealed that the patients' colonized skin (axilla/groin) and mucosal (nares) surfaces had many live *C. albicans* cells. Carriage or infections with these isolates may lead to compromised treatment choices, and high death rates among cases (5 - 10% of known colonized patients develop invasive infections) (28). *C. albicans* mainly cause invasive candidiasis and affects the most vulnerable cases in health care settings (eg, the patients in critical

condition). Patients with infection or colonization should be detected early to manage with a weak immune system and prevent transmission of environmental contamination from patient to patient (29).

Further research is required to fully comprehend the mechanism of colonization. Infection prevention in healthcare settings should be directed by rapid detection because *C. auris* is resistant to various antifungal drugs and is also challenging to treat. Laboratories should use proper procedures to determine all cultured *Candida* species recovered from other body parts to early detect *C. auris* outbreaks. This study was limited by the small number of samples collected, as it was impractical to visit patients regularly and collect samples due to COVID-19 global prevalence and the hospital quarantine conditions.

5.1. Conclusions

According to the results, *C. auris*-specific PCR was a successful fast substitute for culturing to identify *C. auris* in the clinical skin swabs of the axilla/groin. It is possible to obtain results by *C. auris*-specific PCR in less than 2.5 h, with a remarkable improvement compared to culturing with the 14 days needed.

Footnotes

Authors' Contribution: Study concept and design: H.M.; and M.M.; Analysis and interpretation of data: F.S.; and H.M.; Drafting of the manuscript F.S.; and M.M.; Critical revision of the manuscript for important intellectual content: M.M.; H.M.; and M.K.; Statistical analysis: F.S.; and H.M.

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