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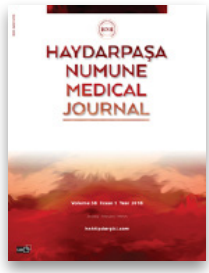

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ORIGINAL ARTICLE

Detection, Screening, and Antifungal Susceptibility of the Current Threat *Candida auris*: A Tertiary Hospital Experience

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Abstract

Introduction: *Candida auris* is currently reported as a global threat. In this study, we aimed to describe antifungal susceptibility, experiences regarding the detection of *C. auris* in clinical and environmental samples, and infection control measures.

Methods: Patients infected or colonized with *C. auris* and all clinical and environmental screening samples between October 2022 and June 2023 were included. Data on demographics, underlying diseases, length of hospital/intensive care unit (ICU) stay, presence of bacterial/fungal co-infection, history of broad-spectrum antibiotic/antifungal use, outcomes, and antifungal susceptibility results were investigated retrospectively. Screening of contact patients and environmental samples was managed according to the instructions prepared by the infection control committee.

Results: *C. auris* was isolated in six patients, four with candidemia and two with colonization. Two of the patients with candidemia were hospitalized in the ICU and two in the internal medicine service. All had at least one comorbid disease, prolonged hospitalization, and a history of broad-spectrum antibiotic use. The crude mortality rate was 50%. Increased minimal inhibitory concentrations (MIC) values for fluconazole and 50% resistance to amphotericin B were detected. No resistance to voriconazole, micafungin, or caspofungin was observed. Two patients with colonization did not develop invasive infections during hospitalization. There was no growth in any of the contact-patient screenings. Growth was detected in one of the environmental samples. As a result of the precautions taken, there was no growth in subsequent environmental cultures.

Discussion and Conclusion: *C. auris* continues to be an important agent of candidemia with high mortality. Although high MIC values were found for fluconazole and amphotericin B, no echinocandin resistance was observed. *C. auris* infection and colonization can be controlled with effective infection control measures and contact screening. Clinicians and microbiology specialists in every health-care institution must be prepared for the possible isolation of *C. auris* to contribute to the reduction of spread and mortality through rapid diagnosis and timely treatment.

Keywords: Antifungal resistance; *Candida auris*; candidemia; environmental screening; infection control measures.

Candida auris is a multidrug-resistant fungal pathogen that causes a wide range of infections, including bloodstream infections, urinary tract infections, otitis, and wound infections[1,2]. This microorganism can survive for a long time, especially in hospital settings, and is resistant to many disinfectants. *C. auris* is reported as

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a current global threat due to its long-lasting hospital outbreaks, difficulties in identification in clinical microbiology laboratories, and high mortality rates[3-5]. It has been reported worldwide since it was first identified in Japan in 2009[1,6-10]. The first case of *C. auris* infection in Türkiye was reported by Kurt et al.[11] in Istanbul in March 2021. After this date, several cases continued to be reported from Istanbul and Izmir[12-18]. However, these studies only evaluated the characteristics of patients, and isolates and did not include the screening results of contact patients or environmental samples. Our study also analyzed these important data. One of the most important features of *C. auris* is its potential to develop resistance to many classes of antifungals. Pan-drug-resistant isolates have been reported in recent studies, resistant to three classes of antifungals: azoles, polyenes, and echinocandins[2]. Resistance to fluconazole and amphotericin B was also observed in isolates detected in our country[13,16].

In our study, we aimed to report the antifungal susceptibility data of *C. auris* strains, thus supporting the treatment management of clinicians in our hospital and contributing to the epidemiology of *C. auris* in our country. In addition, it was aimed to draw attention to the importance of *C. auris* infections, which are an impending threat for our country, and to share our experiences as a microbiology laboratory within the scope of infection control measures in the detection and screening of this fungal pathogen in clinical and environmental samples.

Materials and Methods

Study Design and Patients

In this study, patients with *C. auris* infection or colonization in the intensive care unit (ICU) and other services of our hospital between October 2022 and June 2023, and all patient and environmental screening samples sent to our laboratory were included. Demographic characteristics, underlying diseases, length of hospitalization and/or ICU stay, need for mechanical ventilation, time between hospitalization and *C. auris* growth, history of surgical intervention, presence of bacterial or fungal coinfection, use of broad-spectrum antibiotics, antifungal or immunosuppressive drugs, and patient survival were recorded.

Sample Processing, Identification, and Antifungal Susceptibility Testing

Clinical samples were incubated on sheep blood agar, chocolate agar, and Saboraud dextrose agar (SDA) media for 24–48 h at 37°C and 25°C, and screening samples were incubated for at least 5 days by inoculating only in SDA and checked for growth every day. Matrix-assisted laser desorption ionization-time of flight (VITEK MS [bioMérieux, France]) together with conventional methods (germ tube test and Chrom Agar Candida [Becton Dickinson, USA]) were performed to identify isolates. In vitro susceptibility of the isolates to fluconazole, voriconazole, amphotericin B, caspofungin, and micafungin was determined using the VITEK-2 (bioMerieux, France) automated system and confirmed with the gradient diffusion method (Etest, bioMerieux). Since there are no established susceptibility breakpoints for *C. auris*, minimal inhibitory concentrations (MIC) of fluconazole ≥32 mg/L, voricanazol ≥4 mg/L, amphotericin B ≥2 mg/L, caspofungin ≥2 mg/L, and micafungin ≥4 mg/L were evaluated as resistant, according to tentative breakpoints recommended by the Centers for Disease Control and Prevention (CDC)[19].

Management of Screening Samples and Infection Control Measures

Immediately, after the first infection with *C. auris* was detected in our hospital, a detailed multidisciplinary instruction was prepared in line with the CDC recommendations for the management of *C. auris*[20]. In this instruction, information on isolation of patients and contact persons, disinfection of rooms and devices, patient transfer, education for healthcare professionals, situations requiring screening and management of clinical and environmental samples, and microbiologist-clinician cooperation for rapid diagnosis were presented. When an environmental source of infection was suspected, environmental swab samples were taken for outbreak investigation. Data on the service, sampling time, and *C. auris* growth were retrospectively analyzed.

Results

Patient Characteristics

During the study period, six patients were found to be infected or colonized with C. auris. Among these, four patients with growth in blood cultures were evaluated as having candidemia, and two patients with growth in skin screening samples but without any symptoms or signs were considered to have colonization. The mean age of the patients with candidemia was 55.5, and 75% were female. Two of the patients with candidemia were hospital-

ized in the ICU and two in the internal medicine service. For these four patients, the mean duration of hospitalization was 36.7 days, the ICU stay was 47.5 days, and the mean duration of mechanical ventilation was 40.5 days. All patients had at least one comorbid disease (hypertension, diabetes mellitus, or chronic renal failure), and one had a percutaneous enterogastrostomy. Diabetes was detected in only one patient. It was observed that prior to *C. auris* growth in all patients, broad-spectrum antibiotics were used for different bacterial infections, including fluconazole for *Candida tropicalis* fungemia in one patient and methotrexate for psoriasis in one patient. The mean time between hospitalization and *C. auris* growth was 32.5 days. Two patients died before antifungal treatment could be started; one patient left the hospital with a refusal of treatment before culture results were available; and one was transferred to another health institution while antifungal treatment was continuing. The 30-day crude mortality was 50%.

Regarding the patients with colonization, the mean age was 65.5 years, and 50% were female. Both patients had undergone cardiovascular surgery. Both patients had a history of broad-spectrum antibiotic use before *C. auris* growth. The mean time between hospitalization and *C. auris* growth was 2.5 days. One of the patients died due to cardiac complications, while the other was transferred to another institution. All the characteristics of patients with candidemia and colonization are summarized in Table 1.

Antifungal Susceptibility

Antifungal susceptibility testing was performed on *C. auris* isolates causing candidemia. According to CDC criteria, all isolates had increased MIC values for fluconazole (16 mg/L), and two (50%) were resistant to amphotericin B (8–≥16 mg/L). No resistance was observed against voriconazole (≤0.12 mg/L), micafungin (≤0.06–0.12 mg/L), and caspofungin (0.25 mg/L). The MIC values of the isolates against each antifungal drug are shown in Table 2.

C. auris Screening and Infection Control Measures

C. auris was detected in the blood culture of a patient hospitalized in the ICU for the first time in our hospital on October 25, 2022. Thereupon, the patient's doctor and the hospital infection control committee were immediately contacted and verbally informed. In line with the CDC recommendations, a joint instruction was prepared with all the stakeholders of the infection control committee and implemented quickly. Strict contact isolation measures were taken, and the patient was isolated in a single room during hospitalization. Nasal, axillary, and inguinal swab samples were taken from other patients who had contact with this patient by informing the microbiology laboratory staff. These patients were also isolated until the results were negative. There was no growth in any of the screening samples taken from a total of 52 patients. For environmental source investigation, swab samples were taken from ventilators, perfusers, monitors, equipment, nursing trolleys, computers, USG devices, ECG devices, stethoscopes, and beds. Among a total of 34 samples, *C. auris* growth was detected in one of the nursing trolleys. Then, after detailed disinfection with 1000 ppm sodium hypochlorite, environmental samples were taken again. There was no growth in any of these samples. Approximately 1 month later, on November 25, 2022, *C. auris* growth was detected in the blood culture of a patient hospitalized in the internal medicine service. Skin screening cultures were obtained from a total of 68 patients, and none of them grew. In February and April after this date, *C. auris* candidemia was detected in two more patients, one in the internal medicine service and the other in the ICU. No growth was observed in any of the environmental (n=40) and patient screening samples (n=26) taken during this period. After that, no *C. auris* infection was observed in our hospital until the end of the study. However, *C. auris* was detected in the skin screening cultures of two patients who were transferred from an external center to our hospital ICU in May and June. Since these patients had no clinical symptoms or signs, they were considered as colonized. No *C. auris* infection developed in these patients during their stay in our hospital, and there was no growth in the cultures taken from the contact persons (n=37). A total of 173 patients were screened for colonization during the entire study period, and *C. auris* was detected only in two (1.1%) patients.

Within the scope of infection control measures, each unit was visited daily by the infection control team for 3 months. The isolation rooms of patients (surfaces, tools, equipment, etc.) were disinfected with 1000 ppm sodium hypochloride. Quick-acting disinfectants for the disinfection of screens and surfaces of sensitive devices (based on hydrogen peroxide and alcohol) were used. Each patient area (bed, shelf, monitor, etc.) was cleaned and disinfected as if it were a different room. Cleaning cloths and other cleaning equipment were replaced with new ones when moving from one patient to another. In addition, all hospital staff were informed about strict adherence to hand hygiene.

Table 1. Charasteristics of *C. auris* infected or colonized patients

Patient	Sex Age	Initial Diagnosis	Hospital Stay (days)	ICU Stay (days)	Mechanical Ventilation	Site of Isolation	Comorbidities	Previous Surgery	Previous Broad-Spectrum AB	Previous Antifungal	Immunomodulatory Agents	Pre/co-infection	Antifungal treatment	Outcome
1	Female 61 y	Bacterial pneumoniae	73	73	73	Blood culture	Psoriasis, Encephalopaty	No	Meropenem Teicoplanin	No	Methotreksat (45 days)	UTI: *VRE+K.pneumoniae* *VAP: A.baumannii* *BSI: K. pneumoniae*	No*	Dead
2	Female 75 y	Cholangio-sepsis	35	22	8	Catheter	HT, CRF	No	Piperacillin-Tazobactam Meropenem Teicoplanin	No	No	*BSI: P. aeruginosa* *BSI: VRE+ A.baumannii*	No*	Dead
3	Male 62y	Acute pancreatitis	14	0	No	Blood culture	DM, HT	No	Sefepim Piperacillin-Tazobactam Meropenem	No	No	*BSI: E.coli*	No (treatment rejection)	Discharge
4	Female 24y	Candidal septicemia	25	25	No	Blood culture	VWM, PEG	No	Meropenem	Yes (Flucanazol 4 days)	No	*BSI: Candida tropicalis*	Yes (Anidulafungin 3 days)	Transferred
5	Male 61y	Bacterial pneumoniae	7	7	No	Skin screening	CRF	Aortic aneurysm	Meropenem Teicoplanin Polimiksin B	No	No	*VAP: K. pneumoniae +A.baumannii*	No (colonisation)	Dead
6	Female 70y	Septicemia	47	47	47	Skin screening	HT, PEG	Aortic dissection	Daptomisin Meropenem Polimiksin B	No	No	*BSI: P.stuartii* *BSI: S.aureus (MSSA)*	No (colonisation)	Transferred

*The patients died before antifungal therapy was started. ICU: Intensive care unit; HT: Hypertension; CRF: Chronic renal failure; DM: Diabetes mellitus; VWM: Vanishing white matter; PEG: Percutaneous endoscopic gastrostomy; UTI: Urinary tract infection; VAP: Ventilator-associated pneumoniae; BSI: Blood stream infection; VRE: Vancomisin Resistant Enterococcus; AB: Antibiotic; MSSA: Methicillin sensitive S. aureus.

Table 2. MIC values (mg/L) of antifungal agents for *Candida auris* strains isolated from blood cultures

Antifungal	Case-1	Case-2	Case-3	Case-4
Amphotericin B	8	8	≥16	8
Flucanazole	16	16	16	16
Voriconazole	≤0.12	≤0.12	≤0.12	≤0.12
Micafungin	0.12	0.12	0.12	≤0.06
Caspofungin	0.25	0.25	0.25	0.25

MIC: Minimal inhibitory concentration.

Discussion

As in the rest of the world, reports of patients colonized or infected with *C. auris* continue in our country. In this study, in addition to the data on patients and their isolates, the management of environmental and patient screening samples and the infection control measures taken were also analyzed.

Although *C. auris* causes many invasive infections, it is mostly encountered as a causative agent of candidemia[1,3,6,7]. Underlying diseases and predisposing factors are common in patients with *C. auris* candidemia. Brino et al.[7] reported that long-term hospitalization, history of ICU stay, mechanical ventilation, diabetes mellitus, chronic renal failure, previous surgery, and use of antifungal or broad-spectrum antibiotics are independent risk factors for the development of *C. auris* candidemia. Many of these risk factors were observed in cases of *C. auris* candidemia reported from our country[11-17]. In our study, all four patients with *C. auris* candidemia had at least one comorbid disease, a prolonged hospital stay, and severe bacterial infections, including sepsis, requiring broad-spectrum antibiotics. Among these, the simultaneous detection of multidrug-resistant *K. pneumoniae* and *A. baumannii* in the blood cultures of two patients in whom *C. auris* growth was detected and the additional history of immunomodulatory drug use in one of these patients were also remarkable. One patient developed a *C. auris* infection while on fluconazole treatment for C. tropicalis fungemia, suggesting that the use of fluconazole may lead to the selection of *C. auris*, as reported in other studies[5,9].

In studies conducted worldwide, mortality rates ranging from 0% to 72% have been reported for *C. auris* candidemia. However, due to the presence of other serious diseases in patients, there is no clear information on mortality rates attributable to *C. auris* candidemia alone[1-3,4,8,17]. In a total of sixteen cases of *C. auris* candidemia reported from our country between 2021 and June 2023, for which survival data were available, crude mortality was observed to be between 0% and 100%[11-17]. In these reports, it was stated that some of the patients died despite appropriate antifungal treatment, while others died before antifungal treatment could be initiated or due to severe complications from cardiac disease and malignancy. Similarly, in our study, two of the four patients with concurrent bloodstream infection with multidrug-resistant bacteria died before antifungal treatment was initiated, and crude mortality was determined to be 50%.

In the hospital setting, *C. auris* can cause prolonged outbreaks due to its ability to persist on surfaces and medical devices for long periods of time and to persistently colonize patients[21-23]. Although antifungal treatment is not required in asymptomatic colonization with *C. auris*, timely screening and identification of colonized patients helps to take isolation measures quickly and prevent the spread of *C. auris*. Therefore, the CDC recommends screening every patient with *C. auris* infection, close contacts of the patient, and patients transferred from centers where *C. auris* positivity has been reported[20]. In studies investigating colonization sites, *C. auris* was most commonly isolated from the axilla, groin, and later the nostrils[22,23]. In a study conducted in India, colonization was detected in 21% of patients who had contact with the index case[24]. In recent studies, de St Maurice et al.[21] found *C. auris* colonization in 4.5% and Southwick et al.[22] in 7% of the patients they screened. In our study, screening samples were taken from a total of 173 patients, and colonization (1.1%) was detected only in two patients who were transferred from an external center to our hospital ICU. We think that this result is very valuable in terms of demonstrating the effectiveness and importance of both rapid microbiological diagnosis and infection control measures. Further, when the characteristics of the colonized patients were examined, it was seen that both patients had a history of cardiovascular surgery and similar risk factors to patients with *C. auris* candidemia. However, these patients did not develop an invasive *C. auris* infection during their hospitalization.

The antifungal susceptibility of *C. auris* was evaluated according to the tentative breakpoints recommended by the CDC[19]. In previous studies, it has been reported that 60–100% of *C. auris* strains are resistant to fluconazole, 10–30% show high MIC values for amphotericin B, and 0–7% are resistant to echinocandins[1,2,8,10]. Moreover, it is reported that pandrug-resistant strains are also increasingly isolated[2]. When studies conducted in our country were analyzed, it was observed that all isolates were resistant to fluconazole (≥32–256 mg/L). MIC values ranged between 0.19

and >8 mg/L for voriconazole, 1–4 mg/L for amphotericin B, 0.06–0.25 mg/L for micafungin, 0.06–>8 mg/L for caspofungin, and 0.06–0.25 mg/L for anidulofungin[11-18]. In our study, we found high MIC values (16 mg/L) for fluconazole in all isolates, while two (50%) were resistant to amphotericin B (8–≥16 mg/L). However, it should be noted as a limitation that high MIC values for amphotericin B could be obtained with the VITEK-2 automated system compared to the reference method. Apart from this, we did not find resistance to voriconazole (≤0.12), micafungin (≤0.06–0.12), or caspofungin (0.25 mg/L). Accordingly, we consider that echinocandin-group antifungals can be used in the empirical treatment of *C. auris* candidemia, as recommended in the literature[1,3].

C. auris positivity can be seen on surfaces and equipment used by patients colonized or infected with this pathogen[1,3,24]. Therefore, as soon as *C. auris* is detected in the laboratory, prompt notification to the clinic and infection control committee is very important for the timely initiation of infection control measures. Although routine screening of environmental samples is not generally recommended, it has been stated that screening would be appropriate if an environmental source of contamination is suspected[4,20,25]. In the first *C. auris* case in our country, Kurt et al.[11] conducted source research and collected swab samples from environmental surfaces (mechanical ventilator equipment, patient beds, common objects, supportive care equipment, telephones, door handles, computer keyboards, etc.) but could not isolate *C. auris* from these samples. In other studies conducted in our country, no data on environmental or patient screening were found. When we isolated *C. auris* for the first time in our hospital, we immediately informed the clinician and the infection control committee. On a joint decision, environmental sampling was performed simultaneously with contact patient screening, and *C. auris* was isolated from one of the nursing trolleys in the ICU. Detailed disinfection with 1000 ppm sodium hypochlorite was then performed. There was no growth in any of the environmental samples taken afterward. No growth of *C. auris* was detected in other environmental samples taken as needed during the study period. We should especially point out that this is thanks to the close collaboration with all stakeholders in the infection control committee.

Our study has some limitations. One of them is that the study was retrospective and single-center, while the others were that, due to technical limitations, molecular analysis was not performed on isolates and the antifungal susceptibility test was not performed using the reference broth microdilution method.

Conclusion

C. auris continues to be an important agent of candidemia with high mortality. Although high MIC values were found for fluconazole and amphotericin B, it is pleasing that no echinocandin resistance was observed. *C. auris* infection and/or colonization can be controlled with effective infection control measures and patient screening. For this, clinicians and microbiology specialists in every health-care institution must be prepared for possible isolation of *C. auris* to contribute to the reduction of spread and mortality through rapid diagnosis and timely treatment.

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Ethics Committee Approval: The study was conducted after it was approved by the University of Health Sciences Turkey, Haydarpaşa Numune Research and Training Hospital Ethics Committee (Decision no: KK152, Date: 28.08.2023).

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