Indian Journal of Medical Microbiology 39 (2021) 228–230

Contents lists available at ScienceDirect

Indian Journal of Medical Microbiology

journal homepage: www.journals.elsevier.com/indian-journal-of-medical-microbiology

Original Research Article

Candida auris Fungemia and a local spread taken under control with infection control measures: First report from Turkey

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ARTICLE INFO

Keywords:
Candida auris
Local spread
Turkey

ABSTRACT

Candida auris, draws attention as a new emerging antifungal resistant pathogen, leading to healthcare-associated infections and outbreaks. This is the first report of *C. auris* fungemia in a 81-year-old patient, confirmed by sequential analysis, from Turkey. Although the source of the isolate could not be identified, its spread in the hospital has been taken under control by effective infection control measures.

1. Introduction

Candida auris was first isolated from a Japanese patient in 2009 and is a new emerging antifungal resistant pathogen leading to healthcare-associated infections (HAI) and outbreaks with variable spreading characteristics [1–3]. Although reported in many countries, *C. auris has* not yet been reported in Turkey [2–4]. Here, we present the first *C. auris* case and a limited local spread taken under control with infection control measures in Istanbul, Turkey.

2. Materials

2.1. Index Case

A 81-year-old female patient diagnosed with advanced aortic stenosis was admitted to the cardiovascular surgery (CVS) intensive care unit (ICU) after ascending aortic graft + coronary stent implantation and aortic valve replacement surgery. The central venous catheter (CVC) was removed upon isolation of *Candida parapsilosis* in blood cultures, and a new catheter was inserted and amphotericin-B was initiated. Blood cultures were repeated one week later because there was no clinical improvement, and *Candida parapsilosis was* isolated again. Then caspofungin was added to amphotericin-B accordingly. The source of infection was investigated: imaging modalities did not reveal mediastinitis, no vegetation was detected in transthoracic echocardiography (transesophageal echocardiography could not be performed due to the general condition of the patient) and fundoscopic examination revealed no pathological findings. The blood cultures remained sterile, and antifungal treatment was discontinued on the 36th day. Three weeks later, the body temperature increased and the general condition deteriorated. The blood cultures again revealed *Candida* spp.; CVC was removed, and amphotericin-B + caspofungin was re-initiated. Semiquantitative culture of the removed catheter tip revealed *Candida* spp. as well. Repeated transthoracic echocardiography and fundoscopic examination revealed no pathological findings. The patient was transferred to the CVS ward. *Candida* spp. was identified as *C. auris* by matrix assisted laser desorption ionization-time of flight (MALDI-TOF). Cultures of nasal, axillary and inguinal swab samples revealed very dense growth of *C. auris*.

Infection control precautions were undertaken according to Centers for Disease Control and Prevention (CDC) recommendations after isolation of *C. auris* [4]. The patient was isolated in a single room. The infection control committee visited the CVS unit for information on infection control measures. Contact isolation precautions were applied afterwards in CVS ward and the ICU under the control of the infection

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https://doi.org/10.1016/j.ijmmb.2021.03.007
Received 16 July 2020; Accepted 5 January 2021
Available online 27 March 2021
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control committee. Nasal, inguinal, and axillary swabs from three patients staying in the same ward and of six patients staying in CVS ICU in the same period with the index case were collected. Swab samples from the environmental surfaces were also obtained (mechanical ventilator equipment, patient beds, shared objects, supportive care equipment, telephones, doorknobs, computers' keyboards, etc.). *C. auris* was isolated only in the axillary swab sample of another patient in CVS ICU, and this patient was isolated in a single room as well. No infection developed in this patient during the follow-up period. *C. auris* was not isolated from any environmental surfaces. Alcohol-based hand antiseptics were used for hand hygiene. Environmental disinfection was achieved with 1000 ppm chlorine bleach. The infection control team visited the unit daily to monitor the compliance with control precautions for three months.

The patient was followed for three months. *C. auris* was detected in the nasal, inguinal, and axillary swabs of the patient during weekly controls. A new *C.auris* fungemia developed after three months and was re-treated with amphotericin-B + caspofungin. *C. auris* was not detected in any other patient in the institution.

2.2. Microbiological characteristics

The germ tube test was negative, and culture on the BBL CHROMagar Candida (Becton Dickinson GmbH, Germany) medium revealed pink and smooth colonies. Yeast cells were not producing hyphae in corn meal agar and could grow at 42 °C. The isolates were defined as *C. tropicalis* with API20CAUX (bioMérieux, France) method. These isolates were re-analyzed by MALDI-TOF (Bruker Daltonics, Bremen, Germany) according to the manufacturer's recommendations. The resulting spectra were obtained with the software flexAnalysis 3.4. The mass spectra were analyzed using the Bruker Biotyper 3.1 software, IVD Version 8 (DB-7712MSP) library. The isolates were identified as *Candida auris* with reliable score values (>2). All *Candida* species isolated (4 isolates) from the index patient other than *C. auris as* well as all *Candida* species (24 isolates) isolated from various clinical samples in the hospital were re-evaluated with MALDI-TOF. None of these isolates were identified as *C. auris*.

2.3. Antifungal susceptibility tests

Three different methods were performed: Sensititre YeastONE (SYO) (Trek Diagnostic System, Cleveland; colorimetric liquid microdilution test), E-test (bioMérieux, Marcy l'Etoile, France; agar diffusion gradient test), and the broth microdilution as the gold standard method. Broth microdilution was performed according to Clinical Laboratory Standards Institute (CLSI) M27-A3 recommendations [5]. *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019, and *C. tropicalis* ATCC 750 were used as quality control strains.

In the standard broth microdilution method, amphotericin-B, fluconazole, voriconazole, itraconazole, posaconazole, and anidulafungin (Sigma Aldrich) were used as antifungal agents. Antifungal concentrations were prepared in a range of 32–0.06 mg/L for fluconazole, 16–0.03 mg/L for amphotericin-B, and 8–0.015 mg/L for itraconazole, and 8–0.015 mg/L for voriconazole. The yeast cells were inoculated to have a final concentration of 1–5x10^3 cells/ml. The experiment was repeated three times for each antifungal agent. During the evaluation, no growth was observed with amphotericin-B. For the other antifungals, the concentration of the drug at which ~50% of growth was inhibited at the end of 24 and 48 h relative to the control well was accepted as the minimal inhibitory concentration (MIC).

Commercial tests were performed according the manufacturer's recommendations: In the SYO method, a suspension of 0.5 McFarland turbidity was prepared in demineralized water with colonies obtained after 24 h culture in sabouraud dextrose agar (SDA). Next, 20 µl of this suspension was added to Sensititre YeastONE broth fluid and a final inoculum of 1.5–8 x 10^3 CFU/mL was obtained. Then, 100 µl of the final inoculum was distributed to the SYO plate wells and incubated in 35–37 °C. After 24 h incubation, the color change in the positive control well was checked, and the well with a color change was considered to be the lowest antifungal MIC level in which growth was largely inhibited. For agar diffusion tests, a suspension of 0.5 McFarland turbidity in saline was prepared from colonies in SDA and was swabbed over the surface of RPMI 1640 agar medium. E-test strips of posaconazole, voriconazole, itraconazole, and caspofungin were placed on the agar medium. The results were evaluated on the 24th and 48th hour of incubation (Table 1).

2.4. Molecular biology

We identified isolates by molecular methods, colonies from fresh cultures (24 h culture in SDA) were initially suspended in 1x PCR buffer (Fermentas) and 0.5 McFarland turbidity suspension prepared. Ribosomal RNA ITS1-4 (ITS1 TCCGTAGGTGAACCTGCGG; ITS4 TCCTCCGCTTATTGATATGC),D1/D2(NL1 GCATATCAATAAGCGGAGGAAAAG; NL4GG TCCGTGTTTCAAGACGG) regions, Elongation factor 1a (EF1a-F CATCGAGAAGTTCGAGAAGG; EF1a-R AACTTGCAGGCAATGTGG) and beta tubulin (bTUB-FGGTAACCAAATCGGTGCTGCTTTC; bTUB-RACCCTCAGTGTAGTGACCCTTGGC) gene regions were amplified by direct colony PCR with 1 µl of this suspension. PCR amplification was performed in 25 µl final volume using 2.5 µl 10 × PCR buffer, 2.5 µl, 2.5 mM dNTPs, 2.0 µl 1.5 µM primer pair, 0.25 µl 5.0 U/µl Taq (Fermentas), 16.75 µl water, and 1.0 µl sample. Following the first denaturation at 94 °C for 4 min, 40 cycles of PCR was performed at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min. Final elongation incubation was performed at 72 °C for 5 min. PCR products were imaged with 2% agarose gel electrophoresis. PCR products were sequenced by bidirectional DNA sequence analysis. Edited and aligned sequences were compared with gene bank data and the nucleotide BLAST method used for the comparison. The strains were confirmed as *C. auris* [6].

3. Results & Discussion

C.auris is an emerging fungus. It has been regarded with concern in recent years due to the troubles in identification at the laboratory level, resistance to antifungals, and difficulties in infection control [1,7]. *C.auris* most commonly leads to candidemias, and the most important risk factors are ICU hospitalization, previous antibiotic or antifungal use, use of CVC, history of surgery, and immunosuppression [1,3]. Almost all of these risk factors were present in our patient.

Antifungal resistance is a common feature in *C. auris*. Kathuria *et al.* investigated antifungal susceptibility in 90 *C. auris* isolates according to CLSI standards and determined fluconazole resistance in all of the isolates. Increased MIC levels were seen in the other azoles and increased caspofungin MIC levels were seen in 37% of the isolates. They also evaluated the compatibility of broth microdilution with VITEK 2 and E-test methods and found that the compliance for VITEK 2 and E-tests were as follows: 10% and 81% for amphotericin-B, 90% and 48% for caspofungin, 91% and 79% for voriconazole, respectively (8). In another multicenter study including 54 *C. auris*, resistance rates to fluconazole, amphotericin-B, and caspofungin were 93%, 35%, and 7%, respectively [3].

Table 1
MIC levels of *C. auris* (microg/mL) with different methods.

	CLSI	E-test	Sensititre
Amphotericin-B	1		2
Fluconazole	>32	>256	>256
Voriconazole	0.25	0.19	>8
Posaconazole	0.03	0.064	>8
Anidulafungin	0.06		0.12

MIC: Minimal inhibitory concentration, CLSI: Clinical Laboratory Standards Institute.

The CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST, http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Clinical_breakpoints/Antifungal_breakpoints_v_9.0_180212.pdf) suggest different susceptibility criteria for *Candida* species [5]. Although there are no standardized breakpoints for *C.auris*, cut-off for MIC levels are suggested temporarily. These breakpoints are ≥32 μg/mL, ≥2 μg/mL, and ≥4 μg/mL for fluconazole, amphotericin-B, and caspofungin/micafungin, respectively [1]. In the light of these findings, our isolate was fluconazole resistant and increased MIC levels for voriconazole were determined. The isolate was susceptible to echinocandins according to CLSI criteria. E-test results were found to be compatible with broth microdilution method whereas SYO results were incompatible considering azole susceptibilities.

The percentage agreements between broth microdilution and SYO are not 100% for *C. auris* [9]. Ruitz-Gaitan *et al*. evaluated 73 *C. auris* isolates and determined percentage agreement (±2 dilutions) between EUCAST methodology and SYO as 98% and 95.9% for posaconazole and voriconazole, respectively [10]. Since only one isolate was evaluated in our study, this may lead to a high level of discrepancy between the two methods.

Echinocandins are the first choice for treatment. The addition of liposomal amphotericin-B is recommended in patients unresponsive to echinocandins [1]. Liposomal amphotericin-B was added to caspofungin in our patient due to recurrent attacks of candidemia.

C.auris is a yeast that can lead to nosocomial outbreaks. Difficulties in identification can lead to delays in interventions and the spreading potential in the hospital was determined [8,11]. Previously, an outbreak lasted 16 months in a cardiothoracic ICU affecting 50 patients. A clonal pattern of spread was demonstrated but the source could not be determined. *C. auris* was isolated from environmental surfaces and apart from hand hygiene and isolation measures, the authors emphasized the importance of environmental disinfection with bleach and hydrogen peroxide vapor and screening cultures from the patients for effective infection control [11].

Environmental surfaces may be the source of *C. auris* in outbreaks, but the source of the first case could not be identified in most reports [12]. Similarly, we could not identify the source although all of the patients and the environmental surfaces in the ward were screened.

Infection control measures include contact precautions, single room isolation, cohorts of colonized or infected patients. Close contacts of index cases should be screened for colonization with axilla and inguinal swabs. Environmental cleaning and cleaning of medical devices are among the basic parameters for prevention of transmission in healthcare settings. The CDC recommends EPA-approved disinfectants effective against *C. difficile* spores for environmental disinfection, but other organizations recommend bleach containing 1000 ppm chlorine or an disinfectant with antifungal effect. There is generally no recommendation for decolonization of patients and screening of healthcare workers. Terminal disinfection after discharge of the patients, no use of quaternary ammonium compounds, preference of disposable tools are among other suggestions [7,13]. During the outbreak at our hospital, we isolated the index and colonized patient in single rooms and implemented contact precautions. The screening of environmental surfaces did not reveal *C. auris*. Environmental disinfection was achieved with 1000 ppm chlorine bleach. The unit was visited daily by the infection control team for three months, and the outbreak was controlled.

This is the first report of *C. auris* fungemia and local spread in Turkey. Although the source could not be identified, the spread was stopped with infection control measures.

Conflicts of interest

No conflict of interest in terms of financial and other relationships is present for any of the authors. The manuscript has been read and approved by all of the authors.

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