FIRST CASES IN BULGARIA WITH PROVEN CANDIDA AURIS IN IMMUNOCOMPROMISED PATIENTS

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ABSTRACT

Candida auris is a fungus with pathogenic potential, first isolated in 2009 in Japan. Since then, sporadic cases have been reported, which have increased significantly in recent years.

In Bulgaria, for the first time in 2025, we described almost simultaneously three cases with *Candida auris* strains isolated from hospitalized patients. All of them were immunocompromised, mainly with cardiovascular problems, but in none of them a fungal isolate from blood stream was obtained. It is probably a question of colonization.

In the National Reference Laboratory of Mycoses at the NCIPD, *Candida auris* fungal strains were confirmed and tested for susceptibility to antifungals. All showed resistance to Fluconazole.

Given the frequent misidentification of *Candida auris*, adequate microbiological examination and subsequent appropriate antifungal therapy are necessary, because this fungus is associated with a high mortality rate in immunocompromised individuals, even with initiated treatment.

Keywords: Candida auris , resistance, antifungals

INTRODUCTION

Candida auris is a multidrug-resistant medically significant fungus that can easily spread in healthcare settings and cause severe and deadly infections in immunocompromised patients.

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An epidemiological study conducted by the European Centre for Disease Prevention and Control (ECDC) in 2022, showed an increase in *Candida auris* cases across Europe. The data showed almost doubled cases, from 335 to 655 in 2020 and 2021, respectively . Most cases were reported from Spain and Italy and involved colonization (skin, urine, wounds, nose, axillae, rectum), while systemic and other types of infections accounted for 15.3% and 10.3% of cases, respectively. Reported cases mostly affected immunocompromised COVID-19 patients. [1]

Candida auris was first isolated in 2009 from the external ear canal of a patient in Japan (" *auris* " - ear). [2] It is associated with high mortality rates and persistent candidemia due to resistance to antifungal agents and its persistence in the environment (air, surfaces, disinfectants and other materials). Thermal stability and growth at 42-45 ˚C are used to distinguish it from other Candida spp. [3]. Another feature of this fungus is its high tolerance to osmotic stress (> 10% NaCl), which together with thermal stability contributes to prolonged survival in the environment and on human skin, including in some disinfectants. [4].

Candida auris is difficult to identify due to false positive results from commercially available identification systems (Vitek, Api C Aux, Auxacolor). MALDI-TOF and Real Time PCR have the potential for rapid identification. [5]

The most common resistance is to the following antifungal agents: Fluconazole, AmphotericinB and Voriconazole. Posaconazole, Isavuconazole and Itraconazole, as well as the group of echinocandins (Caspofungin, Micafungin, Anidulafungin), are more active. [6].

Risk factors are associated with immune deficiency (diabetes, sepsis, lung disease, transplantation, solid tumors, chronic otitis media, cardiovascular disease) . However, *Candida auris* has been isolated from the skin of colonized patients for months. The invasive form, even with appropriate treatment, is associated with a high mortality rate (30-70%). [7].

The first three strains were isolated from blood cultures in the UK National Reference Laboratory for Mycoses in 2013. From 2013 to 2017, 225 cases were identified, of which 56% were colonized on the skin and mucous membranes, and 16% (only 9 patients) – with candidemia. [8].

In 2016, four cases of *Candida auris* were reported in Valencia, Spain. From 2017 to 2019, 203 patients were colonized and 30 invasive infections occurred. During the COVID-19 pandemic, *Candida auris* cases continued to increase. [9].

In 2019, the first case of *Candida auris* was reported from Genoa, Italy. This was followed by an increase in cases in 2020 and 2021, and in 2022, 277 cases occurred in Liguria and the neighboring Emilia-Romagna region. [10].

There have been reports from other European countries as well. In Greece, the first case of *Candida auris* was diagnosed in 2019 in a patient with cystic fibrosis, and in 2022 58 new cases were found. In Germany the cases documented by the National Reference Center for Invasive Fungal Infections are also increasing. [12]. Cases were also reported in France, Belgium, Norway, Austria, the Netherlands, Belgium and Poland. [13]. In 2016 and 2017, 49 critically ill patients were reported in Russia [14] . In the United States, the first case was reported in 2013 in a patient from the UAE (United Arab Emirates) . Subsequently, over 250 cases were identified by 2018. As of 2019, a total of 685 confirmed cases were registered according to CDC . In 2021, the cases were already over 1,000. [15]

Candida strains was performed at the National Reference Laboratory for Mycoses at the National Center of Infectious and Parasitic diseases. A total of 132 clinical isolates collected from 2015 to 2021 were included in the study. All strains were re-identified with MALDI-TOF MS (BioTyper , Brucker , Daltonica , Germany) due to the possibility of misidentification with standard mycological procedures. *Candida auris* has been reported to be misidentified as *C. haemulonii*, *C. famata* and *Rhodotorula glutinous* by commercial identification systems such as Vitek 2 and Api 20 C Aux. As expected from our retrospective study, the highest percentage of strains were identified as *C. albicans* (31%), followed by *C. parapsilosis* (19%) and *C. glabrata* (17%). None of the strains were identified as *Candida auris* .

Prevention involves rigorous actions to reduce the risk of developing *Candida auris* outbreaks . Rapid identification, screening of close contacts, implementation of protective measures, and regular cleaning of surfaces and equipment with chlorine-based or other fungicidal disinfectants are required [16].

Regarding therapy and antifungal resistance, there are currently no established interpretative breakpoints for the main antifungal agents. [17]. According to the SANFORD guide, primary therapy includes the echinocandins Caspofungin (70 mg iv , followed by 50 mg iv), Micafungin (100 mg iv) , or Anidulafungin (200 mg iv , followed by 100 mg iv) . Alternative therapy includes liposomal Amphotericin B after determining susceptibility to it (it is believed that at MIC>2 mcg/mL it cannot be used)[18]

The purpose of this study is to see if there are registered cases of C. auris in Bulgaria, how much will they increase over time, and whether they are systemic infections or contaminations?

CASE REPORTS

The first case described in NRL Mycoses is from the beginning of 2025 in a 75-year-old patient, admitted urgently due to echocardiographic data for hemodynamically significant pericardial effusion to a hospital in the city of Sofia. She was treated for multiple organ failure, which developed after heart surgery in 2024. She was admitted to the Cardiac Surgery Department with a diagnosis of pericardial effusion and complaints of shortness of breath with minimal physical exertion and at rest. Her condition was preceded by a mitral tricuspid valve prosthesis with biological valve movements. The concomitant diseases were chronic heart failure, permanent atrial fibrillation, arterial hypertension, dyslipidemia and anemic syndrome, as well as acute renal failure. The patient had been onwith prolonged me-

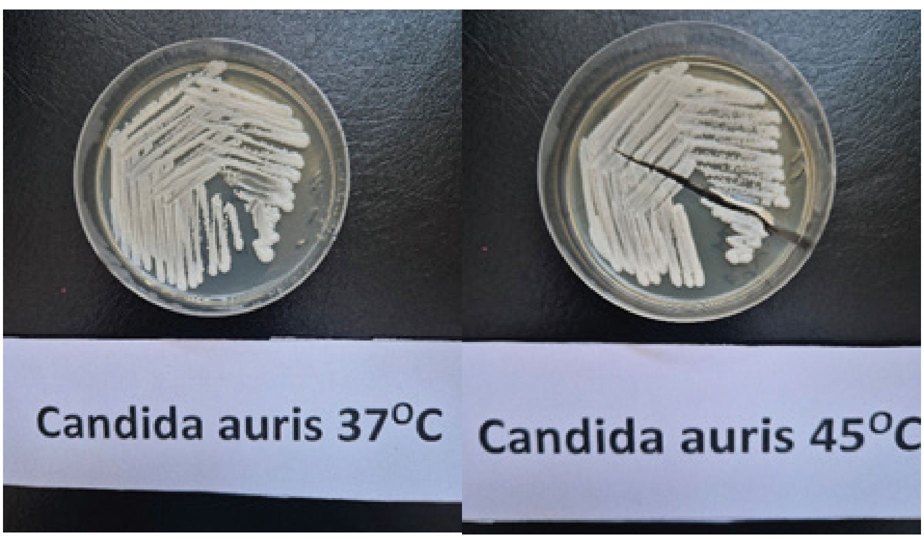

Fig. 1. Macroscopic view of *Candida auris*

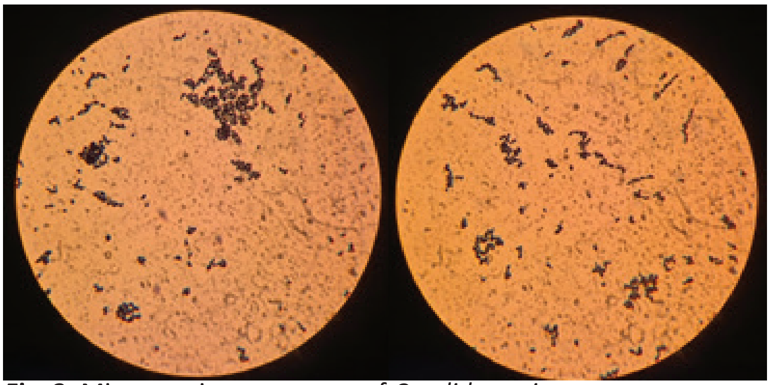

Fig. 2. Microscopic appearance of *Candida auris* , Gram stained

out growth. A little later, a tracheal aspirate was taken due to bilateral pleural effusions, with hypoventilation changes of the underlying lung parenchyma, without X-ray evidence of pneumothorax. This aspirate was also examined microbiologically. A fungal strain was isolated from it, identified as *Candida auris.* (Strain 1). The isolated fungal strain was sent to the NRL Mycoses, at the National Center for Infectous and Parasitic Deseases, Sofia for confirmatory identification.

The second clinical case was in a 78-year-old patient with pericardial effusion again in the Cardiac Surgery Department of a metropolitan hospital. Wound secretion was taken for microbiological examination, from which *Candida auris* was isolated again*.* (Strain 2), and was sent to the NRL Mycoses for confirmation.

The third case was a 73-year-old patient at the Department of Anesthesiology and Intensive Care at the Oncology Hospital in Sofia. He had thromboembolism of the lower extremities arteries. A catheter urine sample was taken for microbiological examination, from which *Candida auris* was isolated*.* (Strain 3), and sent to NRL Mycoses.

At the NRL Mycoses, NCIPD, the reference confirmation of the three fungal strains was performed. The strains were plated on Sabouraud agar medium, followed by cultivation in parallel at two different temperatures: 45 ˚C and 30 ˚C, given the heat resistance of this fungus. At both temperatures, the fungi grew as whitish yeast-like colonies (Fig. 1) .

Microscopic evaluation after Gram staining visualized yeast-like cells that did not differ significantly from other Candida species (Fig. 2).

Microscopy was followed by microbiological species identification using matrix-assisted laser desorption ionization time-of-flight mass spectrometry - MALDI-TOF. MS (BioTyper, Brucker , Daltonica, Germany), (Fig. 3). The identified strains were also confirmed by Real-Time PCR (Candida qPCR Panel, Bio Speedy) after DNA isolation from pure fungal cultures using the Cheleex method (BioRad).

After confirming the strains as *Candida auris,* a test was performed to check the antifungal susceptibility (antimycogram) . For this purpose, the microdilution method (ComASP Antifungal, Liofilchem , Italy) was used to determine the minimum inhibitory concentration (MIC) to the following antifungal agents: Fluconazole, Voriconazole, Itraconazole , Caspofungin , Anidulafungin and Micafungin . A 24-hour yeast culture was turbid to 0.5 McFarland in saline. This solution was diluted 1:20 with commercial saline (solution A), and 100 µl were transferred to a second solution (solution B); 200 µl of the latter were transferred to the wells of an antimycotic susceptibility plate, followed by incubation for 24 hours at 35-37 ˚ C. The MIC was read visually when the wells became turbid (Fig. 4) .

MIC were determined by an E-test (Liofilchem, Italy): strips with a concentration gradient of the antifungal agent (Fig. 5).

DISCUSSION

The three fungal strains were definitively confirmed with a high percentage of identification response as *Candida auris*.

The interpretation of antimicrobial susceptibility has not been not standardized. The European Committee

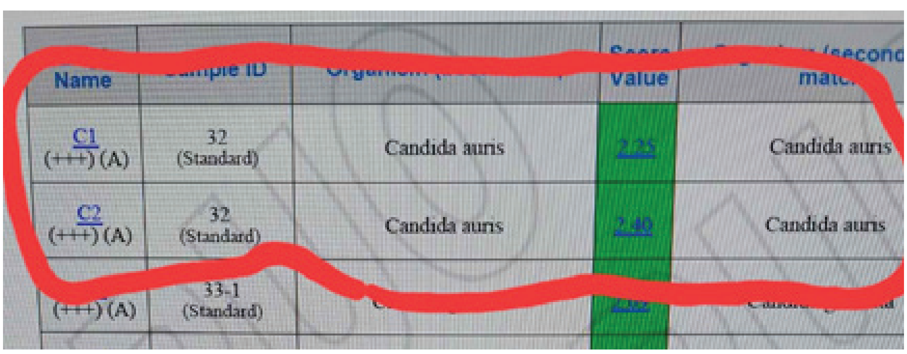

Fig. 3. MALDI-TOF result MS (BioTyper , Brucker , Daltonica , Germany)

Fig. 4. Antimycotic susceptibility plate by microdilution and MIC determination

on Antimicrobial Susceptibility Testing (EUCAST) has no interpretation data yet, while the Clinical Laboratory Standards Institute (CLSI) has set experimental data [19]. Our results are summarized in Table 1 .

The strains showed 100% resistance to Fluconazole. They showed similar susceptibility to the other antifungals. All three strains were sensitive, except for the expected complete resistance to Fluconazole .

CONCLUSION

Candida auris has a proven potential to generate outbreaks in healthcare facilities. Misidentification of the fungus can delay the rapid implementation of necessary measures. Therefore, adequate laboratory testing and correct diagnosis are necessary.

Candida auris cases is a signal that we need to prepare for more and more encounters with this potentially deadly new fungal pathogen.

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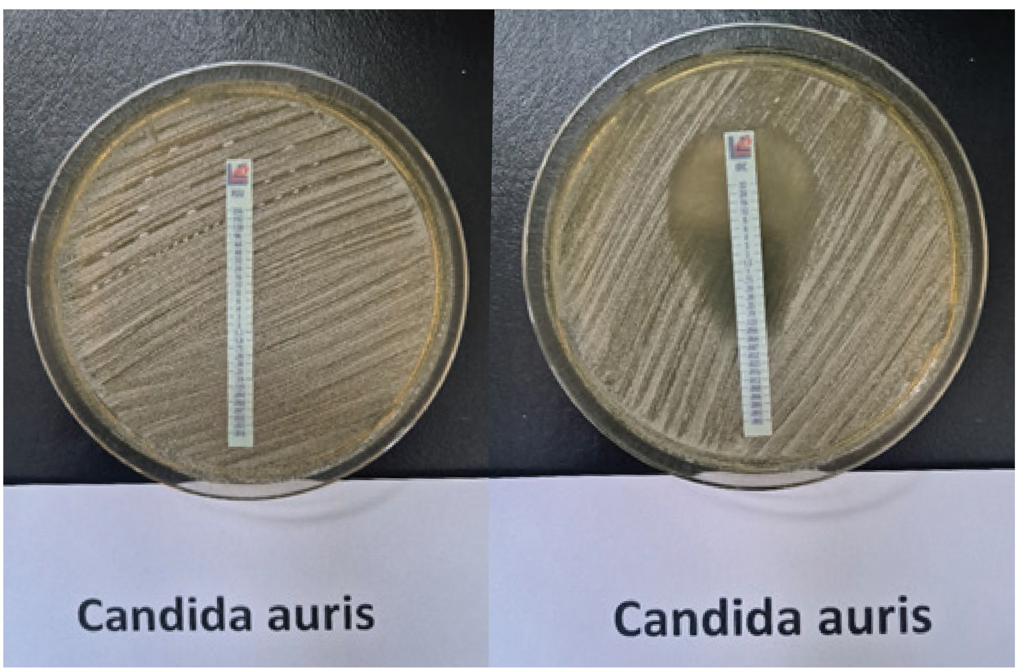

Fig. 5. E-test method for determining the MIC of *Candida auris* to antifungal agents (Fluconazole, Itraconazole)

Table 1. Results of MIC testing and interpretation of antifungal susceptibility of *Candida auris strains*

Antimicrobial	MIC			interpretation		
	Strain 1	Strain 2	Strain 3	Strain 1	Strain 2	Strain 3
Fluconazole	>8	>8	>8	R	R	R
Itraconazoe	>0.5	>0.5	>0.5	S	S	S
Voriconazole	0.25	0.5	0.5	S	S	S
Micafungin	0.25	0.25	0.25	S	S	S
Caspofungin	0.5	0.5	0.5	S	S	S
Anidulafungin	0.25	0.25	0.25	S	S	S
Isavuconazole (E test)	0.016	0.023	0.006	S	S	S
Fluconazole (E-test)	>256	>256	>256	R	R	R
Itraconazole (E-test)	0.19	0.5	1	S	S	S

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