RETROSPECTIVE RE-IDENTIFICATION OF CANDIDA STRAINS BY MALDI-TOF IN SEEK OF *CANDIDA AURIS*

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ABSTRACT

The yeast *C. auris* was first described as a new species in 2009= Since then, this species was recognized as an emerging multi-drug-resistant (MDR) yeast that can cause a wide spectrum of infections, ranging from fungemia to deep-seated infections, especially in intensive care settings. It can be not- or misidentified by commercial identification systems. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has recently been considered as a convenient, rapid and accurate technology in the identification of yeast isolates to species level. A total of 132 clinical isolates, collected from 2015 to 2021 in the National Reference Laboratory of Mycology and Sexually Transmitted Infections were included in the study. The isolates were mainly from patients with candidemia and other specimens from invasive Candida infections.

The isolates were identified by standard mycological procedures, the assimilation profile was done by commercially available strips and with the automated VITEK2 Compact system. All the strains were identified with MALDI-TOF MS. Antifungal susceptibility testing was conducted to some of the strains by agar disk-diffusion (according to CLSI M44-A2), E-test and VITEK2.

Some of the strains which could not be identified or were misidentified by standard mycological procedures were correctly determined by MALDI-TOF MS. None of them was identified as *C. auris.* There were no large deviations in the antifungal susceptibility profiles of the tested strains.

We assess MALDI-TOF MS as convenient, rapid, cost-effective and accurate technology in the identification of fungal strains which are difficult to determine with the traditional procedures. Up to now, isolation of *C. auris* has not been reported in Bulgaria.

Keywords:

C. auris, MALDI-TOF, candidemia, antifungal resistance, Bulgaria

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INTRODUCTION

The yeast *C. auris* was first described as a new species in 2009, isolated from the external ear canal of a Japanese patient (1). Since then, this species was recognized as an emerging multi-drug-resistant (MDR) yeast that can cause a wide spectrum of infections, ranging from fungemia to deep-seated infections, especially in intensive care settings (2). As it is spread over five continents, CDC is concerned about *C. auris* for three main reasons. First, t is often resistant to multiple antifungal drugs commonly used to treat *Candida* infections and some strains are resistant to all three available classes of antifungals= Second, it is difficult to identify with standard laboratory methods, and it can be misidentified in labs lacking specific technology. Misidentification may lead to inappropriate management. Finally, it has caused outbreaks in healthcare settings. Therefore, it is important to quickly identify *C. auris* in a hospitalized patient so that healthcare facilities can take special precautions to stop its spread (https://www.cdc.gov/fungal/candida-auris/index.html). *C. auris* is reported to be misidentified as *C. haemulonii, C. famata* and *Rhodotorula glutinis* by commercial identification systems, such as Vitek2 and API20C-AUX. In clinical microbiology laboratories the conventional methods for yeast identification are based on biochemical methods. However, these procedures are time-consuming, and occasionally the results may be difficult to interpretate because of indistinct reactions or outdated databases. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has recently been considered as a convenient, rapid and accurate technology in the identification of various microorganisms to species level (3). The process

compares the proteomic fingerprint of an unknown microorganism, which mainly consists of ribosome proteins, to those in a reference spectral database. The peak profiles that are generated by Bruker MALDI-TOF MS are matched to reference libraries using the integrated patterns matching algorithm, BioTyper software (Bruker Daltonics, Bremen, Germany).

In case of difficulties in the correct identification of some yeast strains, the best choice of techniques is to combine ITS-region DNA-sequencing, MALDI-TOF MS and assimilation profile determination by commercially available API strips (API 20C AUX; bio Mérieux, Marcy l'|Etoile, France) and AuxaColor (BIO RAD)(4).

In April 2019, a report on the first isolation of *Candida auris* in Greece from a sputum culture of a cystic fibrosis patient was published. After the pathogen was misidentified as *C. duobushaemulonii* by VITEK2YST, it was identified as *C. auris* by MALDI-TOF MS (5). Greece is the closest place to our country where this emerging species has been isolated. Based on all the recent information about *C. auris,* we have decided to conduct a retrospective re-identification with MALDI-TOF of isolates collected through the years at the National Reference Laboratory of Mycology as well as to identify newly isolated strains, some of which – with reduced antifungal susceptibility.

MATERIAL AND METHODS

Isolates. In the study were included a total of 132 clinical isolates, collected from 2015 to 2021 at the National Reference Laboratory of Mycology and Sexually Transmitted Infections. Some of the strains were isolated in the laboratory and the others were sent from big hospitals in the country. The isolates were mainly from patients with candidemia (blood; n=49). Other specimens from invasive Candida infections included: sputum (n=21), transtracheal aspirate (n=11), bronchoalveolar lavage (n=7), venous/arterial catheter lavage (n=5), peritoneal fluid (n=2) and pleural fluid (n=2) = Another 35 isolates were associated with colonization and carriage (urine, vaginal swab, buccal mucosa, feces and others).

Phenotypic characterization. The isolates were identified by standard mycological procedures, including colony color on CHROM agar Candida medium (Difco, Becton Dickinson & Company, Baltimore, MD, USA) and morphology on corn meal agar. Additionally, the assimilation profile of all yeast isolates was done by commercially available API strips and AuxaColor 2, which were read and interpreted at 48 and 72 h. Some of the strains were identified with the automated VITEK2 Compact system using a VITEK 2 YST card.

MALDI-TOF MS. Prior to sample preparation, all yeasts were grown for 24 hours (plus additional 24h if necessary) at 30°C on Sabouraud dextrose agar. For the identification of yeast isolates the ethanol-formic acid extraction procedure was followed according to the manufacturer's protocol.

The process in MALDI TOF MS compares the proteomic fingerprint of an unknown microorganism, which mainly consists of ribosome proteins, to those in a reference spectral database. The peak profiles that are generated by Bruker MALDI-TOF MS are matched to reference libraries using the integrated patterns matching algorithm, BioTyper software (Bruker Daltonics, Bremen, Germany). This gives an arbitrary score value of 0 to 3.0 representing the similarity between the sample and the reference spectrum. A log(score) of ≥2.0 represents successful identification of a species, and a score of 1.7 to 2.0 is acceptable at the genus level. Scores higher than 1.8 represent highly-accurate yeast identification (6,7). Cultivation is inevitable when yeasts are identified by MALDI-TOF MS, but the process only requires a minute number of cells, and limited cultivation time. The spectra were analysed using MALDI Biotyper OC version 4.1 (Bruker Daltonik GmbH) and online software MSI (Mass Spectrometry Identification platform)(8).

In case of discrepancies between MALDI-TOF and biochemical testing results, we confirmed the ID by DNA-sequencing of ITS region as previously described by our scientific team (9).

Antifungal susceptibility testing. It was conducted by agar disk-diffusion (according to CLSI M44-A2) and to some of the strains with automated system VITEK2.

RESULTS AND DISCUSSION

As expected, the highest percentage of strains were identified as *C. albicans* (31%), followed by *C.*

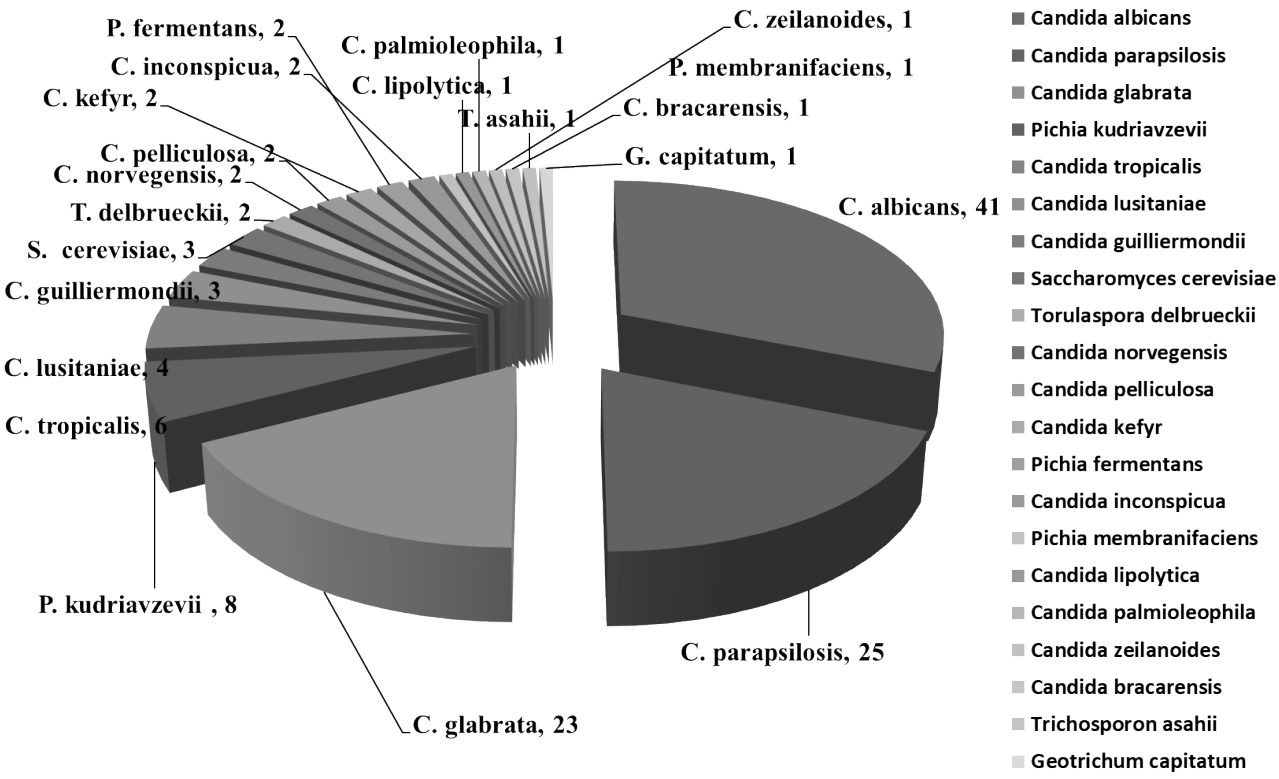

Figure 1. Species distribution of all the strains identified with MALDI-TOF MS

Table 1. Species identification of yeast isolates using three approaches: MALDI-TOF MS, VITEK2 Compact and API 20C AUX/AuxaColor 2.

Species (number)	Correctly identified			Misidentified/Not-identified		
	MALDI-TOF MS	VITEK2 Compact	API 20C AUX/ AuxaColor 2	MALDI-TOF MS	VITEK2 Compact	API 20C AUX/AuxaColor 2
Candida albicans (n=41)	41	41	41	-	-	-
Candida parapsilosis (n=25)	25	25	25	-	-	-
Candida glabrata (n=23)	23	23	23	-	-	-
Pichia kudriavzevii (n=8)	8	7	7	-	1	1
Candida tropicalis (n=6)	6	5	5	-	-	1
Clavispora lusitaniae (n=4)	4	4	4	-	-	-
Candida guilliermondii (n=3)	3	3	3	-	-	-
Saccharomyces cerevisiae (n=3)	3	3	3	-	-	-
Pichia fermentans (n=2)	2	-	-	-	2	2
Torulaspora delbrueckii (n=2)	2	-	-	-	2	2
Candida norvegensis (n=2)	2	2	2	-	-	-
Candida pelliculosa(n=2)	2	2	2	-	-	-
Candida kefyr (n=2)	2	2	2	-	-	-
Candida inconspicua (n=2)	2	2	-	-	-	2

parapsilosis (19%) - *C. glabrata* (17%), and *Pichia kudriavzevii/C. krusei* (6%) (Fig. 1). There was a shift in species distribution towards *Candida* non-*albicans* isolates some of which had increased minimal inhibitory concentration (MIC) to one or more antifungal agents. This may be a result of the increased consumption of antifungal agents.

We have compared three approaches for species identification of yeast isolates: MALDI-TOF MS, VITEK2 Compact and API 20C AUX/AuxaColor 2. As a reference method for correct identification to the species level we used ITS DNA sequencing. Some of the strains which were not identified or were misidentified by standard mycological procedures

Table 3. The results of MICs determination of 4 antifungals (FLU, VOR, MICA and Amp B) to 17 yeast isolates with VITEK® 2 AST-YS08 Ref. 420739.

Species	Fluconazole	Voriconazole	Micafungin	Amphotericine B
Candida albicans (n=8)	MIC≤0.5 – S, n=7 MIC=1 – S, n=1	MIC≤0.12 – S, n=7 MIC=1 – R, n=1	MIC≤0.06 – S, n=8	MIC≤0.25 – S, n=8
Candida glabrata (n=6)	-	-	MIC≤0.06 – S, n=6	MIC≤0.25 – S, n=1 MIC=0.5 – S, n=5
Candida tropicalis (n=1)	MIC≤0.5 - S	-	-	MIC≤0.25 - S
Candida lipolytica (n=1)	MIC=1 - S	-	-	MIC≤0.25 - S
Candida lusitaniae (n=1)	MIC≤0.5 - S	-	-	

Table 2. Misidentified or not-identified isolates using API 20C AUX/AuxaColor 2 compared to MALDI-TOF MS.

Species	Not-identified or misidentified as		
	MALDI-TOF MS	VITEK2 Compact	API 20C AUX/ AuxaColor 2
Pichia kudriavzevii (n=8)	-	Candida spherica(n=1)	Candida spherica(n=1)
Candida tropicalis (n=6)	-	-	Candida parapsilosis(n=1)
Pichia fermentans (n=2)	-	Candida glabrata (n=2)	Candida glabrata (n=2)
Torulaspora delbrueckii (n=2)		Not-identified(n=2)	Candida kefyr(n=2)
Candida inconspicua (n=2)	-	-	Candida glabrata (n=2)
Pichia membranifaciens (n=1)	-	Candida krusei(n=1)	Candida krusei(n=1)
Candida zeilanoides (n=1)	-	-	Candida krusei(n=1)
Candida bracarensis(n=1)	-	-	Candida glabrata (n=1)

were correctly determined by MALDI-TOF MS (Table 1.). There were difficulties in the correct identification of some Candida non-albicans species when using VITEK2 Compact and API 20C AUX/AuxaColor 2. E.g, one strain of *P. kudriavzevii* was misidentified as *C. spherica*, *P. fermentans* - as *C. glabrata, P. membranifaciens* - as *C. krusei*, and so on (Table 2). None of them was identified as *C. auris*.

There are no breakpoints for all yeast species and the antifungal agents tested and this hampers the interpretation of MICs. This was observed when using the automated system VITEK 2 for antifungal susceptibility testing of 17 strains. MIC interpretation for all antifungal agents is available only for *C. albicans* and a couple of other species (Table 3). There were no large deviations in the antifungal susceptibility profiles of the tested strains. Only one strain of *C. albicans* was interpreted as resistant to voriconazole. Using the agar disk-diffusion method, 21.2% fluconazole-resistance rate was determined. MALDI-TOF MS identification revealed that fluconazole resistant strains belonged to species with expected resistant phenotypes (i.e. *P. kudriavzevii* and *C. glabrata*) and none of the resistant isolates was identified as *C. auris*.

CONCLUSION

To date, there is a slight increase in the number of yeast isolates which can not be identified or are misidentified with the traditional and commercial

Table 3. The results of MICs determination of 4 antifungals (FLU, VOR, MICA and Amp B) to 17 yeast isolates with VITEK® 2 AST-YS08 Ref. 420739.

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Candida glabrata (n=6)	-	-	MIC≤0.06 – S, n=6	MIC≤0.25 – S, n=1 MIC=0.5 – S, n=5
Candida tropicalis (n=1)	MIC≤0.5 - S	-	-	MIC≤0.25 - S
Candida lipolytica (n=1)	MIC=1 - S	-	-	MIC≤0.25 - S
Candida lusitaniae (n=1)	MIC≤0.5 - S	-	-	

identification systems. According to our results, MALDI-TOF MS as convenient, rapid, cost-effective and accurate technology for the identification of fungal strains which are difficult to determine with the traditional procedures. Until now there has been no report on *C. auris* isolation in Bulgaria. Because of the high clinical significance of *C. auris*, we recommend identification to species level of every yeast isolate with decreased antifungal susceptibility. Clinical microbiologists have to expect and be aware of the possibility of *C. auris* isolation.

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