ORIGINAL ARTICLE

Investigation of *Candida auris* in Tanta University Hospitals, Egypt

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ABSTRACT

Background: *Candida auris is an emerging multidrug-resistant (MDR) fungus of global significance. It causes invasive life-threatening infections associated with treatment failure.* ***Objectives:*** *To investigate the occurrence of C. auris species in Tanta University Hospitals, and its susceptibility profile to different antifungal agents.* ***Methodology:*** *Candida was isolated from different specimens. Screening for C. auris was performed using CHROMagar Candida medium. Species identification and antifungal susceptibility testing were performed by VITEK2 system. Molecular confirmation was done by PCR.* ***Results:*** *Out of 414 Candida isolates, 295 (71.3%) isolates were C. albicans, 48 (11.6%) were C. tropicalis, 42 (10.2%) were C. parapsilosis, 20 (4.8%) were C. krusei, 8 (1.9%) were C. glabrata and one (0.2%) isolate was C. haemulonii. Two isolates were MDR. No C. auris was identified by VITEK2 system or by PCR.* ***Conclusion:*** *No C. auris has been detected in Tanta University Hospitals. Nationwide studies are required for early detection of this superbug and limit its spread.*

INTRODUCTION

Candida auris is one of the multidrug-resistant species of *Candida*, that was first detected in 2009, when it was isolated from the external ear of a Japanese patient, and hence its name "auris, ear in Latin"[1]. However, a retrospective South Korean report demonstrated that the earliest *C. auris* species was actually dated back to 1996, when isolated from a child having blood stream infection[2].

Since then, detection and isolation of *C. auris* have been reported from many countries all over the world, including the United States, Canada, Venezuela, the United Kingdom, Russia, South Africa, Saudi Arabia, Kuwait, Pakistan, Malaysia, South Korea, and China[3]. However, no clear data are yet available concerning *C. auris* epidemiology and its antifungal susceptibility profile in Egypt.

Recognition of *C. auris* species among hospital fungi is very essential challenge, since spread of drug-resistant *C. auris* represents a great problem[4]. The mortality rate of *C. auris* candidemia cases has reached 30 to 72%, with most infections appear in adults, and in critically ill cases in intensive care units[3]. In June 2016, Centers for Disease Control and Prevention (CDC) released several alerts to warn the clinicians, and the infection control practitioners of the emerging multidrug-resistant fungus *C. auris* and to give the laboratorians certain recommendations concerning the methods of its identification[5].

C. auris has been known by its ability to cause invasive diseases. This invasive power may be due to its products of proteinase and phospholipase[6]. It does not form pseudohyphae or germ tubes *in vitro*[5]. Other virulence determinants were observed in *C. auris* such as its ability to adhere to materials like plastic, forming biofilms[7]. An additional interesting observation is that some strains of *C. auris* can form buddings but are not able to release daughter yeasts, producing large clumping aggregates of these fungi difficult to be disrupted by vigorous vortexing or by detergent application[8].

The exact way of *C. auris* transmission is still unknown, however it seems to be through direct and indirect contact i.e., from the colonized patients or environmental surfaces in the hospitals to the hands of health care staff[9]. This may be related to the finding that *C. auris* can persist on dry plastic surfaces for 14 days at normal conditions of hospital rooms (57% relative humidity and 25°C)[10].

Several research have been carried out to characterize the resistance of *C. auris* to different disinfectants. It is well known that quaternary ammonium compounds have fungicidal activities against different yeasts including *Candida*[11], however these disinfectants have shown poor activity against *C. auris*[12]. Recent studies suggest that chlorine-based disinfectants and non-sporocidal hydrogen peroxide are effective against *C. auris*[13,14]. However, despite frequent

hand washes with chlorhexidine antiseptic every day, *C. auris* continued to colonize patients' skin[9].

Considering the tendency of *C. auris* to cause hospital outbreaks, the CDC has affirmed the adherence to standard and contact precautions, isolation of infected patients in separate rooms if possible, and thorough daily cleaning of these rooms with a disinfectant that is effective against sporing organisms particularly *Clostridioides difficile*[15]. In addition, CDC has recommended continuing these precautions for the entire period of the patient's stay in the hospital, since patients remain colonized with this fungus for many months and may be indefinitely, even after acute infection has been treated[15].

Emergence of *C. auris* is alarming because of the ability of this organism to develop multidrug resistance (MDR). Some isolates have shown resistance to all available antifungal classes, with poor outcome of associated infections[16]. Objectives of this study were to evaluate the occurrence of *C. auris* among different clinical specimens collected from Tanta University Hospitals and investigate the *in vitro* antifungal susceptibility profile of this fungus.

METHODOLOGY

Screening for *C. auris*:

This cross-section study was conducted between February 2020 and August 2021. A total of 414 *Candida* isolates were collected from the Clinical Pathology Department of Tanta University Hospitals during the study period. The collected isolates were included according to the related specimen type. Since *C. auris* is characterized by causing biofilms and invasive infections, these features affected our choice of specimen types (from invasive and device-associated infections). This study was approved by the ethical committee of Faculty of Medicine, Tanta University (No: 33665/1/20).

All isolates were reidentified to genus level by conventional mycology methods in the laboratory of Microbiology Department, Faculty of Medicine, Tanta University. Screening for *C. auris* species was done using chromogenic agar medium (CHROMagar™ Candida, Paris, France). According to the manufacturer's instructions, *C. albicans* colonies appear green, *C. tropicalis* colonies appear metallic-blue, *C. krusei* colonies appear pink and fuzzy, and *C. glabrata* colonies appear mauve. According to CDC, *C. auris* may develop white, pink, purple, or red colors[17].

After *Candida* isolation on CHROMagar, *C. auris* species was excluded by germ tube test. A colony of *Candida* isolate was added to 0.5 ml of human serum in a small tube. After incubation at 37°C for 3 hours, a drop of serum was examined under high power lenses. A germ tube appeared as short filamentous extension (3-4 times the length of yeast cell), arising laterally from the yeast body, without any constriction at the point of arising[18].

Species identification using VITEK2 system:

Isolates with uncertain species identifications were identified to species level using the VITEK®2 Compact 15, software version 8.01 (bioMérieux, France), with a VITEK2 YST ID card according to the instruction of the manufacturer.

Antifungal susceptibility test using VITEK2 system:

All isolates identified by VITEK2 system were reexamined for evaluating their *in vitro* antifungal susceptibility profile using VITEK2 AST-YS08 card following the manufacturer's instructions. This card tests 6 antifungal drugs: amphotericin B (1- 32 µg/ml), flucytosine (0.06-4 µg/ml), two azole drugs [fluconazole (2-64 µg/ml), voriconazole (0.5-8 µg/ml)], and two echinocandin drugs [caspofungin (0.12-8 µg/ml), and micafungin (0.06-4 µg/ml)].

Molecular confirmation using polymerase chain reaction (PCR):

All isolates identified by VITEK2 system were confirmed genotypically using PCR. DNA was extracted from all tested isolates using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Extracted DNA was amplified using a pair of primers, which amplify specific sequences within the internal transcribed spacer regions (ITS-1 and ITS-2) of ribosomal DNA of *C. auris.* The forward primer was (CAURF, 5'-ATTTTGCATACACACTGATTTG-3') and the reverse primer was (CAURR, 5'-CGTGCAAGCTGTAATTTTGTGA-3') as described previously[19].

Statistics:

Data were analyzed using SPSS software version 26. The data were described by numbers (n) and percentages.

RESULTS

Fungal isolates on CHROMagar:

Out of 414 *Candida* isolates tested on CHROMagar Candida medium, 295 (71.3%) isolates developed the green color of *C. albicans*, 48 (11.6%) isolates developed the blue color of *C. tropicalis*, and the remaining 71 (17.1%) isolates showed white, pink, or purple color and were categorized as "**Suspected Species**". These isolates were subjected to further examination to detect *C. auris* among them.

The 71 suspected species were isolated from different specimens as follows: 29 (40.8%) catheter specimens of urine, 21 (29.6%) tracheal aspirates, 10 (14.1%) ear swabs, 10 (14.1%) blood specimens, and one (1.4%) oropharyngeal swab (Table 1). All of the suspected isolates were negative for germ tube formation.

Species identification by VITEK2 system

The suspected isolates were identified to species level using the VITEK2 yeast identification system as shown in Table 1. VITEK2 identified 42 (59.2%) isolates as *C. parapsilosis*, 20 (28.2%) isolates as *C. krusei*, 8 (11.2%) isolates as *C. glabrata* and one (1.4%) isolate as *C. haemulonii*. No *C. auris* isolates were identified by VITEK2 system.

Table 1. Distribution of the 71 isolates of suspected *Candida* species detected by VITEK2 among the different clinical specimens:

Specimens	Related medical diseases	*Candida parapsilosis*	*Candida krusei*	*Candida glabrata*	*Candida haemulonii*
Urine (catheter) (n=29)	CAUTI	17/29 (58.6%)	5/29 (17.2%)	7/29 (24.2%)	-
Tracheal aspirates (n=21)	Pneumonia	10/21 (47.6%)	11/21 (52.4%)	-	-
Ear swabs (n=10)	Otitis media	10/10 (100%)	-	-	-
Blood (n=10)	Candidemia	5/10 (50%)	3/10 (30%)	1/10 (10%)	1/10 (10%)
Oropharyngeal swab (n=1)	Oropharyngeal candidiasis	-	1/1 (100%)	-	-
Total (n=71)		42/71 (59.2%)	20/71 (28.2%)	8/71 (11.2%)	1/71 (1.4%)

CAUTI: catheter-associated urinary tract infection

Antifungal susceptibility testing by VITEK2 system

As presented in Table 2, the highest non-susceptibility was against fluconazole (32.4%), followed by voriconazole, caspofungin, and micafungin (2.8% for each). No resistance against amphotericin B or flucytosine was detected among the 71 isolates. Two isolates (1 *C. parapsilosis*, 1 *C. krusei*) were non-susceptible across two antifungal classes (echinocandins, and azoles).

Table 2. *In vitro* susceptibilities of the 71 isolates of suspected *Candida* species as determined with the VITEK2 system:

Antifungal Drug	*Candida parapsilosis* (n = 42)			*Candida krusei* n = 20			*Candida glabrata* n = 8			*Candida haemulonii* n = 1			Total non-susceptible isolates[c]
	S	I	R	S	I	R	S	I	R	S	I	R	
Amphotericin B	**42**	0	0	**20**	0	0	**8**	0	0	**1**	0	0	0
Flucytosine	**42**	0	0	**20**	0	0	**8**	0	0	**1**	0	0	0
Fluconazole	**41**	0	**1**	-	-	**20**[a]	**7**	0	**1**	0	0	**1**	23/71(32.4%)
Voriconazole	**41**	0	**1**	**20**	0	0	-	-	-[b]	0	0	**1**	2/71 (2.8%)
Caspofungin	**41**	**1**	0	**19**	**1**	0	**8**	0	0	**1**	0	0	2/71 (2.8%)
Micafungin	**41**	**1**	0	**19**	**1**	0	**8**	0	0	**1**	0	0	2/71 (2.8%)

S: sensitive; I: intermediate sensitive; R: resistant

[a] No fluconazole breakpoints for *C. krusei* because of its intrinsic resistant to it.

[b] Voriconazole breakpoints for *C. glabrata* are not yet established by the Clinical and Laboratory Standards Institute (CLSI).

[c] Non-susceptible isolates include resistant and intermediate sensitive isolates.

Molecular confirmation by PCR

Using CAURF and CAURR primers, no amplicon was detected during PCR amplification of DNA isolated from the suspected 71 isolates i.e. no *C. auris* were detected using the molecular confirmation method.

DISCUSSION

At the beginning of this study, no clear data were provided regarding the rate of *C. auris* in Egypt and their antifungal susceptibility profile. Only one report published in 2019 denoted that a case of *C. auris*

appeared in Egypt[20]. It was a case of a 53-year-old male admitted to a hospital in Cairo just after returning from Saudi Arabia in December 2017. He complained of abdominal pain, vomiting, and bone aches. After investigations, he was diagnosed as renal failure and received a hemodialysis.

After 40 days of admission, he was transferred to a tertiary care facility in Alexandria with renal, respiratory, and cardiovascular failure. Blood culture showed *Candida* growth which was identified as *C. auris* with VITEK2 system and confirmed by Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) device. The isolate showed high resistance to fluconazole and amphotericin B, but it was susceptible to echinocandins. The patient died after 4 days of the new admission. After this case, strict contact precautions were taken and no *C. auris* was isolated later in this facility[20].

All over the world, only four distinct *C. auris* clades were identified after the analysis of the whole genome sequences of the isolated *C. auris* during the last decade. These clades are associated with geographical regions: South America (Venezuela), South Africa, East Asia (Japan), and South Asia (India and Pakistan)[21]. This strain isolated from Egypt was clustered within South Asian *C. auris* clade[20].

This report has put Egypt for the first time on the CDC global map for the countries documented *C. auris* cases[22]. Since this report, only one recent research published in 2021, has studied the impact of *C. auris* in Egypt conducted by Khairat *et al* [23]. They reported that no *C. auris* was detected in Cairo University Hospitals after two years of searching. This supports our result that no *C. auris* was found in Tanta University hospitals. This could be explained by the hypothesis suggesting that *C. auris* spreads from existing clades present in endemic countries to new countries, not emerges as new strains following the misuse of antifungal drugs[24]. According to the last update of CDC report, *C. auris* has been detected in 47 countries, of which 11 countries reported only one case of *C. auris*[22]. In Africa, only four countries have reported *C. auris* (Egypt, Sudan, Kenya, and South Africa)[22].

In the current CDC's identification algorithm for *C. auris*[25], no more testing is needed if a *C. auris* identification is performed by the VITEK2 8.01 system, as the update of VITEK2 identification software to version 8.01 included the addition of the *C. auris* taxon. However, a later study performed by Ambaraghassi *et al*.[26] reported that the VITEK2 (software version 8.01) correctly identified only half of *C. auris* isolates and the ability of the system to discriminate between *C. auris* and *C. duobushaemulonii* was low. All these data directed us to use VITEK2 8.01 system for the identification of suspected *Candida* isolated on CHROMagar, and then confirm the results with PCR.

In the current study, 28.7% of the *Candida* isolates were non-albicans species. This percentage is relatively low when compared with the rates of non-albicans *Candida* in other Egyptian studies. A rate of 43.25% was detected by a study in Cairo University Hospitals[23] in blood, urine, wounds, and ear specimens, and a rate of 74% was detected in only blood specimens by another study at Cairo University Hospitals too[27]. This discrepancy could be related to the differences in the sample size, specimen types and the region of the study. On the contrary, an earlier study at Tanta University Hospitals recorded a rate of 31.5% for non-albicans *Candida*[28], which is consistent with our result.

In the present study, the *in vitro* antifungal susceptibility was performed using VITEK2 AST-YS08 card which is compliant with the last update in CLSI breakpoints. Since MDR is defined according to the non-susceptibility of the isolates, we presented and estimated the non-susceptibility rate (intermediate sensitive isolates + resistant isolates) in Table 2.

In this study, the highest non-susceptibility rate against fluconazole correlates well with many other studies[23,29]. This high rate could be explained by the fact that some non-albicans *Candida* species such as *C. krusei* exhibits intrinsic resistance to fluconazole[30]. In addition, fluconazole is the most frequently prescribed drug for treating *Candida* infections[31].

In the current study, high susceptibility of non-albicans *Candida* species to amphotericin B, flucytosine, voriconazole, caspofungin, and micafungin is consistent with other studies[29,32]. On the contrary, individual resistance against each of these drugs were reported by some studies[33,34,35]. Generally, antifungal resistance is still uncommon, however resistance mechanisms are increasingly emerging worldwide[30].For example, resistance to flucytosine develops rapidly if used as monotherapy due to mutations in the relevant genes[36]. In addition, cross-resistance among the azoles and echinocandins continue to be reported in *C. glabrata*[37]. Also, cross-resistance among the azoles and amphotericin B was reported[38].

Finally, MDR *Candida* is defined as an isolate non-susceptible to more than 1 drug in ≥2 drug classes[16]. Accordingly in the present study, two isolates (1 *C. parapsilosis*, 1 *C. krusei*) were considered MDR, they were non-susceptible to two antifungal classes (echinocandins, and azoles).

CONCLUSION

C. auris is an important emerging organism causing life-threatening nosocomial infections. It has become a global threat that cannot be ignored. *C. auris* has not yet been identified in Tanta University Hospitals. Further studies are needed all over the country for early detection of this dangerous organism and limit its spread.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

Author's contribution: Marwa M.E. Abd-Elmonsef and Sara Y. Maxwell contributed to the study design, laboratory work and data interpretation. Both authors drafted, revised, and approved the final manuscript.

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