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Setting a Protocol for Identification and Detecting the Prevalence of *Candida auris* in Tertiary Egyptian Hospitals Using the CDC Steps

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Abstract

BACKGROUND: *Candida* is considered the most common cause of opportunistic infections in the world. Increased use of antifungal agents may have led to increasing resistance of *Candida* for antifungals and may be related to therapeutic failures. Recently, a multidrug-resistant *Candida auris* has immerged causing outbreaks in several countries all over the world. This discovered superbug is widely spread causing a broad range of health care-associated infections.

AIM: This study aims to set a protocol for the identification and detection of the prevalence of *C. auris* in tertiary Egyptian hospitals following the center of disease and control (CDC) methodology.

METHODOS: Over almost 2 years, 400 *Candida* isolates were collected from different wards of Cairo University Hospitals. Identification of species of all isolates was done by germ tube test followed by sub-culturing on chromogenic agar media for confirmation. *Candida* non-albicans isolates were further subjected to thermotolerance. Isolates that grew in 42°C were further identified by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry for definite species identification. Antifungal susceptibility using E-test was done for isolates identified by MALDI to detect resistance patterns.

RESULTS: Among the 400 isolates, 227 (56.75%) were *Candida albicans* while 180 (43.25%) were non-albicans *Candida*. *Candida* non-albicans was classified by Chromagar as following; 25 (13.8%) were *Candida tropicalis*, 43 (23.8%) were *Candida krusei,* and 112 (62.2%) were other *Candida* spp. (*Candida glabrata, Candida kefyr, Candida parapsilosis,* and *Candida lusitaniae*). Using thermotolerance, 10 isolates grew at 42°C suspecting *C. auris*. MALDI-TOF was used for definite and final identification; five isolates were identified as *C. glabrata*, four as *C. krusei,* and one *C. kefyr*. Antifungal susceptibility testing of the 10 identified isolates revealed total resistance to fluconazole.

CONCLUSION: Following the set protocol for identification based on CDC guidelines, *C. auris* is not prevalent in Egyptian hospitals. Fluconazole resistance is on the surge among candida isolates. Further studies on a bigger scale including larger number of hospitals are recommended.

Introduction

Fungal infections are becoming more widely accepted as a global health problem. A fungal infection affects approximately 1.7 billion people worldwide, the majority of which are superficial infections of the skin and mucosa [1]. *Candida* species are the most common cause of nosocomial fungal infections and the fourth most common source of hospital-acquired infections. Globally, *Candida* species cause approximately 400,000 bloodstream infections per year, with mortality rates exceeding 40%. *Candida albicans* is the most common *Candida* species, although non-albicans species such as *Candida tropicalis*, *Candida parapsilosis*, and *Candida glabrata* have become more common due to long-term use and reduced antifungal treatment choices [2].

Candida auris, one of non-albicans species, is an emerging invasive multidrug-resistant fungal infection. It has been reported from 42 countries and presents a serious global health threat [3]. It causes infections in hospital settings around the world. Such hospital-acquired infections have proven to be very difficult to diagnose and treat. Individuals, exposed to this pathogen, are at a high risk for systemic infections resulting in high mortality rates [4]. It has been reported as a major rapidly emerged cause of candidemia worldwide, surpassing the number of cases caused by *C. glabrata* and *C. tropicalis* in South Africa [5].

C. auris is difficult to be identified with standard phenotypic laboratory methods, and only specific technology such as matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry and DNA sequencing can distinguish it effectively from other yeasts. Misidentification may lead to inappropriate management and spread of infection in health-care settings [6].

Furthermore, there is a significant genetic heterogeneity among *C. auris* strains from different parts of the world. *C. auris* has been divided into five distinct clades using whole-genome sequencing-based phylogenetics [7].

For this reason, it is important to set a protocol to rapidly identify it in hospitalized patients; thus, health-care facilities can take special precautions and implementing effective infection control measures to stop its spread especially because some commonly used health-care disinfectants are not sufficiently effective against *C. auris* leads to its persistence in health-care environments for several weeks [8].

However, even as knowledge about *C. auris* grows, notable gaps remain in the understanding of Candida auris emergence, spread, and resistance [9]. Especially from countries such as Qatar, Jordan, and Egypt [10].

The purpose of this study is to set a protocol and detect the prevalence of *C. auris* in tertiary Egyptian hospitals following the center of disease and control (CDC) in detection [11].

Methods

The study was conducted on all samples sent to Cairo University Hospitals with positive *Candida* growth, during the period from February 2018 to December 2019.

Clinical data

Patients' wards and types of samples of candida non-albicans were collected from the associated routine request form of patients' samples delivered to the laboratories. The research protocol was approved by the Research Ethical Committee and following the ethical rules of Faculty of medicine Cairo University Hospitals under ID I-251015.

Selection of samples and isolates

Samples shown growth for *Candida* on blood, chocolate, or MacConkey agar plates were collected, subcultured on Sabouraud dextrose agar for isolation of *Candida* (Biomerieux, France REF, 43 651) [12].

Isolates either pathogenic or colonizers were collected as *C. auris* could be detected as pathogen or as a colonizer [13].

Primary identification of isolates to species level

Germ tube method was used to categorize candida isolates to *C. albicans* and *Candida* species. The method is as following; incubating some *Candida* colonies in 400 ul of human serum for 3 h in 37°C then examined with a wet KOH films for filamentous outgrowth extending from yeast cells. It is positive for *C. albicans* and Candida dubliniensis *and negative for other species* [14].

Confirmation of isolates identification by chromogenic agar medium

Further species identification to *C. albicans*, *C. tropicalis*, *Candida krusei,* and other species was performed using chromogenic media (Oxoid England, Ref CM100) [15]. Agar was prepared and result interpreted according to the manufacture guidelines in Table 1.

Table 1: Identification of candida based on substrate color change in chromogenic media

Candida type	Typical colony color appearance
C. tropicalis	Dark blue
C. albicans	Green
C. krusei	Dry, irregular pink-brown
C. glabrata, *C. kefyr*, *C. parapsilosis*, *C. lusitaniae*	Beige/yellow/ brown

C. tropicalis: *Candida tropicalis*, *C. albicans*: *Candida* albicans, *C. krusei*: *Candida krusei*, *C. glabrata*: *Candida glabrata*, *C. kefyr*: *Candida kefyr*, *C. parapsilosis*: *Candida parapsilosis*, *C. lusitaniae*: *Candida. lusitaniae*.

Thermotolerance to detect suspected isolates

Isolates that appeared non-albicans on chromogenic medium were further subjected to thermotolerance. This was done by subculturing isolates on chromogenic medium as discussed before, but the plates were incubated at 40–42°C for 24–48 h. Only *C. albicans* and few species including *C. auris* can grow at 42°C. *C. albicans* was used as a control [11].

MALDI-TOF for definite diagnosis of C. auris

Isolates that appeared non-albicans and grew on 42°C were subjected to bioMérieux VITEK (MALDI-TOF) MS research use only libraries (with Saramis Ver 4.14 database and Saccharomycetaceae update) according to CDC [16].

Resistance pattern of MALDI-TOF identified isolates

Resistance in identified candida species was tested using three antifungal E tests, amphotericin B, caspofungin, and fluconazole (Biomerieux). The MIC results were interpreted according to CLSI guidelines M60, 2017 [17].

The European Committee on Antimicrobial Susceptibility Testing was used for interpretation of amphotericin B because it is not yet identified by CLSI [18].

Quality control strains

C. parapsilosis ATCC 22019 was used to ensure the quality of antifungal E tests used in detection of the antifungal resistance pattern [16].

Statistical analysis

Statistical calculations were done using computer programs Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows. Percentage from total analysis was used using the following equation part/whole ×100.

Results

This is a cross-sectional study that was conducted on 400 *Candida* isolates collected from different samples sent to microbiology laboratories of Cairo University Hospitals during the period from February 2018 to December 2019.

All *Candida* isolates were subjected to germ tube and cultured on chromogenic agar medium which were classified as follows; 227 (56.75%) *C. albicans* and 180 (43.25%) were non-albicans *Candida*. *Candida* non-albicans was further classified by chromogenic agar as following; 25 (13.8%) were *C. tropicalis*, 43 (23.8%) were *C. krusei,* and 112 (62.2%) were other *Candida* species (*C. glabrata, Candida kefyr*, *C. parapsilosis*, and *Candida lusitaniae*), as shown in Table 2.

Table 2: Classification of candida non-albicans by chromagar

Candida species		Number		Percentage		Patient location		
						ICU (N%)	Wards (N%)	OP (N%)
Candida non albicans	Candida Spp (*C. glabrata, C. kefyr, C. parapsilosis, C. lusitaniae*)	180	112	100	62.2	152 (84.4%)	27 (15%)	1 (0.5%)
	C. krusei		43		23.8			
	C. tropicalis		25		13.8			

C. glabrata: *Candida glabrata*, *C. kefyr*: *Candida kefyr*, *C. parapsilosis*: *Candida parapsilosis*, *C. lusitaniae*: *Candida. lusitaniae*, *C. krusei*: *Candida krusei*, *C. tropicalis*: *Candida tropicalis.*

Isolates of non-albicans *Candida* were collected from different patient population in different wards. Most of candida isolates were collected from ICU wards 83.7% followed by in patient's wards 15.4%, then 0.8% from outpatient samples as shown in Table 2.

Classification of sample types of *Candida* non-albicans was as follows; candiduria represents 89.4%, followed by candidemia 5.6, then *Candida* infection in wounds 4% while the least type of samples was candida isolated from ear 0.8.

After subjecting all isolates n = 180 to high temperature at 42°C, 170 (94.4%) showed no growth, while only 10 (5.6%) isolates were grown at 42°. The 10 isolates were cultured from urine samples (candiduria) and were identified by Chromagar as 90% *Candida* species (*C. glabrata, C. kefyr, C. parapsilosis,* and *C. lusitaniae*) and 10% *C. krusei.*

The accurate identification of thermotolerant isolates was done using MALDI-TOF. Identification of the organism being tested is automatically determined using software that compares the spectral profile of the unknown organism with a reference database. Five of them were identified as *C. glabrata*, four were identified as *C. krusei,* and one was identified as *C. kefyr*.

The antimicrobial susceptibility of MALDI-TOF identified isolates to three antifungals using E test is shown in Table 3; caspofungin and amphotericin were highly effective while flicin were highly effective while ALDI-TOF isolates. The lowest MIC values were recorded for amphotericin B with *C. krusei* with a susceptible range of 0.01–0.1; for caspofungin with *C. glabrata*, with a susceptible range of 0.0–0.02; and *C. kefyr* with amphotericin 0.02.

Table 3: Antimicrobial resistance of thermo-tolerant isolates

Identified isolate	Antifungal	MIC values (ug/ml)	Interpretation
C. glabrata (n=5)	Amphoteracin	0.02–0.3	Sensitive
	Fluconazole	48–64	Resistant
	Caspofungin	0.0–0.2	Sensitive
**C. krusei* (n=4)	Amphoteracin	0.01–0.1	Sensitive
	Caspofungin	0.01–0.6	Sensitive
C. kefyr (other species) (n=1)	Amphoteracin	0.02	Sensitive
	Fluconazole	16	Resistant
	Caspofungin	0.15	Sensitive

**C. krusei* is intrinsically resistant to Fluconazole. The MICs for fluconazole, caspofungin and amphotericin B for C. Parapsilosis ATCC 22019 ranged from 1 to 3 μg/mL, from 0.3 to 0.5 μg/mL, and from 0.6 to 1 μg/mL, respectively and all met the expected ranges according CLSI. *C. glabrata*: *Candida glabrata, C. krusei: Candida krusei*, *C. kefyr*: *Candida kefyr.*

After excluding the four intrinsically resistant *C. krusei* isolates, fluconazole resistance was recorded in 100% of other non-albicans isolates.

The MICs for fluconazole, caspofungin, and amphotericin B for *C. parapsilosis* ATCC 22019 ranged from 1 to 3 μg/mL, from 0.3 to 0.5 μg/mL, and from 0.6 to 1 μg/mL, respectively; the results met expected ranges according to CLSI, as shown in Figure 1 [19].

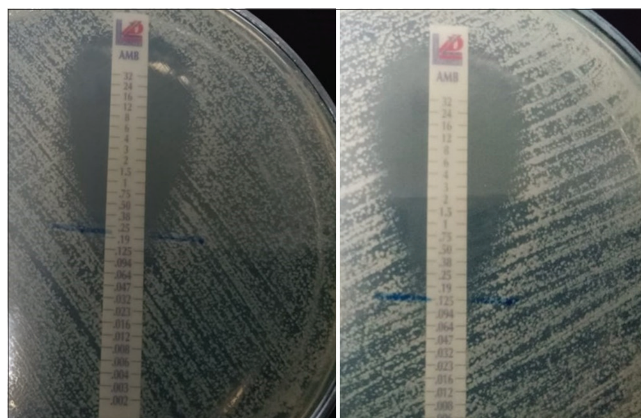

Figure 1: Amphotericin E test result of two isolates of Candida glabrata

Discussion

C. auris is a newly emerged *Candida* species which presents a serious health threat and outbreaks in health-care settings. Isolates are resistant to at

least one antifungal drug; in addition, it could not be identified easily by standard laboratory methods [2]. Consequently, it is crucial to detect the prevalence in each country, and setting a protocol for rapid and accurate diagnosis of *C. auris*.

In this study, 400 clinical candida isolates were collected to detect the prevalence of *C. auris* strains among candida isolates in Cairo University Hospitals from February 2018 to December 2019 using a set of sequential steps for identification based on CDC guidelines.

Our study showed that 227 isolates (56.75%) were *C. albicans* and 180 (43.25%) were non-albicans *Candida* as detected by germ tube and confirmed by Chromagar. Non-albicans *Candida* were isolated at high frequency from patients and this is in agreement with Ferreira *et al*. who stated that *Candida* species are the most frequent fungi isolated from hospitals; this is in contrast to few years ago where *C. albicans* was the predominant organism in health-care settings [20]. Different non-albicans species were isolated by Chromagar ranging from 25 (13.8%) *C. tropicalis*, 43 (23.8%) were *C. krusei,* and 112 (62.2%) were other *Candida* species (*C. glabrata, C. kefyr, C. parapsilosis,* and *C. lusitaniae*). Most of non-albicans *Candida* 84.4% were collected from ICUs, while 15% from inpatients wards. This indicates the prevalence of non-albicans *Candida* and its pathogenicity in ICU patients which could be explained by the frequent usage of antibiotics, and indwelling devices, especially in immune-compromised and severely ill patients as stated by Deorukhkar *et al*. [21].

The most common specimen for non-albicans *Candida* isolated in this study was urine (89.4%), followed by candidemia (5.6%), then wounds (4%). This was in accordance with Singla *et al*. [22] who declared that candiduria is the most common specimens for non-albicans *Candida*, especially in ICUs; however, urine specimens represents the least to isolate *Candida* in healthy individuals [23].

After segregating *Candida* isolates using two methods for confirmation, we followed the set steps for identification, the 180 isolates of non-albicans were subjected to high temperature at 42°C. Phenotypical characteristics showed that most *Candida* species are unable to grow at high temperature with exception to few including *C. auris* [24]. Ten isolates of the 180 were thermotolerant and grew at 42°C and were suspected to be *C. auris.* For accurate identification according to CDC, MALDI-TOF was used for the definite identification, five isolates were identified as *C. glabrata*, and four isolates were identified as *C. krusei* and one *C. kefyr*. This result was in accordance with Bezjak and Chandy who stated that species of *C. glabrata, C. krusei,* and *C. kefyr* can grow at high temperature as *C. auris* [25].

Antimicrobial resistance of the 10 isolates showed high efficacy of amphotericin B and echinocadins, but high resistance against fluconazole this was in agreement with Berkow and Lockhart [26] who detected the high resistance of fluconazole among *Candida* non-albicans isolates. The discrepancy shown between the identification by Chromagar, the most commonly used method for *Candida* identification, and MALDI-TOF was alarming. Nine of the thermotolerant isolates were detected as *Candida* species (*C. glabrata, C. kefyr, C. parapsilosis,* and *C. lusitaniae*) while one isolate detected as *C. krusei* by Chromagar; in contrast, MALDI-TOF detected five isolates as *C. glabrata*, four isolates as *C. krusei,* and one *C. kefyr*. Missed identification of *C. krusei* by Chromagar represents a pit fall in diagnosis and selection of treatment options for patients as *C. krusei* is intrinsic resistant to fluconazole. This defect in phenotypic identification highlights the importance of MALDI-TOF in rapid and accurate identification of fungal isolates, especially in ICU patients. This study detected that *C. auris* is not prevalent in Egypt and confirmed the reliability of the set protocol for identification and this was in concordance with CDC case tracking in January 2021 which tracked 1625 cases worldwide including only one isolate reported from Egypt [27].

These findings also confirmed by Lu *et al*., 2018, who conducted a detailed search for *C. auris* in Taiwan of more than 5000 archived *Candida* isolates from the period 1999 to 2016 and no isolates detected [28]. Moreover, Lockhart *et al*., 2017, looked for misidentified or overlooked candida before the recent emergence, they queried the international surveillance program SENTRY which contained 15,271 *Candida* isolates collected from 2004 to 2015. Four isolates only were identified as *C. auris* supporting its scarcity which is in accordance with our findings [29].

However, despite scarcity of *C. auris* isolates [30], clinical microbiologists should be aware and have a definite protocol for prevention, prompt, and early identification due to the high resistance and aggressiveness of this bug.

Conclusion and Limitation of Work

It is concluded from this study that *C. auris* is not prevalent in Egyptian hospitals. In addition, Chromagar could be used as a screening method; however, definite diagnosis of isolate species for follow-up of treatment requires a more advanced technique, such as MALDI-TOF. The strength of this study is in the number of isolates collected where it reflects a wide sampling range of *Candida* organisms. One limitation is the limited number of hospitals from where *Candida* was collected. Isolates were collected from three Cairo University Hospitals; however, larger number of hospitals is preferred in further studies to reflect a bigger image of the situation in Egypt.

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