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Original article

Detection of *Candida auris* in Patients Attending Four Healthcare facilities West of Tripoli, Libya.

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Citation: *Altter A, Altayf A, Salem G, Moman R, Alsagher M. Detection of Candida auris in Patients Attending Four Healthcare facilities West of Tripoli, Libya. Libyan Med J. 2024;16(2):90-94.*

Received*: 29-06-2024*
Accepted*: 19-08-2024*
Published*: 22-08-2024*

Funding*: This research received no external funding.*

Conflicts *of Interest: The authors declare no conflict of interest.*

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Abstract

Candida auris (C. auris) has emerged as a significant global health threat, particularly in regions like Asia, Africa, the Middle East, and recently North America, due to its virulence, multidrug resistance, and potential for causing prolonged nosocomial outbreaks, especially in critically ill patients. This study aimed to isolate and identify C. auris in patients from outpatient clinics at two hospitals, a sanctuary clinic, and a cancer institute west of Tripoli, Libya, and to assess antifungal susceptibility in Candida species isolates. Swab samples were taken from various sites including the vagina, urine, wounds, ears, throat, and blood. These samples were cultured on Sabouraud Dextrose Agar (SDA) and incubated at 25°C, followed by microscopic identification and antifungal susceptibility testing. Out of 119 positive samples, the majority were C. albicans, with 53.34% from vaginal swabs and 23.33% from nasal swabs. Only one sample (0.83%) was identified as C. auris, which exhibited resistance to fluconazole, nystatin, and clotrimazole. The findings underscore the importance of accurate identification and strict infection control measures, particularly given C. auris's resistance profile, which contrasts with the susceptibility of C. albicans to common antifungals.

Keywords: *Candida auris, Candida albicans, Fungal contamination, Hospital Acquired Infection.*

Introduction

Candida species are responsible for invasive infections that are notably associated with severe outbreaks, including candidemia. These infections are recognized as a significant cause of morbidity and mortality in healthcare settings worldwide. A novel *Candida* species was cataloged after its isolation from a patient's ear canal secretion in Japan in 2009 [1,2]. To date, at least 15 distinct *Candida* species have been identified as causing human disease [3]. Candidiasis, a well-known disease caused by yeasts of the Candida genus, remains a global health threat, with disseminated candidiasis often resulting in significant mortality [4]. International population-based studies estimate the annual incidence of invasive candidiasis to be between 1.5 and 8 cases per 100,000 people. Bloodstream infections (BSIs) caused by *Candida* species are associated with a global mortality rate ranging from 30% to 50%. These BSIs predominantly occur in hospitalized patients, particularly those in intensive care units, and are linked to factors such as broad-spectrum antifungal agents, internal vascular catheters, parenteral nutrition, abdominal surgery, and immunosuppressants [5]. Candidiasis, in its various clinical forms, is also considered a sexually transmitted disease (STD), with the gastrointestinal mucosa, genitourinary system, and skin surface being the primary sites of colonization for *Candida* species [6].

Hospital outbreaks caused by less common *Candida* species, particularly *Candida auris*, have recently garnered significant attention [7-9]. Although *C. auris* can lead to severe infections, there is still limited understanding of its transmission mechanisms, which contributes to challenges in controlling the microorganism and reducing the associated high mortality rates [5]. In Europe, hospital outbreaks of *C. auris* have been most prominent in the

UK and Spain, leading to invasive infections, particularly among patients with severe immunocompromised conditions. Notably, most *C. auris* isolates have been found to be resistant to the three main classes of antifungal agents, including fluconazole. The transmission of *C. auris* in healthcare settings is likely facilitated by its high propensity for environmental contamination, transient colonization of individuals or devices, and its persistence in hospital environments [10].
The virulence and multi-drug resistance profile of *C. auris*, combined with its potential for causing prolonged nosocomial outbreaks, pose a serious threat, especially among critically ill patients [1].

Methods

Setting and study design

This study focuses on the isolation and identification of fungal pathogens. The research was conducted on patients attending outpatient clinics at four healthcare facilities located in the western region of Tripoli, Libya: SbTH, SrTH, NCI, and SSC. The study was carried out from April to June 2018.

Microbiological techniques

Swab samples of vagina, urine, wounds, blood, ear, and throat were taken from patients attending outpatient clinics in SbTH, SrTH, NCI, and SSC. Swabs were transferred into Sabouraud culture media and then incubated at 25°C for 24-48 hours. After incubation loopfuls were taken from culture media and streaked onto SDA a relatively rich medium for growing a wide range of fungi. SDA was then incubated at 25°C for further 72 hours and colony characteristics of isolates were then identified morphologically and then examined microscopically after being stained with Lactophenol Cotton Blue Stain. Antifungal susceptibility testing was carried out for all identified isolates of *Candida* species.

Antifungal susceptibility assay

The in vitro antifungal susceptibility profile of *Candida* isolates to fluconazole, nystatin, and clotrimazole was assessed by agar cup cut diffusion method. Briefly, the *Candida inoculum* was adjusted to appropriate number to match 0.5 McFarland. The fungal inoculum was swabbed on the surface of SDA plates using sterile cotton swab. Cups were cut by sterile cork borer (6 mm), then the cups filled with 0.2 ml solutions of antifungal drugs (antifungal solution was prepared by dissolving the contents of one antifungal capsule in 5 ml sterile water). The agar plates were incubated at 25 oC for 24–48 hours. At the end of incubation period the diameter of inhibition zones was measured. Table 3.2 shows the inhibition zones of the three antifungal drugs tested against *Candida* isolates. All three antifungal drugs demonstrated no activity against *C. auris* which is the only fungus isolated from the ear swab of female patient in SSC, whereas the other isolates of C. albicans were fully susceptible to the three antifungal agents.

Results

The results of this study indicate that a total of 120 patients participated, with 94 (78%) being female and 26 (22%) males. Regarding the number of fungal-contaminated samples (Table 1), 52 samples were collected from SbTH (30 vaginal, 11 nasal, 5 urine, 2 ear, 3 throat, 1 wound, and 1 blood); 41 from SSC (22 vaginal, 12 nasal, 6 urine, and 1 ear); 22 from SrTH (8 vaginal, 5 nasal, 4 urine, 4 ear, and 1 wound); and 5 from NCI (4 vaginal and 1 wound).

Table 1. The number (No.) and percentage (%) of fungal contamination in healthcare facilities.

Health Care Facilities	Samples	Fungal isolates from different body sites								Total
		C. auris	*C. albicans*							
		Ear	Vaginal	Nasal	Urine	Ear	Wound	Throat	Blood	
Sabratha Teaching Hospital	No.	1	30	11	5	1	1	2	1	52
(SbTH)	%	0.83%	25%	9.17%	4.17%	0.83%	0.83%	1.67%	0.83%	43.33%
Sabratha Sanctuary Clinic	No.	0	22	12	6	1	0	0	0	41
(SSC)	%	0	18.3%	10%	5%	0.83%	0	0	0	34.17%

Surman Teaching Hospital (SrTH)	No.	0	8	5	4	4	1	0	0	22
	%	0	6.6%	4.17%	3.3%	3.3%	0.83%	0	0	18.33%
National Cancer Institute, Sabratha (NCI)	No.	0	4	0	0	0	1	0	0	5
	%	0	3.3%	0	0	0	0.83%	0	0	4.17%
Total	No.	1	64	28	15	6	3	2	1	120
	%	0.83%	53.34%	23.33%	12.5%	5.0%	2.5%	1.67%	0.83%	100%

Antifungal susceptibility testing (Table 2) identified various *Candida* species and tested their resistance to antifungal drugs. Notably, one of the two ear swabs taken from patients at SbTH was found to be contaminated with an organism fully resistant to all three tested antifungal drugs, identified as *C. auris*.

A total of 119 C. albicans isolates were obtained from seven different sites in patients across the four healthcare facilities mentioned above. The distribution of C. albicans isolates was as follows: 64 (53.34%) vaginal swabs (30 [25%] from SbTH, 22 [18.3%] from SSC, 8 [6.6%] from SrTH, and 4 [3.3%] from NCI); 28 (23.33%) nasal swabs (11 [9.17%] from SbTH, 12 [10%] from SSC, and 5 [4.17%] from SrTH); 15 (12.5%) urine samples (5 [4.17%] from SbTH, 6 [5%] from SSC, and 4 [3.3%] from SrTH); 6 (5%) ear swabs (1 [0.83%] from SbTH, 1 [0.83%] from SSC, and 4 [3.3%] from SrTH); 3 (2.5%) wound swabs (1 [0.83%] from SbTH, 1 [0.83%] from SrTH, and 1 [0.83%] from NCI); 2 (1.67%) throat swabs (2 [1.67%] from SbTH); and 1 (0.83%) blood sample (1 [0.83%] from SbTH). In contrast, *C. auris* was isolated from 1 (0.83%) ear swab taken from a female patient at SbTH. In terms of microbial contamination, the overall fungal contamination in the healthcare facilities occurred in the following descending order: SbTH 52/120 (43.33%), SSC 41/120 (34.17%), SrTH 22/120 (18.33%), and NCI 5/120 (4.17%). Of the 120 samples tested, 119 (99.17%) were isolates of C. albicans, while only one sample (0.83%) was identified as *C. auris.*

Table 2. Antifungal susceptibility test of *Candida* isolates using cup-cut agar diffusion assay

Fungal isolates Inhibition zones (mm) of antifungal drugs					
Type	Number	Site of isolation	Clotrimazole	Nystatin	Fluconazole
C. albicans	119	Vagina, Nasal, Urine, Ear, Wound, Throat, and Blood	+++	++	+
C. auris	1	Ear	-	-	-

Discussion

In recent years, *Candida* species have increasingly been reported as causes of uncommon human infections, with *Candida auris* being particularly recognized for leading to hospital outbreaks [7,8,9]. These outbreaks often involve *Candida* species that are difficult to identify using phenotypic methods, such as *Candida orthopsilosis, Candida metapsilosis, Candida bracarensis,* and *Candida nivariensis* [9,11].

In 2009, *C. auris* was identified as a novel opportunistic fungal pathogen genetically close to the *C. haemulonii* complex and capable of causing candidemia. Just a few years after its initial isolation and identification, *C. auris* emerged as a significant fungal pathogen responsible for healthcare-associated infections worldwide, noted for its high antifungal resistance, difficulties with laboratory identification, and potential for horizontal transmission [11]. Reports indicating an increase in *C. auris* cases in European countries, especially following the European Centres for Disease Control and Prevention (ECDC) rapid risk assessment on *C. auris* in December 2016, highlighted the need for focused research on the possibility of its occurrence and spread in healthcare facilities. This study was conducted in response to these reports, aiming to investigate the presence and spread of *C. auris* in healthcare facilities located in the western region of Tripoli, Libya. The study was carried out in four healthcare facilities (SbTH, SrTH, NCI, and SSC) in the west of Tripoli from April to June 2018.

A high rate of contamination with potential fungal infections was observed, particularly in vaginal samples (53.3%), followed by nasal (23.3%) and urine samples (12.5%).

Approximately 45%, 34%, 17%, and 4% of swabs taken from SbTH, SSC, SrTH, and NCI, respectively, were found to be contaminated with at least one fungal pathogen. Notably, an ear swab sample taken from a female patient at SbTH initially showed a fungal isolate identified as *Candida* species by morphological appearance and microscopic examination. However, antifungal susceptibility testing later identified the organism as *C. auris*, demonstrating complete resistance to fluconazole, nystatin, and clotrimazole, which are among the most effective antifungal drugs. This appears to be the first report of *C. auris* isolated from patients in Libya and serves as a significant alert to health authorities in Libya regarding the potential danger posed by *C. auris* in healthcare facilities. Consequently, there is an urgent need to establish up-to-date medical laboratories, adopt advanced testing strategies, and implement stringent infection control and prevention measures. Finally, no direct connection was found between the cases presented in this study and the epidemiological outbreaks of *C. auris* reported in India, Venezuela, the UK, Spain, and Oman [12-17].

It is well known that critical-care settings within hospitals are particularly vulnerable to the transmission of *C. auris* [12]. The transmission and spread of infection are often linked to hand or mucosal colonization of affected patients and healthcare workers with *C. auris.* Moreover, *C. auris* has been isolated from various patient contact points, such as mattresses, furniture, sinks, and medical equipment [12,18]. The low percentage of *C. auris* isolates observed in this study might be due to the challenges in identifying *C. auris* using conventional phenotypic systems and the lack of molecular biology tools in routine laboratories. Additionally, it is possible that the patients involved in this study had not received prior treatment with antifungal drugs, which could reduce the likelihood of infection with *C. auris.*

Conclusion

In conclusion, usion, the alert issued by the US-CDC in 2016 [19] regarding the spread of *Candida auris* as an emerging multidrug-resistant pathogen should be taken as a very serious threat to healthcare settings worldwide [13,20,21]. It is crucial to strengthen measures for the accurate and rapid identification of *C. auris* using MALDI-TOF MS or molecular biology methods, which should be adopted as routine laboratory practices in all healthcare facilities in Libya. Additionally, implementing stringent infection control measures and promoting effective antifungal strategies are essential. Furthermore, maintaining strong adherence to hand hygiene, particularly through the use of alcohol-based hand rubs, must be prioritized at all times in healthcare settings to prevent the dissemination of *C. auris*.

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