Original Article

Detection of *Candida auris* and its antifungal susceptibility: first report from Bangladesh

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Abstract

***Background and objectives*:** *Candida auris* is an emerging multidrug-resistant fungal pathogen that has been associated with nosocomial infections with a high mortality. The organism has been reported from several countries of the world except Bangladesh. The present study describes the presence of *C. auris* in clinical samples obtained from a large hospital of Dhaka city, Bangladesh.

***Materials and methods*:** The A total of 100 *Candida* species isolated from different clinical samples were purposively included in the present study. Samples were obtained from patients attending a 750 bed hospital of Dhaka city. *C. auris* was identified by growth characteristics, biochemical and carbohydrate assimilation test and further confirmed by polymerase chain reaction and sequencing using ITS1 and ITS2 targeting the conserved regions of 5.8S rRNA. Antifungal susceptibility of identified *C. auris* was performed by disk diffusion and minimum inhibitory concentration (MIC) methods.

Results: Out of 100 *Candida* sp. tested, 21 isolates were identified as *C. auris*. Of the 21 *C. auris*, 14 (66.7%) were isolated from blood samples and the remaining 7 (33.4%) were from urine. Most of the *C. auris* isolated were from patients admitted in intensive care units. Out of 21 *C. auris*, 17 (81.0%), 7 (33.3%) and 3 (14.3%) were sensitive to amphotericin B, fluconazole and voriconazole respectively by disk diffusion method. Out of 14 fluconazole resistant isolates, 5 were susceptible dose-dependent (SS-D) by MIC method.

Conclusion: The present study is the first report demonstrating the presence of *C. auris* in clinical samples obtained from a large hospital of Bangladesh. Majority of isolates showed resistance to fluconazole and variable susceptibility to other antifungal agents. Further study is suggested to find its true magnitude and its susceptibility pattern to a range of antifungal agents.

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Introduction

Candida is now recognized as a major agent of hospital-acquired infection [1]. Although, most infections are attributed to *C. albicans*, the shift towards treatment resistant non-*albicans Candida* (NAC) species is increasingly evident in recent years [2,3]. *C. auris* is an emerging NAC species which is first reported in Japan in 2009 [4]. Studies from several countries have documented that *C. auris* is causing severe illness in hospitalized patients and difficult to control hospital outbreaks [5, 6]. The organism is extremely transmissible between patients,

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inter healthcare facilities and from contaminated environments [7-9]. Infection by *C. auris* requires proper attention as it shows resistance to many commonly used antifungal agents [10-12]. A study from India has reported that 90%, 15% and 8% of *C. auris* isolated between 2009 and 2017 were resistant to fluconazole, voriconazole and amphotericin B, respectively [12].

Identification of *C. auris* is not usually done in routine microbiology practice due to lack of awareness about the organism and limited laboratory facilities. Moreover, *C. auris* is often difficult to differentiate from other NAC species in laboratories with limited biochemical tests. No study has yet been done in Bangladesh with regard to the detection and antifungal susceptibility of *C. auris*.

The present study investigated the presence of *C. auris* in different clinical samples obtained from patients attending a hospital of Dhaka city. Its susceptibility to common antifungal agents was also determined.

Materials and methods

***Study samples and place*:** The study was carried out at a 750 bed-hospital of Dhaka city over a period of one year. A total of 100 *Candida* species isolated from different clinical samples were purposively included in the present study for detail species identification. Clinical samples included urine, blood, sputum, pus, high vaginal swab and body fluid from patients admitted in wards, intensive care unit (ICU) and neonatal intensive care unit (NICU).

Isolation and identification of *C. auris*: All clinical samples were inoculated on the Sabouraud Dextrose Agar (SDA) media. Phenotypic features of *C. auris* were identified by wet film, (oval or round shape yeast or budding yeast cell), Gram staining (Gram positive yeast cell), and incubation at 37-42^{0}C temperature [13,14,]. Carbohydrate assimilation test was performed as described earlier [15]. *C. auris* identified by growth characteristics, biochemical reactions and carbohydrate assimilation tests were further confirmed by polymerase chain reaction and sequencing using ITS1 and ITS2 targeting the conserved regions of 5.8S rRNA [16].The purified PCR product was sent to McLab, California, USA for sequencing. The sequence was used as probes in NCBI blast search database in order to retrieve similar sequences.

Determination of antifungal susceptibility

a. ***Disk diffusion method (DDM)*:** The isolates were tested for susceptibility to amphotericin B (10µg), fluconazole (25µg) and voriconazole (1µg) by disk diffusion method as described in NCCLS manual M44-A, 2004 [17]. The zone of inhibition around the disc was recorded and interpreted as susceptible (S), susceptible -dose dependent (S-DD), and resistant (R) as mentioned in Table-1. All disks were obtained from HIMEDIA, India Ltd.

b. ***Minimum inhibitory concentrations (MIC) method:*** MIC of amphotericin B, fluconazole and voriconazole against isolated *C. auris* was determined by broth dilution method following the NCCLS approved guideline M27-A3 [18]. All reading was visually taken between 24 and 48 h of incubation at 35 °C in aerobic condition and interpreted according to the values mentioned in Table-1. Each isolate was tested in duplicate by both disk diffusion and MIC methods.

Table-1: *Interpretative breakpoints for C. auris by disk diffusion and MICs (µg/mL) methods as per M44-A and M27-A3 CLSI documents*

Antifungal agent	Disk content (µg)	Zone diameter (mm)			MIC (µg/ml)		
		S	S-DD	R	S	S-DD	R
AMB	10	≥15	10-14	≤10	≤ 1.0	-	≥ 2.0
FLZ	25	≥19	15-18	≤14	≤ 8.0	16-32	≥ 64
VOR	1	≥17	14-16	≤ 13	≤ 1.0	2.0	≥ 4.0

Note: *AMB= amphotericin B; FLZ = fluconazole; VOR = voriconazole; S= susceptible; S-DD=susceptible dose dependent; R= resistant.*

Results

Out of 100 *Candida* sp. tested, 21 isolates were identified as *C. auris* by growth characteristics and carbohydrate assimilation tests. Representative isolates of 21 *C. auris*, as identified by growth characteristics and carbohydrate assimilation tests were confirmed as *C. auris* by sequencing (5.8S rRNA gene sequences). Sequence analysis of our isolates showed 99%-100% similarity with those of *C. auris* KP326583, KP131674 and MF167535 5.8S ribosomal RNA gene. Out of 21 isolates, 8 and 13 were isolated from samples of adult and neonate patients respectively (Table-2). Of the 21 *C. auris*, 14 (66.6%) were isolated from blood samples and the remaining 7 (33.4%) were from urine samples of adult patients. Except one, all *C. auris* were isolated from blood of neonates admitted in intensive care unit.

The susceptibility pattern of *C. auris* to different antifungal agents by disc diffusion and MIC method is shown in Table-3. Out of 21 *C. auris*, 14 (66.7%) were resistant to fluconazole by disk diffusion method. However, out of these 14 resistant isolates 5 were found susceptible dose-dependent (SS-D) by MIC method (Table-3). Most of isolates were sensitive to amphotericin B by both disk diffusion (81.0%) and MIC (76.2%) methods. Out of total 21 *C. auris* tested, 18 (85.7%) was resistant to voriconazole. The detail MIC, MIC_{50} and MIC_{90} of all isolates are shown in Table-4.

Table-2: *Rate of Isolation of C. auris according to samples and locations (n= 21)*

Location	Adult		Neonate	
	Samples			
	Urine	Blood	Urine	Blood
ICU	5 (24%)	1 (5%)	-	-
CCU	1 (5%)	-	-	-
Ward	1 (5%)	-	-	-
NICU				13(61.9%)

Note: *ICU= intensive care unit; CCU= coronary care unit; NICU= neonatal intensive care unit*

Table-3: *Susceptibility pattern of C. auris to amphotericin B, fluconazole and voriconazole by DD and MIC methods*

Antifungal agent	Susceptibility by					
	DDM			MIC		
	S n (%)	S-DD n (%)	R n (%)	S n (%)	S-DD n (%)	R n (%)
AMB	17 (81.0)	-	4 (19.0)	16 (76.2)	-	5 (23.8)
FLZ	7 (33.3%)	0	14 (66.7)	7 (33.3)	5 (23.8)	9 (42.8)
VOR	3 (14.3)	0	18 (85.7)	3 (14.3)	0	18 (85.7)

Note: *AMB= amphotericin B; FLZ = fluconazole; VOR = voriconazole; S= susceptible; S-DD=susceptible dose dependent; R= resistant; DDM= disk diffusion method.*

Table-4: *MIC of amphotericin B, fluconazole and voriconazole against isolated C. auris (n=21)*

	Number of isolate with MIC (µg/ml)													
	.12	.25	.5	1	2	4	8	16	32	64	128	256	MIC_{50}	MIC_{90}
AMB	0	6	7	3	5	0							0.5	2.0
FLZ	0	0	0	0	0	0	7	0	5	0	0	9	32	256
VOR	1	2				4	10	0	4				8	32

Note: *AMB= amphotericin B; FLZ = fluconazole; VOR = voriconazole*

Discussion

C. auris is an emerging fungus and has become a global nosocomial problem. It causes candidiasis ranging from superficial skin infection to severe invasive bloodstream and multi organs infections. It is variably resistant to multiple antifungal drugs commonly used to treat *Candida* infections. *C. auris* was first isolated from the ear canal of a 70-year-old Japanese woman in Japan in 2009 [4]. In 2011, the first three cases of disease-causing *C. auris* were reported from South Korea [19]. The first report of a *C. auris* outbreak in Europe was in 2016 [20]. Up till 2019, C*. auris* in clinical samples has been documented in more than 30 countries of the world [21].

This is the first study of *C. auris* in Bangladesh. The study has revealed the presence of *C. auris* infection in the hospitalized patients of Bangladesh. About 62% and 29% of *C. auris* were found in pediatric and ICU adult patients respectively and most frequently it is isolated from blood (67%). A recent study in USA documented 77 clinical cases of *C. auris* from seven states of which 45 were bloodstream isolates and the remaining were from urine (n=11), respiratory tract (n=8), bile fluid (4), wound (4), CVC tip (2), bone, ear and jejunal biopsy specimens [14].

Antifungal susceptibility of *Candida* species varies from place to place and species to species. Susceptibility of *C. auris* to fluconazole, voriconazole and amphotericin B found in the present study was similar to the findings of other studies reported from different countries of the world [11,12, 21].

In the present study, 5 (23%) *C. auris* found resistant by disk diffusion method to fluconazole were actually dose dependent susceptible by MIC method. These *C. auris* isolates exhibited slightly hazy zone of growth within the zone of inhibition in disk diffusion method and were recorded as 'resistant' by disk diffusion test. Therefore, any strain showing hazy zone of growth within the zone of inhibition should be confirmed by MIC method as higher dose of fluconazole could be used to treat infection by such S-DD strains of *C. auris*. It is important because fluconazole is a cheaper drug compared to other more expensive and toxic antifungal agents.

In our study, almost all (20/21) *C. auris* were isolated from patients admitted either in adult or neonatal intensive care units of the hospital. The finding of the study emphasizes the need for quick detection of this organism in clinical samples to prevent its spread in the hospitals. So, special attentions are needed to quickly detect *C. auris* and its antifungal susceptibility for appropriate treatment and for the prevention of its nosocomial transmission. Present method of identifying *C. auris* by biochemical and sugar assimilation tests is time consuming and is often fraught with difficulties. Rapid technique is needed for quick diagnosis of *C. auris* infection to initiate timely and appropriate treatment. Further study is warranted to determine its true magnitude in the hospitals of Bangladesh. Also, measures should be taken to create awareness among the microbiologists and clinicians regarding the importance of *C. auris* infection in severely ill patients requiring long hospital stay or admission in intensive care units.

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