Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 4 Issue 8 August 2021

Research Article

Species Level Identification of Yeast and Yeast Like Fungus for Prompt Infection Control Measures in Prevention of Outbreaks: With Special Reference to *Candida auris* in Pre-covid Era

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Received: June 03, 2021

Published: July 09, 2021

Abstract

Background: *Candida auris* (*C. auris*) is emerging as a multi-drug resistant (MDR) strain of Candida amongst Non-albicans Candida (NAC) which poses a serious risk of nosocomial spread with high mortality rate.

Aim: Aim of the study is to give emphasis on species level identification of all yeast and yeast like fungus (YYLF) for the implementation of infection control practices (ICP) to prevent outbreak, with special reference to *C. auris.*

Methods: The study was done over a period of 12 months in a tertiary care hospital. YYLF isolated from primary culture were further sub-cultured on Sabouroud Dextrose Agar (SDA) and incubated at 25°C and 37°C for 72 hours. Identification and antifungal susceptibility was done using Vitek 2 Compact system 8.01 (bioMérieux, North Carolina/USA). The 1st two isolates were confirmed by molecular method (D1-D2 sequencing). *C. auris* specific containment measures were implemented in the early part of the study. Clinical data and outcome were evaluated at end of the period.

Findings: Out of 2,487 non-duplicate samples processed over 12 months, YYLF were isolated from 209 (8.40%) samples. Amongst the YYLF, predominant isolate was *C. albicans* (109/209, 52.15%), followed by *C. tropicalis* (35/209, 16.74%). Predominant source was urinary samples (115/209, 55%) followed by respiratory samples (60/209, 28%). *C. auris* was isolated in 7 non-duplicate samples (7/209, 3.35%). Out of these 7 cases, 5 had history of cancer (72.42%). Measures for containment of *C. auris* were placed prior to the study and implemented at each time of its isolation. No nosocomial spread was detected during this study period.

Conclusion and Clinical Significance: Species level identification following proper quality control in microbiology laboratory along with appropriate sample collection can act as a primer in controlling outbreaks by YYLF. Pre-emptive implementation of hospital infection control (HIC) policies can help in controlling the MDR *C.auris*, even amongst immunocompromised patients.

Keywords: *C. auris*; Multi Drug Resistant; Non-albicans Candida; Infection Control Practices; Yeast and Yeast-like Fungus; Hospital Infection Control

Abbreviations

C. auris: Candida auris; MDR: Multi Drug Resistant; NAC: Non-albicans Candida; ICP: Infection Control Practices; YYLF: Yeast and Yeast-Like Fungus; HIC: Hospital Infection Control; BAL: Bronchoalveolar Lavage; CSF: Cerebrospinal Fluid; CCA: Critical Care Areas; ICU: Intensive Care Unit; BMT: Bone Marrow Transplant; KTU: Kidney Transplant Unit; CLED: Cystine Lactose Electrolyte Deficient Agar; SDA: Sabouraud's Dextrose Agar; ATCC: American Type Culture Collection; WHO: World Health Organization; UWP: Universal Work Precautions; SUD: Single Use Device

Introduction

In 2009, a novel *Candida* species, *Candida auris (C. auris)* was isolated from external ear canal of a Japanese patient in Tokyo Metropolitan Geriatric Hospital [1]. In the same year, 15 cases of *C. auris* were isolated in South Korea from the ear canals of patients suffering from chronic otitis media [2]. During 2013 to 2014, two larger series of cases of persistent candidemia and deep-seated infections with high mortality rate were reported by multi drug resistant (MDR) strains of *C. auris* from 3 hospitals in India [3]. In Chicago, a hospital has been dealing with an outbreak of *C. auris* since March 2017, when its first case was confirmed. Since then, despite implementing all ICP, the proportion of residents testing positive has climbed to 71% [4].

Whole genome sequencing and epidemiological analysis proved that the MDR *C. auris*-related disease appeared independently on three continents (Indian subcontinent, Venezuela, and South Africa during 2012 to 2015) simultaneously [5]. One of the hypotheses predicts its emergence might have been linked to global warming effects on wet-lands where it prevailed as a plant saprophyte [6]. This study was done with the objectives to understand the prevalence and epidemiology of yeast and yeast like fungus (YYLF) with special reference to *C. auris* in a tertiary care hospital over 12 months along with the containment strategies by infection control measures.

Materials and Methods

Study design

The study was carried out in the department of microbiology in a tertiary care hospital in Delhi, India over a period of 12 months, from January 2018 to December 2018. The various clinical samples included in the study comprised of urine, blood, respiratory [sputum, bronchoalveolar lavage (BAL) etc.] and others [pus, wound swab, Cerebrospinal fluid (CSF), Ascitic fluid etc.] The samples were received from Critical care areas (CCA) [Intensive Care Unit (ICU), Bone marrow transplant (BMT) unit, Kidney Transplant unit (KTU) etc.], various indoor departments of the hospital and outdoors.

Inclusion criteria of the samples

- **Urine:** Wet mount showing pus cells >=10 WBC/cubic mm or pure growth in culture with colony count >=10^3 CFU/ml irrespective of pus cells in routine examination [8].
- **Sputum**: Samples showing Bartlett's score more than 1 [9].
- **Bronchoalveolar lavage**: Colony count >=10^4 CFU/ml in quantitative culture [10].
- **Blood**: Repeated isolation of same species in paired samples [10]. *C. auris* specific containment measures were implemented in the early part of the study and clinical data and outcome were evaluated at the end of this study.

Microbiological testing

All the samples were inoculated on routine culture media like blood agar, MacConkey agar, Cystine Lactose Electrolyte Deficient Agar (CLED). After growth in routine culture media, for cultural morphology, colonies were inoculated on two Sabouraud's Dextrose Agar (SDA) slants; one was incubated at room temperature and other at 37°C for 24 - 48 hrs. The growth on the slope was processed for identification of the fungus.

From the isolated colony, Gram staining was done. Germ tube test was performed to differentiate *Candida albicans* from Non-albicans Candida (NAC); slide culture and Dalmau plate [7] techniques were performed for speciation, using Corn meal agar. The final identification and antifungal susceptibility tests were performed by YST Identification cards and YST AST cards respectively using Vitek 2 Compact System 8.01 (bioMérieux, Inc. Durham, North Carolina/USA). Control strains used were: *C. albicans* ATCC (American Type Culture Collection) 14053, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 14243.

Two of the *C. auris* isolates from Case1 and Case 2 were further confirmed by sequencing of internal transcribed spacer and D1/D2 regions in Reference lab, PGI, Chandigarh. This showed homology with the type *C. auris* reference strains. This helped us in inter-laboratory comparison. Once it was confirmed, all Vitek identified *C. auris* were considered as confirmed case and accordingly prompt infection control measures were implemented (Figure 1 and 2).

Results

Out of 2487 non-duplicate samples processed over 12 months, 209 samples grew YYLF (8.40%). Majority of the culture positive samples for YYLF were from IPD (155/209, 74%), followed by critical care areas and OPD (Figure 3). This is contributed to the immunocompromised status of the patients, mainly cancer patients admitted in the institute. Four (4/7, 57.14%) isolates of *C. auris*

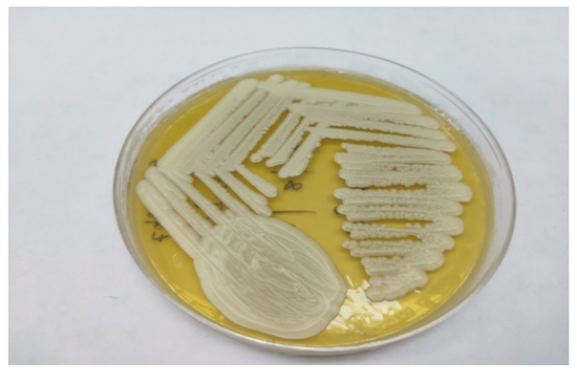


Figure 1: Colony morphology of *C. auris* in Sabouraud Dextrose Agar (SDA).

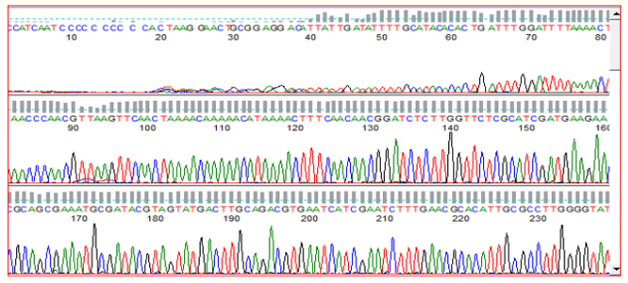
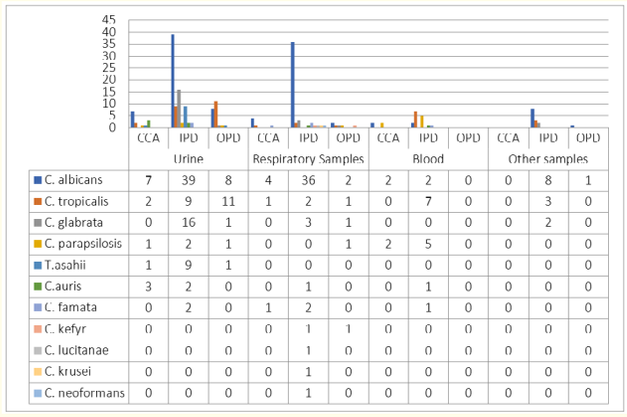


Figure 2: Sequencing of D1-D2 region of *C. auris* isolate 1.



	CCA	IPD	OPD	CCA	IPD	OPD	CCA	IPD	OPD	CCA	IPD	OPD
	Urine			Respiratory Samples			Blood			Other samples		
C. albicans	7	39	8	4	36	2	2	2	0	0	8	1
C. tropicalis	2	9	11	1	2	1	0	7	0	0	3	0
C. glabrata	0	16	1	0	3	1	0	0	0	0	2	0
C. parapsilosis	1	2	1	0	0	1	2	5	0	0	0	0
T.asahii	1	9	1	0	0	0	0	0	0	0	0	0
C.auris	3	2	0	0	1	0	0	1	0	0	0	0
C. famata	0	2	0	1	2	0	0	1	0	0	0	0
C. kefyr	0	0	0	0	1	1	0	0	0	0	0	0
C. lucitanae	0	0	0	0	1	0	0	0	0	0	0	0
C. krusei	0	0	0	0	1	0	0	0	0	0	0	0
C. neoformans	0	0	0	0	1	0	0	0	0	0	0	0

Figure 3: Distribution of Yeast and Yeast like fungus as per clinical samples and patient care area.

Note: Respiratory sample includes sputum, induced sputum, endotracheal aspirate, bronchoalveolar lavage etc. Other samples include pus, wound swab, stool, high vaginal swab etc. YYLF: Yeast and yeast like fungus, CCA: Critical care areas, IPD: Indoor patient Department, OPD: Outdoor patient Department.

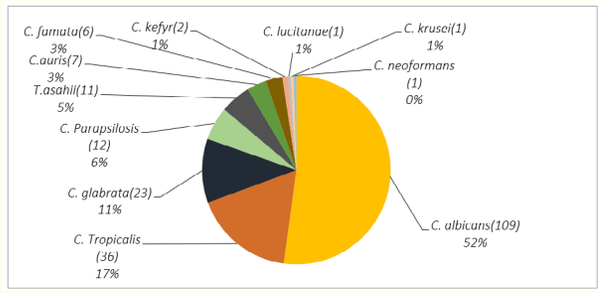


Figure 4: Distribution of Yeast and Yeast-like isolates from various samples.

were retrieved from indoor patients while three (3/7, 42.86%) from intensive care unit (Figure 3).

The predominant organism isolated was *C. albicans* (109/209, 52.15%), followed by *C. tropicalis* (35/209, 16.74%) and *C. glabrata* (23/209, 11%). The isolation rate of *C. auris* was 7 (7/209, 3.35%) (Figure 3 and 4).

No *C. haemulonii* or *C. duobushaemulonii* was isolated which is supposed to be misidentified by Vitek 2 YST which was used for identification [11].

YYLF isolates were mostly retrieved from urinary samples (115/209, 55%) followed by respiratory samples (60/209, 28%) and blood (20/209, 9.57%). Five of the isolates of *C. auris* were from urinary samples (5/7, 71.43%) with colony count >=100,000 CFU/ml. One sample of Bronchoalveolar lavage fluid (BAL fluid) with colony count >=10,000 and the other sample of paired blood culture was found to be positive for *C. auris* (Figure 3 and table 1).

On analysis of the cases, it was found that out of seven cases, five patients had history of cancer, one had history of chronic kidney disease (CKD) on dialysis and testicular abscess with type 2 diabetes mellitus (Type 2 DM) and the rest other had history of ruptured liver abscess with sepsis (Table 1).

Location of the patients were in different areas of the hospital, while their isolation was in different months of the year except two cases in the month of February and two in the month of December, 2018. Each case was identified promptly and infection control

measures were taken. No nosocomial spread was detected during the study period. Out of 7 identified cases, one patient expired with mortality rate 14.28% (Table 1).

Out of seven isolates, six were resistant to fluconazole (MIC>=32) and amphotericin-B (MIC>=2). No isolate was found to be resistant to caspofungin or micafungin [12,13] (Table 1).

Policies of infection control implemented for each isolate of *C. auris* [13,14]:

- To rule out colonization, surveillance samples in the form of nasal swab/swab from axilla were collected from nurses associated with the infected patients. A total 16 swabs were collected and processed in Microbiology laboratory for culture. However, no swab was found to be positive for *C. auris*.
- Environmental swabs were collected from door handles, bed rails, cannula hubs etc. in each case of *C. auris* isolate. Bed rail of one case (Case 3) (Table 1) was found to be positive for *C. auris*. Stringent cleaning protocol with hydrogen peroxide and sodium hypochlorite with proper concentration and contact period as per manufacturers' recommendation was followed. Due to the fixed cleaning protocols, no swab was found to be positive for *C. auris*.
- Revision of Isolation policy: The list of contact isolation policy of the hospital has been extended including *C. auris*.
- Two dedicated rooms with dedicated staff, Bio-medical waste dust bins and separate bed pans were allocated for patients positive for *C. auris*.
- Signage of Contact isolation was displayed outside the room.

	Patient's Details	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
1	Age(years)	65	67	66	61	65	26	48
2	Gender	Male	Male	Female	Male	Female	Male	Female
3	Area of admission	2nd floor	1st floor	ICU	2nd floor	ICU	ICU	2nd floor
4	Month (2018)	Feb	Feb	April	June	Oct	Dec	Dec
5	Sample positive for *C. auris*	BAL	Urine	Urine	Blood	Urine	Urine	Urine
6	Colony count	>=10,000	>=100,000	>=10,000	NA	>=100,000	>=100,000	>=100,000
7	Predisposing factor	Type 2 DM with lower respiratory tract infection	CKD (StageIV), Hypocontractile bladder, HTN, Type 2 DM, Hypothyroidism, UTI	On and off hospital admission since Oct, 2017; Patient was on ventilator and urinary catheter	Patient was on chemotherapy since April, 2018	T2DM, HTN and paraparesis	Nil	Pituitary adenoma and aneurysm diagnosed in 1997
8	History of surgery	Decompessive craniotomy with tumour excision in 2017	Orchidectomy (March, 2015)	Post-operative case of TAH with B/L RPLND, omentectomy (Oct, 2017); on CT	Nil	Nil	Craniotomy and Tumour decompression	Craniotomy and Clipping of Aneurysm done in 2018
9	Diagnosis	Glioblastoma multiforme	CKD(StageIV) with recurrent UTI	Recurrent CA Right Ovary with ileal perforation with sepsis	Carcinoma Lung	Ruptured Liver abcess with B/L pneumonia with sepsis with recurrent UTI	Esthesioneuroblastoma	Subarachnoid hemorrhage (SAH)
10	No. of Hospital days	24	10	Day care treatment for Chemotherapy	20	37	12	35
11	H/O use of Broad spectrum antibiotic antibiotics	Colistin, Linezolid, Tigecycline	Meropenem, Teicoplanin	Meropenem, Teicoplanin	Colistin, Fosfomycin	Polymyxin B, Fosfomycin, Minocycline, Linezolid	Colistin, Vncomycin	Colistin, Vancomycin, Linezolid

12	Outcome	Recovered from UTI	Recovered from UTI	Died due to severe sepsis and Cardio-pulmonary arrest	Recovered from blood stream infection	Recovered from UTI	Recovered from UTI	Recovered from UTI
13	Minimum inhibitory concentration (MIC) of *C. auris* against Anti-fungals							
a	Amphotericin B	>=16	8	8	0.5	>=16	32	>=16
b	Caspofungin	0.25	0.25	0.25	0.25	0.25	0.5	0.25
c	Flucytosine	>=64	<=1	<=1	<=1	<=1	<=1	<=1
d	Fluconazole	32	32	32	32	16	32	32
e	Micafungin	0.12	0.12	0.12	0.12	0.25	0.12	0.12
f	Voriconazole	4	0.25	0.5	2	4	1	2

Table 1: Details of cases infected with *C. auris.*

Similarly, in ICU also, two rooms were allocated for such positive patients for contact isolation:

- **Stringent policy on hand hygiene:** 5 moments of Hand Hygiene and Steps of Hand hygiene recommended by World health organization (WHO) was followed stringently. Audit of Hand hygiene was done regularly using hand hygiene data sheet provided by WHO. One hourly alarm for hand washing is being followed in ICU area. This is followed by all staff including doctors in ICU.
- **Universal Work precautions (UWP):** Apart from hand hygiene, other measures of UWP were followed like wearing gowns, gloves, proper sterilization and disinfection of instruments etc. Mostly Single use device (SUD) preferred for patient care; high level disinfection by ethylene oxide or by autoclave was done for other items.

Discussion

In the year 2018, total 209 non-duplicate samples were found to be positive for YYLF in various samples with positivity rate 2.34% (209/8918). Amongst the YYLF, *C. albicans* was the predominant yeast (52%) and the rest were NAC (48%). Among all NAC, *C. tropicalis* predominated with 17%, followed by *C. glabrata*, 11%; rate of isolation of *C. auris* was only 3% despite a notorious for nosocomial spread.

Similar data was found in a study done from National Hospital Discharge Survey (NHDS), United States which revealed rate of invasive Candidiasis was predominated by *C. albicans* ranging from 73.3% in 1997 to 62.3% in 2003. No *Candida auris* was isolated during these periods; however unidentified spp. of Candida ranged from 3.9% in 1997 to 4.9% in 2003 [15].

Candidemia was seen in 9.57% (20/209) cases in our study. NAC candidemia 80% (16/20) predominated; 35% of which was contributed by *C. tropicalis* and *C. parapsilosis* each. *C. auris* also contributed to candidemia in one indoor case (5%) [Table 1]. Similar finding was detected from a 13-year long study on candidemia from a tertiary care hospital in Thailand showing prevalence rate of 6.14% for *Candida* species. NAC candidemia 134 (57.1%) predominated over *C. albicans* 101 (42.9%) [16]. In another study, prevalence of candidemia in infants was found to be 1.4%. *NAC* was responsible for 56.5% of neonatal candidemia; *C. glabrata* 33.3% followed by *C. tropicalis* 20.3% [17]. No *C. auris* or unidentified *Candida spp.* was mentioned in these studies [16,17].

In Kenya, C. auris was considered as the most common pathogen responsible for candidemias in a reference hospital accounting for 45 (38%) episodes over a period of 3 years (2010 to 2013) [18]. During 2009–2011, 12 cases of candidaemia by *C. auris* were identified 2 hospitals (a tertiary care general hospital and a pediatric center) in Delhi, India [3]. Similarly, a study of 27 ICUs across India found 5.7% of *C. auris* candidemia from April 2011 to September 2012 [19].

Urinary tract infection (UTI) was predominated by *C. albicans,* 25.83% (54/209) followed by *C. tropicalis* 10.52% (22/209) and *C. glabrata* 8.13% (17/209). *C. auris* contributed to 2.39% (5/209) of UTI in indoor patients. In one study from Pakistan revealed that 193 isolates of *C. auris* among 92 patients; out of which 38 had candideamia and 19 patients had UTI [20]. Globally, few cases of UTI have been reported by *C. auris*. This might be because of under-reporting of the isolates due to lack of proper microbiology laboratory all over the globe especially in lower to middle income group countries. Secondly, lack of amenities for species level identification in most of the other laboratories. Third, under reporting can be a cause of false reporting of the cases as many different automated systems falsely identify it as various different *Candida spp.* like *Candida haemulonii*, *Candida duobushaemulonii* by Vitek 2

Compact; *Candida haemulonii*, *Candida catenulate by* BD Phoenix yeast identification system; Microscan misidentifies it as *Candida famata, Candida guilliermondii, Candida lusitaniae and Candida parapsilosis [14].*

C. auris can spread in healthcare settings through contact with contaminated environmental surfaces or equipment, or from person to person [13,14]. However, due to stringent IPC, no outbreak was seen in our hospital. Cluster of seven cases were seen in different months of the year (except 2 cases in February and 2 cases in December, 2018) at different locations amongst these immunocompromised patients (Table 1).

Out of seven isolates, six were resistant to fluconazole (86%) which is similar to the findings of other studies [93% [5] and 100% [20]]. In our study, amphotericin-B resistance was 86% which was not in concordance with the findings of other studies [35% [5] and 7.9% [20]]. No isolate was found to be resistant to echinocandins which is dissimilar to the result found by echinocandins [(5-10%) [18] and 7% [5]] (Table 1).

Conclusion

With the use of broad spectrum antibiotics, NAC has emerged as an important cause of infection in hospitalized patients with associated risk factors. Species level identification following proper quality control of microbiology laboratory with proper sample collection can act as a primer for prevention of outbreak by YYLF. Continuous monitoring and surveillance is necessary for all YYFL for the changing epidemiological pattern. *C. auris* is emerging MDR yeast which spreads readily between patients in healthcare set-ups. In our set-up, although isolation rate of *C. auris* infected patients were less in number, it was significant because of immediate and efficient containment with no nosocomial spread among the immunocompromised patients. Therefore, it is now essential that microbiology laboratories should identify clinical isolates of *Candida* upto the species level along with the antifungal susceptibility pattern. Stringent IPC focusing on source isolation, hand hygiene and disinfection are the most effective measure to prevent nosocomial spread by *C. auris.*

Acknowledgements

The authors acknowledge Dr. Arunaloke Chakrabarti, Prof. and Head, National Culture Collection for Pathogenic Fungi, Department of Medical Microbiology, PGIMER, Chandigarh, India, for reconfirming the isolates by sequencing.

Conflict of Interest

All authors report no conflicts of interest relevant to this article.

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