



# *Candida auris*: A New Emerging Fungal Monster

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## Abstract

The incidence of invasive fungal infections (IFI) caused by unusual pathogens is on the rise, partly driven by the increased population of immunocompromised patients. The emerging multidrug-resistant yeast pathogen *Candida auris* (*auris* means ear in Latin) has been a source of concern as an agent of healthcare-associated infections. Some strains of *Candida auris* isolates are multi-resistant to the main classes of conventional antifungal drugs, and their identification using standard laboratory protocols has been proved difficult. Many of these strains have been misidentified to be other yeasts such as *Rhodotorula glutinis*, *Saccharomyces cerevisiae* or *Candida haemulonii*. In fact, specialized laboratory procedures are required for their proper identification such as molecular techniques based on sequencing the D1-D2 region of the 28 s rDNA or matrix-assisted laser desorption ionization time of flight (MALDI-TOF). Misidentification might result in inappropriate treatment. Furthermore, *C. auris* has the tendency to cause outbreaks in health-care settings as has already been reported from several countries worldwide. Finally, it is important to emphasize that *C. auris* is emerging as an important nosocomial pathogen in many parts of the world, which highlights the need for developing rapid and reproducible methods for its identification and typing.

**Keywords:** Invasive Fungal Infections, *Candida auris*, Multi-Drug Resistance, MALDI-TOF, D1-D2 Region, 28s Ribosomal DNA

## 1. Context

The incidence of invasive fungal infections (IFI) caused by unusual pathogens is on a growing trend, partly driven by the increased population of immunocompromised patients. Among other *Candida* species, widespread resistance to conventional antifungal drugs, high treatment failure, and high mortality rate are the characteristics of fungemia due to *C. auris*. (1). The word ‘*auris*’ is derived from Latin and means ‘ear’. *Candida auris* is an emerging fungal superbug characterized by high multidrug resistance and as such is regarded as a serious threat and source of concern among healthcare givers.

During 2004 - 2006, unconventional yeast isolates phenotypically just like *Candida haemulonii* were reclaimed in culture from scientific samples collected from 23 patients in five Korean hospitals. Almost all of these isolates were significantly less susceptible to amphotericin B (AMB) or even fluconazole (FZU) compared to most other *Candida* species (2). In 2009, yeast isolates from the external auditory canal of Japanese patients were identified to be *Candida auris* using sequence analysis of the nuclear rRNA gene (3). In the same year, about 15 *C. auris* isolates were

retrieved from otitis media patients in South Korea (4). In 2011, it was described that three bloodstream infections (BSIs) were caused by *C. auris* in South Korea, and *C. auris* was later isolated from patients in India, South Africa, Kuwait, Venezuela and Pakistan (5-7). These isolates have been reported in Germany and Norway as well (8).

The first episode of candidemia outbreak due to *Candida auris* in Venezuela occurred between early 2012 and mid 2013 in pediatric intensive care unit (ICU) (9). In Columbia, a few episodes of the infection have been reported sporadically since year 2013. Between 2015 and 2016, less than 30 isolates of the yeast were verified in Barranquilla city of Columbia. Similarly, in Cartagena, another episode of outbreak occurred in pediatric ICU patients in August 2016. It involved disseminated yeast infections caused by initially misidentified yeast species that were later verified to be *C. auris*.

*Candida auris* infections have also been reported in several parts of England since 2013. Between 2015 and 2016, a cardiothoracic center experienced the episodes of outbreak (9, 10). The isolated species were found to be evolutionary and related to some rare *Candida* species such as *C. haemulonii*. *Candida haemulonii* or *Torulopsis haemu-*

*lonii* is recognized as one of the rare yeast species that can be isolated from human clinical samples (11). The draft genome of *C. auris* has a genome size of approximately 12.3 Mb (12, 13). The phospholipase (PL) activity and secreted proteinase were detected in 37.5% and 64% of the tested isolates, respectively (14, 15).

Comparison of whole-genome sequencing (WGS) data demonstrated that *C. auris* is phylogenetically closely related to *C. lusitania*, a species recognized for innate antifungal resistance. Using WGS, three different amino acids have been identified in the *ERG11* gene of *C. auris*. These substitutions were exclusively associated with geographic clades, namely Y132F from Venezuela, F126T from South Africa and Y132F or K143F from India and Pakistan. Each mutation was linked to isolates from a different country, indicating that resistance to FCZ might be acquired rather than inherent (6, 9, 16).

The emergence of *Candida auris* as a significant pathogen in tertiary and pediatric centers is alarming. Recently, about 8.6% to 30% of candidemia cases have been reported in India (17). Healthcare-based epidemics caused by *C. auris* have occurred across Asia and South America, as highlighted in a 2016 clinical warning (18). Hospital-acquired infections are gaining momentum with estimates of about 99000 deaths a year, according to the Centre for Disease Control (CDC) in the USA (19). Hospital-acquired IFI is of public health concern and candidemia is becoming very relevant in European countries (13). In India, the national prevalence of candidemia in ICUs due to *C. auris* was around 5% and as high as 30% of the *Candida* isolates were identified to be *C. auris* in some centers (20, 21).

## 2. Who Is at Risk?

Available data show that the risk factors for acquiring *C. auris* infections are not very different from those for other *Candida* species. These include immunosuppressive conditions such as diabetes mellitus (DM), cancers and chemotherapy, the presence of central line catheters, the use of broad-spectrum antibiotics, neutropenia, total parenteral nutrition (TPN), hemodialysis, blood transfusion, major surgery within one month, critical care, previous therapy with antifungal agents within one month, concomitant bacteremia or candidemia, candiduria, indwelling urinary catheter and chronic kidney diseases (9).

*C. auris* infection is defined as any clinical case with clinical signs and symptoms of infection and a positive culture sample from a non-sterile site (e.g., urine or sternal wound,) requiring treatment with antifungal agents (10). The emerging MDR yeast pathogen *Candida auris* has attracted considerable attention as a source of healthcare-

associated infections (9). This pathogen has been associated with life-threatening invasive diseases such as candidemia and wound infections. About 20% of cases of colonization with *C. auris* resulted in candidemia among ICU-hospitalized patients in the UK (7, 22, 23). Other reports showed that mortality following *C. auris* candidemia ranged from 30% to 60%, involving larger groups of adult patients (24).

Transmission of infection was observed in intensive care, surgical, medical, neonatal, oncologic, and pediatric wards, which were mutually exclusive with respect to healthcare staff. Biofilm formation is an important phenomenon for *C. albicans* pathogenicity and is associated with patient death. Genome analysis is equally important in identifying the key proteins involved in slime formation (22, 25, 26). The clinical resistance of *C. auris* to FCZ is distinctly alarming. Alterations at azole-resistance codons have been detected in both *C. albicans* and *C. auris* isolates by comparing *ERG11* amino acid sequences between the two species (6).

## 3. Mode of Transmission *Candida auris*

- Person to person
- Spread through contact with contaminated environmental surfaces
  - Direct contact with contaminated fomites (such as blood pressure cuffs, stethoscopes, and other equipment in contact with the patient)
  - Indirect transmission from the hands of health care staff
  - Invasive procedures or exposure to an indwelling device
- Hospitalization of susceptible patients (9, 22)

## 4. *Candida auris* Virulence Factors

- Histidine kinase-2 component system
- Iron acquisition
- Tissue invasion
- Enzyme secretion
- Phospholipase and proteinase production
- Multidrug efflux
- Genes/pathways involved in cell wall modeling
- Nutrition acquisition
- Salt tolerance and cell aggregation
- Thermotolerance up to 42°C
- Biofilm formation (15)

### 5. Clinical Conditions and Mortality

*C. auris* has been identified as an agent of serious invasive fungal infections associated with high mortality rate (up to 72%) (6, 18, 23).

It has been isolated from various clinical conditions including:

- Candidemia
- Urinary tract infection (UTI)
- Otitis
- Surgical site infections
- Skin lesions associated with catheter insertion
- Cardiac muscles infection
- Central nervous system infections such as meningitis
- Bone infections (BI)
- Burns wound infection and colonization (9).

### 6. Diagnosis of a *Candida auris* Infection

- Fungal culture of clinical specimen the affected site such as blood, pus and body fluids,
  - Identification of the yeast isolates based on the phenotypic characteristic such as oval yeast cells without pseudohyphae or germ tube formation.
    - *C. auris* does not form chlamyospore
    - The pink colony on CHROMagar *Candida* medium
    - Growth at 37°C and 42°C
    - Assimilated N-acetylglucosamine (NAG) (*C. auris* isolates from India)
      - Ability to ferment glucose, weak fermentation of sucrose and trehalose, but with inability to ferment galactose, maltose, lactose, or raffinose
        - Starch formation, urease activity and diazonium blue B reaction are negative
- Commercially available biochemical-based tests
- VITEK-2 (misidentified isolates as *C. haemulonii* and *C. famata*)
- API20C (misidentified them as *R. glutinis* and *C. sake*)
- MicroScan AutoSCAN 4 and MicroScan Walkaway
- Molecular identification (ITS and D1/D2 regions)
- The phenotypic divergence of these- isolates (M13 and AFLP typing)
  - Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF) (2, 9, 27).

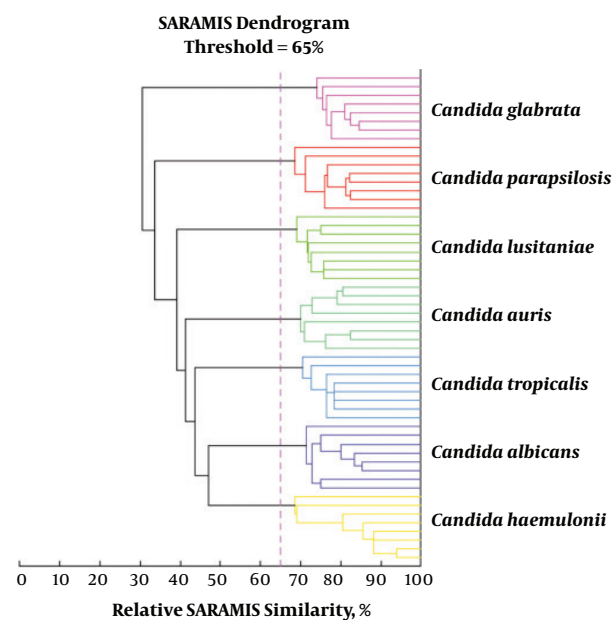
### 7. Decolonization

To date, clinical studies have shown that colonization with *C. auris* is tenacious and difficult to eradicate making infection prevention and control strategies even more important. For patient decolonization, the following methods have been suggested:

**Table 1.** Laboratory Diagnosis and Misidentification of *Candida auris*<sup>a</sup>

Diagnostic System	Comment
API-20C <i>glutinis</i> , <i>Candida</i>	May misidentify <i>Candida auris</i> as <i>Rhodotorula sake</i> , or <i>Saccharomyces cerevisiae</i>
Vitek-2 <i>Candida famata</i>	May misidentify <i>C. auris</i> as <i>Candida haemulonii</i> or
MALDI-TOF the database	Will identify <i>C. auris</i> if appropriate sequences are in
DNA sequencing subunit rRNA	Sequencing of the ITS and D1-D2 domain of the large gene has been performed most commonly
Clinical and laboratories standards Institute broth microdilution method	May give falsely elevated caspofungin MICs
Etest	May give most consistent results

<sup>a</sup> Abbreviation: MIC, minimum inhibitory concentrations.



**Figure 1.** Cluster based on the relative similarity of spectra from representative isolates for the most common yeast species present in the VITEK MS database showing clear distinction between *Candida* species. A similarity below 65% for two spectra means that the strains belong to different species (29)

- Twice daily 2% chlorhexidine gluconate wipes twice daily or 4% chlorhexidine aqueous formulations on skin.
- Oral decolonization can be achieved by using 0.2% chlorhexidine mouthwash or 1% chlorhexidine dental gel in patients on ventilator support.
  - Use of oral nystatin in oropharyngeal colonization.
  - Chlorhexidine-impregnated protective disks for central vascular catheter exit sites (9).

**Table 2.** Biochemical Identification

Isolate No.	Species Tested	API 20C AUX <sup>a</sup>	BD Phoenix <sup>b</sup>	Vitek-2 <sup>c</sup>	MicroScan <sup>d</sup>
1	<i>C. auris</i>	<i>R. glutinis</i>	<i>C. catenulate</i>	<i>C. haemulonii</i>	<i>C. famata</i>
2	<i>C. auris</i>	<i>R. glutinis</i>	<i>C. haemulonii</i>	<i>C. haemulonii</i>	<i>C. famata</i>
3	<i>C. auris</i>	<i>R. glutinis</i>	<i>C. haemulonii</i>	<i>C. haemulonii</i>	<i>C. famata</i>
4	<i>C. auris</i>	<i>R. glutinis</i>	<i>C. haemulonii</i>	<i>C. haemulonii</i>	<i>C. lusitanae</i>
5	<i>C. auris</i>	<i>R. glutinis</i>	<i>C. haemulonii</i>	<i>C. haemulonii</i>	<i>C. guilliermondii</i>
6	<i>C. auris</i>	<i>R. glutinis</i>	<i>C. haemulonii</i>	<i>C. haemulonii</i>	<i>C. famata</i>
7	<i>C. auris</i>	<i>R. glutinis</i>	<i>C. haemulonii</i>	<i>C. haemulonii</i>	<i>C. guilliermondii</i>
8	<i>C. auris</i>	<i>R. glutinis</i>	<i>C. haemulonii</i>	<i>C. haemulonii</i>	<i>C. parapsilosis</i>
9	<i>C. auris</i>	<i>R. glutinis</i>	<i>C. haemulonii</i>	<i>C. haemulonii</i>	<i>C. guilliermondii</i>
10	<i>C. auris</i>	<i>R. glutinis</i>	<i>C. haemulonii</i>	<i>C. haemulonii</i>	<i>C. guilliermondii</i>
11	<i>C. duobushaemulonii</i>	<i>R. glutinis</i>	<i>C. parapsilosis</i>	<i>C. haemulonii</i>	<i>C. guilliermondii</i>
12	<i>C. duobushaemulonii</i>	<i>R. glutinis</i>	<i>C. parapsilosis</i>	<i>C. haemulonii</i>	<i>C. guilliermondii</i>
13	<i>C. haemulonii</i>	<i>R. glutinis</i>	<i>C. haemulonii</i>	<i>C. haemulonii</i>   <i>K. ohmeri</i>	<i>C. catenulata</i>
14	<i>C. duobushaemulonii</i>	<i>R. glutinis</i>	<i>C. parapsilosis</i>	<i>C. haemulonii</i>	<i>C. parapsilosis</i>
15	<i>C. haemulonii</i>	<i>R. glutinis</i>	None	<i>C. haemulonii</i>   <i>K. ohmeri</i>	<i>C. parapsilosis</i>

<sup>a</sup> Identification at 48 and 72 hours of incubation; API 20C AUX does not have *C. auris*, *C. haemulonii*, or *C. duobushaemulonii* in its library.

<sup>b</sup> *C. haemulonii* is in the BD Phoenix library, but *C. auris* and *C. duobushaemulonii* are not.

<sup>c</sup> The Vitek-2 library has *C. haemulonii* but not *C. auris* or *C. duobushaemulonii*.

<sup>d</sup> MicroScan does not have *C. auris*, *C. haemulonii*, or *C. duobushaemulonii* in its library (28).

### 8. Drug Resistance

The commonly used methods for testing antifungal sensitivity are the CLSI-BMD, E-test method and the VITEK-2 antifungal susceptibility test (30-32). There are reports of *C. auris* resistance to the major classes of anti-fungal drugs (i.e., azoles, polyenes, and echinocandins). For the purpose of epidemiological studies, the CDC has maintained the conservative breakpoints already in use for other *Candida* spp. than *C. auris*. The breakpoint sets were  $\geq 32 \mu\text{g/mL}$  for FCZ,  $\geq 2 \mu\text{g/mL}$  for AMB,  $\geq 2 \mu\text{g/mL}$  for voriconazole (VOR),  $\geq 128 \mu\text{g/mL}$  for flucytocine (FLU) and  $\geq 8 \mu\text{g/mL}$  for the echinocandins. Resistance to FCZ exceeds 90%, while resistance to VOR may approach 50% among isolates of *C. auris*. Low MICs for the newer triazoles, such as posaconazole and isavuconazole, suggest that these antifungals may be effective against *C. auris*.

Resistance to AMB has been reported in up to 35% of isolates, while resistance to echinocandins has been between 2% - 8%. About 4% of isolates have been resistant to all the three major classes of anti-fungal drugs (20). SCY-078 is a triterpene glucan synthase inhibitor (GSI) that has exhibited both in-vitro and in-vivo activity against the most common *Candida* species (antifungal/antibiofilm), including echinocandin-resistant isolates. This drug is the only 1, 3-glucan synthase inhibitor with both oral and intravenous (IV) formulations in development.

It can be concluded that SCY-078 is a promising novel antifungal agent against *Candida auris*, although further investigations are warranted (15, 33). Combination therapy coupled with VOR and micafungin highlighted synergistic activities towards multidrug-resistant *C. auris*, indicating a great substitute approach to handle its antifungal drug resistance (34).

### 9. Case Reports

1- The first report of donor-derived *C. auris* transmission was in a lung transplant recipient. A 71-year-old male with end-stage chronic obstructive lung disease due to idiopathic pulmonary fibrosis (35).

2- Fungal otomastoiditis is a rare disease, which can be fatal in immunocompromised patients. Recently, there have been increasing cases of otologic infection caused by *Candida auris* (36).

3- A case report of vulvovaginal candidiasis caused by *C. auris* characterized by its virulence traits and drug resistance profile using molecular methods (37).

4- A rare case of fungal pericardial effusion caused by *Candida auris* in a patient with chronic liver disease (CLD) (38).

## 10. Conclusions

Numerous difficulties have been reported with regards to the identification of *C. auris*. Identification based on molecular methods is not commonly performed in diagnostic laboratories, leading to the underestimation of the actual incidence of *C. auris* infections. Currently, the diagnosis of *C. auris* infections should be confirmed using acceptable methods such as MALDI-TOF or molecular identification techniques like polymerase chain reaction (PCR), sequencing and amplified AFLP-PCR (39). The inherent sturdiness of *C. auris* to survive and persist in the environment and its high transmissibility make its infections highly tenacious. To date, clinical studies have revealed that colonization is difficult to eradicate and tends to persist, making infection prevention and control strategies even more important. Poor susceptibility to chlorhexidine could be a possibility, but not evident enough to establish whether *C. auris* is susceptible or resistant to chlorhexidine, which necessitates further studies in this area. However, methods such as ultraviolet light (UV), hydrogen peroxide vaporization and halide-based agents are shown to be effective for environmental cleaning.

Attention should be given specifically to the cleaning of non-disposable devices (e.g., blood pressure cuffs, thermometers and ultrasound machines), beds and surrounding areas occupied by an infected/colonized patient. Finally, it is pertinent to emphasize that *C. auris* is emerging as an important nosocomial pathogen in many centers in the world and this obliges developing rapid and reproducible methods for its identification and typing. Therefore, further evaluation of MALDI-TOF MS as an acceptable typing method for this yeast is warranted. Effective methods of decolonization, surface decontamination and hand hygiene are essential in preventing nosocomial infections, especially outbreaks in healthcare settings.

## Footnotes

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