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Original article

Understanding the Distribution of Candida auris and Its Antifungal Susceptibility in Diverse Samples

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Abstract

Background: This study aims to assess the prevalence and susceptibility to antifungal drugs of Candida auris, a pathogen known for causing both invasive and noninvasive infections associated with high mortality rates. This yeast, classified as an emergent superbug fungal pathogen, has garnered global attention due to its resistance to azole antifungal agents. Variations in susceptibility patterns underscore the need for heightened awareness and systematic surveillance efforts. ***Results:*** Over 4 years, there were 8,351 (41.5%) reported cases of Candida infections, including 268 (3.2%) attributed to C. auris, identified across various clinical samples such as respiratory (45.9%), urine (23.8%), blood (11.9%), sterile body fluids (8.2%), pus (6.7%), and tissue (3.3%). In Delhi, Candida auris, an emerging infection, predominantly affected respiratory samples like BAL (66), endotracheal tip (32), and sputum (25), totalling 268 (3.2%) positive isolates, with a higher incidence observed in males (64.5% male vs. 35.5% female). This study emphasizes the notable sensitivity of Caspofungin and Micafungin against C. auris isolates, alongside the concerning development of high-level resistance to Azoles and Amphotericin B in clinical settings in North India. ***Conclusion:*** In conclusion, the emergence of multidrug-resistant (MDR) C. auris poses significant challenges in treatment of invasive candidiasis, drawing significant concern from policymakers and underscoring the urgent need for stringent antifungal prescription policies. Echinocandins remain pivotal in the treatment of C. auris infections, proving highly effective despite increasing resistance to other antifungal classes. This study aims to raise public awareness, effective management strategies about C. auris, advocate for increasing knowledge and vigilance among physicians and healthcare workers, especially in critical care settings, to monitor resistance patterns and the development of straightforward, rapid, accurate, and cost-effective diagnostic methods, and promote future research, including whole genome analysis.

Keywords: ***Candida auris (C. auris), Echinocandins (Micafungin, Caspofungin), Azoles resistance, Amphotericin B resistance.***

Introduction

Candida auris (C. auris) is an emerging fungal superbug that poses a significant threat to human health due to its resistance to multiple drugs, which complicates its diagnosis and treatment. *C. auris* is highly transmissible, leading to numerous outbreaks worldwide [1-3,6,8,10,11-13].

The fungus can infect or colonize humans, especially those with compromised immune systems in intensive care units (ICUs and predominantly found in patients who have had prolonged hospital stays in ICUs and is recognized as a major cause of nosocomial infections in many countries [2,3,5,6,9,13]. These patients often require diverse treatment approaches, resulting in varying clinical outcomes that require careful differentiation. It has been isolated from various types of specimens, including sterile body fluids, respiratory secretions, urine, bile, tissues, wounds, and mucocutaneous swabs [1,5,6]. Distinguishing between colonization and infection is challenging, except in cases of bloodstream infections. The mortality rate for *C. auris* fungemia ranges from 30% to 60% [2,6,8]. These statistics highlight the urgent need for effective strategies in both preventing and treating this challenging pathogen.

C. auris was first identified from external ear discharge of a Japanese patient in 2009 [1-13], the fungus now represents a major global public health threat and has been documented in over 40 countries across six continents [1,4,5]. According to the genome sequences, *C. auris* isolates were divided into four clades of *C. auris* have unique geographical characteristics which were reported to emerge simultaneously from different continents. Clade I was mainly reported in India, Pakistan, Kuwait, Russia, United States, United Kingdom, Germany, Malaysia, Netherlands, Italy, etc.; And Clade II were mainly in Japan and South Korea. Clade III was mainly found in South Africa, United States, United Kingdom and China, whereas Clade IV mainly distributed in Colombia and Venezuela. Clade I and III were the most prevalent clades which have more reported cases and wider geographical distribution [2,6,10,13].

Before 2009, *C. auris* was thought to be a rarely observed microorganism. It is now deemed an emerging human pathogen (du).

C. auris can colonize different sites, such as the skin, axilla, nose, and groin, and is transmitted by contact or through feces. In addition, the pathogen can survive on inanimate object surfaces for more than 7 days [5,10]. Therefore, hospital beds, Sphygmometers, thermometers, and other reusable equipment are potential infection sources for inpatients. This has resulted in relatively high rates of *C. auris* transmission in inpatients, particularly in ICU rooms [5,9,13]. It is difficult to eradicate *C. auris* once it has colonized a patient. Even though many countries have implemented infection prevention and control (IPC) strategies, *C. auris* transmission is still a problem that warrants attention.

Thus, traditional identification methods such as Vitek 2 and API 20CAUX tend to incorrectly identify *C. auris* as these two close relatives *Candida haemulonii* and *Candida pseudohaemulonii* and it is difficult to distinguish them by phenotype. It is characterized as white to cream colonies with a smooth edge on the Sabouraud-dextrose agar and pink colonies on CHROMagar *Candida* medium [1,4,6,7,10,13]. Thus, *C. auris* is usually misidentified by routinely used conventional methods in clinical microbiology laboratories. Biochemical assays as one of the most widely used phenotypic approaches, besides of being time consuming and expensive, cannot properly identify [1,13]. Therefore, the prevalence of *C. auris* infections in the global population remains unknown.

Although the development of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) strategies, development of specific PCR assays for *C. auris* have helped in the rapid and accurate diagnosis of *C. auris* particularly in outbreak settings, but it is not routinely used in most parts of the world because do not have the infrastructure to carry out these techniques [5].

The appearance of multidrug resistance (MDR) in *C. auris* is another problem that has attracted global attention in recent years [7]. *C. auris* is usually resistant to several drugs, such as fluconazole, voriconazole, amphotericin B, so it may be difficult to start an adequate empirical therapy without accurate species identification. However, resistance rate varies between studies [2,4,7-11].

Due to the low incidence of *C. auris*, no large-scale epidemiology studies were reported by now. Therefore, in this present study, we performed a retrospective study to estimate the prevalence of *C. auris* case count and their susceptibility pattern, in Delhi, North India. We analyzed cases from the emergence of *C. auris* infections up until the July of 2019. It is hoped that the results of this analysis will raise awareness in scientists to promote protection and control research pertaining to this pathogen.

Materials and methods

This retrospective study conducted on 20,106 samples, tested during July 2019 to December 2023, performed at Microbiology department of *Dr Lal Path Labs*. The samples subjected to direct microscopy-using KOH wet mount, and India ink preparations depending on the type of specimen. Fungal culture done on SDA agar, with chloramphenicol (16µg/mL) and with cycloheximide (0.3µg/ mL) plus chloramphenicol (16µg/mL), Specimens were cultured in duplicate; one set of inoculated slants incubated at 22°C and the other incubated at 37°C, and they were examined every day for growth up to 28 days before discarding as negative. Fungal growth as identified by colony morphology and use of more advanced and standardized methods, such as MALDI TOF-MS (Bruker, Daltonics) were included in this study. VITEK-2 (Biomerieux) system used for the antifungal susceptibility testing of isolates from the pure culture of isolated colonies of the Candida species on CHROM agar Candida, and antifungal susceptibility was carried out on YST 07 cards respectively. The results interpreted according to CDC [8].

At present, there are no antifungal clinical breakpoints reported for *C. auris*. Studies examining the susceptibility of this organism to antifungals have used a variety of methods, including Clinical and Laboratory Standards Institute (CLSI) broth microdilution, Etest, and the Vitek 2 yeast susceptibility system. Antifungal breakpoints for *C. auris* are not yet defined by the Clinical Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing. The US Centers for Disease Control and Prevention (CDC) set up, as it were, provisional breakpoints as follows: Fluconazole (FLC) >=32 µg/mL, Amphotericin B (AMB) >=2 µg/mL, Micafungin (MCFG) >=4 µg/mL, and Caspofungin (CPFG) >=2 µg/mL.

Statistical analysis

For the evaluation of the Data analysis, Myla statistical program (BioMerieux, India, Pvt. Ltd) used.

Results

Over the 4 years period, 8351(41.5%) Candida cases reported, 268 (3.2%) were due to *C. auris* isolated from various clinical specimens like Respiratory (BAL, sputum, endotracheal secretion) (45.9%), urine (23.8%), blood (11.9%), sterile body fluids (8.2%), pus (6.7%), tissue (3.3%) respectively [**Figure 1**].

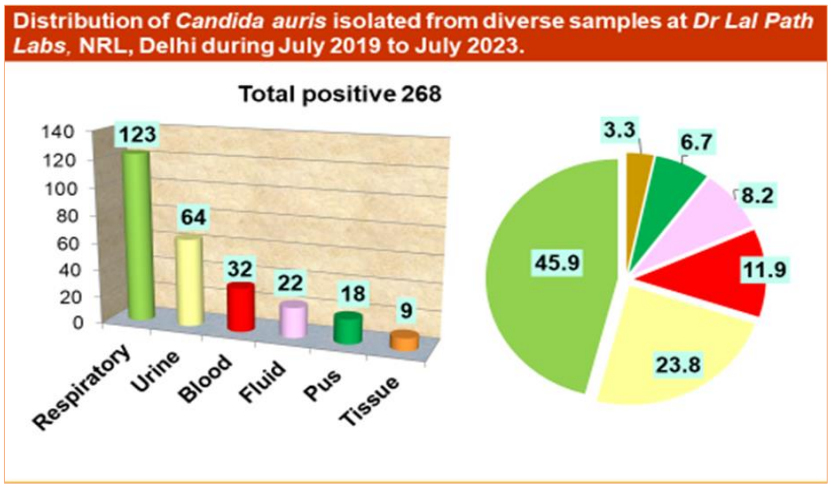

Figure 1: Distribution of Candida auris isolated from diverse samples during four year period 2019-2023.

In Delhi Candida auris is also, an emerging infection reported in respiratory samples and were isolated from BAL (66), endotracheal tip (32), Sputum (25) specimen respectively.

Table 1: Age and Gender wise prevalence of C. auris isolated from diverse sample during July 2019- July 2023.

Age group	Total No. of *C. auris* isolates (N=268) (%)	Male N=173 (64.5%)	Female N=95 (35.5%)
21-40	34 (12.7%)	25	9
41-60	76 (28.3%)	55	21
>=61	158 (58.9%)	93	65

In total, 268 (3.2%) positive isolates of Candida auris were obtained, with males accounting for 173 (64.5%) and females for 95 (35.5%), indicating a higher proportion of infections in males. Table 1: illustrates the age distribution of study participants, ranging from 21 to 100 years, with C. auris not detected in individuals under 20 years. Participants were categorized into three groups: young adults (21-40 years) with 34 (12.7%) positive cases, middle-aged adults (41-60 years) with 76 (28.3%) positive cases, and elderly adults (>61 years) with more than half, 158 (58.9%) positive cases [**Table 1**]. Specimens were primarily collected from Delhi and neighbouring states in North India.

Table 2: Cumulative interpretation with Cumulative MIC (50/90) of Antifungal susceptibility pattern among Candida auris in diverse samples during 4 year period.

Antifungal	S (%) N=157	R (%)	Ranges $MIC_{50/90}$	Blood N=29	Urine N=34	Pus N=14	Fluid N=18	Tissue N=8	Respiratory N=54
				S%	S%	S%	S%	S%	S%
Fluconazole	4.5	95.5	32/>=32	10.5	5.8	0	0	0	11.1
Voriconazole	13.6	86.4	4/16	31	0	28.6	0	0	22.2
Amphotericin B	11.6	88.4	8/>=16	15.2	17.6	0	0	11.1	25.9
Caspofungin	95.5	4.5	0.12/0.25	93.1	94.1	100	100	100	85.1
Micafungin	95.9	4.1	<=0.06/0.25	96.5	97	100	100	100	83.3

The United States Centers for Disease Control (CDC) defined conservative cutoff points to determine whether *C. auris* is resistant to antifungals: Fluconazole (FLC): >=32 µg/mL, Amphotericin B (AMB): >=2 µg/mL, Micafungin (MFG): >=4 µg/mL, and Caspofungin (CAS): >=2 µg/mL. A MIC < cutoff was defined as sensitive while a MIC ≥ cutoff was defined as resistant [8].

Among the isolates that were included in the analysis, drug resistance information was available for 157 isolates; the proportion of isolates exhibiting FLC resistance was the highest (95.5%), while isolates exhibiting AMB (88.4%) and VRC (86.4%) resistance were the second and third most prevalent, respectively [**Table 2**].

In addition, this study describes for the cumulative MIC interpretation of antifungal sensitivity patterns among *Candida auris* with help of Myla statistical analysis (Biomerieux, India) which causes complicated infection such as *Candida auris* shown highly resistant to Fluconazole (95.5%), Amphotericin B (88.4%) and Voriconazole (86.4%) respectively [**Table 2**]. Voriconazole activity ($MIC_{50/90}$ 4/16 µg/ml) against *Candida auris* demonstrated that 50% of isolate were within 4µg/ml MIC and 90% isolates were within 16µg/ml. The distribution of Antifungal drugs with MIC values against sensitivity and resistant patterns of *Candida auris* followed in [**Table 2**].

Out of 268 total *Candida auris* isolates tested, 157 were evaluated for their susceptibility to various antifungal agents. The results indicated that Caspofungin and Micafungin showed high sensitivity, with 95.5% and 95.9% of *Candida auris* isolates being susceptible respectively, at MIC50/90 values of 0.12/0.25µg/ml and <=0.06/0.25µg/ml.

This study highlights the significant sensitivity of Caspofungin and Micafungin to *C. auris* isolates, while also underscoring the critical issue of developing high-level resistance to Azoles and Amphotericin B in clinical settings in North India.

Discussion

Candida auris, a recently emerged global fungal pathogen, is recognized as a formidable superbug. Initially reported in 2009 from a patient's external ear in Japan, it has since spread to 44 countries, causing nosocomial infections across East Asia, the Middle East, Africa, and Europe [6,10]. In India, the first case was documented in 2011 [3]. Known for its resilience on hospital surfaces like mattresses and windowsills, *C. auris* can survive for extended periods, up to 7 days on steel disks and over 14 to 28 days on plastic surfaces, facilitating transmission between patients [10,11,13].

Accurate identification of *C. auris* is challenging with conventional methods, often leading to misidentification as *Candida haemulonii* or *Rhodotorula glutinis* in routine laboratories. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has emerged as a rapid diagnostic tool for precise species identification, aiding in prompt treatment decisions and potentially reducing hospital stays. The findings underscore the urgent need for early diagnostic methods and effective treatments tailored to *C. auris* infections [10,11,13].

According to our data, *Candida auris* exhibits a significant resistance rate to fluconazole (FLC), reaching as high as 95.5% over the past four years. Consequently, fluconazole is not recommended for empiric therapy due to elevated fluconazole MICs (>64 mg/liter) observed across various geographic clusters and our study was concordance with susceptibility testing on Indian isolates has consistently shown nearly universal resistance to fluconazole, initially suggesting intrinsic resistance to this azole drug [1,3,7,8,11] who have reported resistant to fluconazole and amphotericin B are common.

Our finding indicates a notably high resistance rate of 88.4% to Amphotericin B, substantially exceeding the rates reported in other studies [1,9]. This elevated level of resistance is especially alarming given that both Indian and international studies have reported lower rates of resistance [1,7,8]. The increasing prevalence of multidrug-resistant strains, particularly high resistance to Azoles and Amphotericin B, highlights the evolving challenge in clinical management.

The clinical presentation of *Candida auris* infection is similar to that of other Candida species. *C. auris* has been detected in several body sites, such as sputum, BAL, ET, blood, fluid, urine, wounds/surgical tissue, and pus [12]. Isolates from non-sterile sites, including the genitourinary tract, skin, soft tissues, and lungs, are

more likely to signify colonization rather than an active infection [12]. In our study, 45.9% of *C. auris* isolates were obtained from respiratory specimens (Sputum, Bronchoalveolar lavage, and Endotracheal), with the remainder from other sources. This retrospective analysis revealed a predominance of male patients and a significant proportion of cases originating from hospital settings.

Notably, the majority of infections caused by *C. auris* isolates in our study were susceptible to echinocandins, consistent with previous research highlighting echinocandins as effective antifungal agents against *C. auris* infections [1-2,4,6,11]. Have prompted the recommendation to prioritize echinocandins as empirical treatment for invasive *Candida auris* infection in many regions [1,2,11].

However, the increasing use of echinocandins has also revealed *C. auris* isolates with reduced susceptibility to this class of drugs [1,3,7,8]. Therefore, there is a critical need to develop new antifungal agents capable of combating the high levels of echinocandin resistance observed in these isolates [7,8]. Continued research and development efforts are crucial to address the evolving challenges posed by drug-resistant *C. auris* strains worldwide, challenges also exist in selecting appropriate antifungal agents for invasive infections, particularly those involving the central nervous system, genitourinary system, where echinocandins may have limited efficacy [9]. Notably, certain strains of *C. auris* have exhibited diminished susceptibility to multiple classes of antifungal medications, suggesting the potential for pan-drug resistance [7,8].

According to many study, colonization is difficult to eradicate, and it tends to persist for months. Doctors and healthcare workers need to be very knowledgeable and alert, especially in critical care settings. Strict infection control measures should include isolating patients in single rooms, improving hand hygiene, using precautions for contact, removing bacteria from the environment and patients, and monitoring for new cases. Washing hands with sanitizer or soap and water before and after wearing gowns and gloves, and before touching clean areas or other patients, can help control the spread and improve diagnostic and therapeutic strategies [1,13].

This study is limited by several factors. Being retrospective, it did not capture all potential risk factors relevant to *Candida auris* infections, which may have influenced the study's outcomes. Additionally, the study included a small, specific group of patients. These limitations emphasize the need for careful interpretation of the study's results and implications. The rise of more resistant strains is worrisome. Future research should focus on filling these gaps to gain a more comprehensive understanding *of Candida auris* epidemiology and treatment outcomes.

Conclusion

In conclusion, the emergence of multidrug-resistant (MDR) *C. auris* presents substantial challenges in treating invasive candidiasis, raising considerable concern among policymakers and highlighting the urgent need for strict antifungal prescription guidelines. The increasing prevalence of *Candida auris* infections, along with their potential to lead to severe invasive infections and sepsis, is exacerbated by multidrug resistance and possibly higher mortality rates, emphasizing the necessity for increased awareness. Echinocandins remain pivotal in the treatment of *C. auris* infections, proving highly effective despite increasing resistance to other antifungal classes. This study aims to raise public awareness, effective management strategies about *C. auris*, advocate for increasing knowledge and vigilance among physicians and healthcare workers, especially in critical care settings, to monitor resistance patterns and the development of straightforward, rapid, accurate, and cost-effective diagnostic methods, and promote future research, including whole genome analysis.

List of Abbreviations

AMB: Amphotericin B
BAL: Bronchoalveolar Lavage
CDC: Centers for disease Control and Prevention
CLSI: Clinical and Laboratory Standards Institute.
CPFG: Caspofungin
ET: Endotracheal
FLC: Fluconazole
ICU: Intensive care unit
MDR: Multi-drug Resistant
MALDI-TOF MS:
MCFG: Micafungin
VRC: Voriconazole

Data Availability

All the data is available on records and can be obtained by contacting the corresponding author.

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No funding was received.

Ethical Approval

It is not applicable.

Conflicts of Interest

There are no conflicts of interest.

Authors' contributions

PS developed the study concept, designed the methodology, and supervised data collection and analysis. Additionally, PS made substantial contributions to drafting the manuscript, with a focus on the theoretical framework and literature review.**SM** was instrumental in shaping the study design and provided critical feedback on the manuscript. All authors reviewed and approved the final manuscript.

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