Candida auris for the clinical microbiology laboratory: Not your grandfather’s Candida species

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Abstract

Candida auris is a newly emerging species that was first identified in Asia in 2009 but has rapidly spread across the world. C. auris differs from most other Candida species in that antifungal resistance is the norm rather than the exception, it is a commensal of human skin rather than the human gut, and it can be easily transmitted from person to person in a healthcare setting. This review discusses the emergence of C. auris, global epidemiology, identification, antifungal susceptibility testing, and precautions to be taken when it is identified from a patient specimen.

Introduction

“Have you heard about this new Candida species, Candida auris?”

If you haven’t gotten that question yet, chances are you soon will. Candida auris was first described as a species in 2009 but it is still a relatively new to clinical microbiology laboratories [1]. The first described isolate was from the ear canal of a patient in Japan, but C. auris soon started showing up as a cause of bloodstream infection in other parts of Asia including Korea, India, and Kuwait [2–5]. Over the past eight years it has slowly started to emerge on other continents; first in Africa, then South America, and most recently in North America and Europe [6–12]. C. auris is closely related to three other rare Candida species, C. haemulonii, C. duobushaemulonii, and C. pseudohaemulonii in the family Metschnikowiaceae along with the more common Candida lusitaniae [13]. Although very few members of this family are known to cause human infection, those that do are known for their ability to develop resistance to fluconazole and amphotericin B. As will be mentioned below, C. auris can be difficult to distinguish from isolates of the C. haemulonii species complex in the clinical microbiology laboratory.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
Where in the world did *C. auris* originate?

Although the first reports of *C. auris* came out of Asia, shortly after there were subsequent reports of isolates from South Africa as well as Venezuela [6, 7]. One of the major questions surrounding *C. auris* was how it was able to emerge in so many countries in such a short time when it was essentially unheard of prior to the first report in 2009; did it emerge independently in each country or did a single outbreak strain spread from an original source? Whole-genome sequencing (WGS) and single-nucleotide polymorphism (SNP) analysis allow the analysis of *C. auris* isolates in high resolution, by characterizing genetic diversity of the 12.5 million base pairs in the genomes, which helps to differentiate even the most closely related isolates. *C. auris* reference genomes were assembled using two different types of whole genome sequencing systems, Illumina and PacBio, and those sequences were published and made publically available [9, 14, 15]. Using these methods and resources, phylogenetic trees that reveal population dynamics and structure were constructed, so that when coupled with the epidemiology, a better understanding of the spread of *C. auris* could be obtained.

In a study that was initiated by the Centers for Disease Control and Prevention, *C. auris* isolates from Japan, India, Pakistan, South Africa, and Venezuela were sequenced and the results were startling [9]. Supporting previous studies that employed more traditional molecular and proteomic methods, isolates were found to cluster by geography [2, 3, 16] (Figure 1). The remarkable finding was that isolates grouped to clusters that were nearly identical within the cluster, but the clusters themselves were genetically unrelated to each other. Specifically, four clades characterized by region (East Asian, South Asian, African, and South American) were identified. Each clade was separated by 40,000 – 140,000 single nucleotide polymorphisms (or SNPs). Within each clade, the average number of SNP differences between any two isolates was <70 (with many being <10), indicating a high degree of clonality within clades. Together, these results suggest an independent, nearly simultaneous emergence of four populations on three continents. Why and how this occurred remains unknown. Multiple independent laboratories with international culture collections subsequently reviewed older isolates to see if *C. auris* had been isolated previously and was either misidentified or not identified at all. While a single isolate from 1996 in Korea had been misidentified and a single isolate from Pakistan in 2008 had been unidentified, no other isolates of *C. auris* were identified from over 30,000 isolates from more than 40 countries that were reviewed [4, 9] (CDC, unpublished data). This corroborates the recent clinical emergence of *C. auris* within the last 10 years.

Combining phylogenetic analysis with epidemiology can help public health officials track the spread of *C. auris*. This has been particularly true in the United States. As of August 31, 2016, seven U.S. cases of *C. auris* infection were reported to the CDC [10]. Cases were reported from four states; two from a single hospital in Illinois, one from Maryland, one from New Jersey, and three from three different hospitals in New York. WGS was performed on isolates from six cases, and surprisingly, isolates did not form a new clade distinct from the four known clades. Instead, isolates from the east coast of the United States (Maryland, New Jersey, and New York) clustered with the South Asian clade with a pairwise difference of around 60 SNPs when compared to the Indian and Pakistani isolates. As for the cases
from Illinois, isolates clustered with the South American clade, differing by around 150
SNPs from the Venezuelan isolates. Although none of the cases from which isolates were
sequenced had known travel or other direct links to South Asia or South America, the data
suggests an original transmission link between these regions and the United States. Since
the time of that report, the case count in the United States has increased to 33 (https://

However, all of the additional cases have been identified either in Illinois or New York, and
each can be linked epidemiologically to a previous case. The majority of the cases of C.
auris that have been reported either show multiple cases in a single institution, or report the
clonal relatedness of isolates from multiple institutions [2, 3, 7–9, 11, 12, 17]. This suggests
that there is transmission within the healthcare setting. This is unusual for Candida.

Although outbreaks of Candida do occasionally occur, it is thought that the majority of
Candida infections are endogenous; patients are infected with their own commensal flora.

However, data from patient and environmental isolates suggests that transmission of C. auris
occurs within healthcare settings [12]. During an outbreak at a hospital in England, patients
were colonized with C. auris in multiple body sites including axilla, groin, nares, ear,
rectum, and in urine. With the exception of C. parapsilosis, Candida is generally thought of
as gut flora and not a skin colonizer. In addition, C. auris can be detected in the environment
of the room of a colonized or infected patient, including on the bed, windowsills, tables
equipment monitors, and the floor [10, 12], more like what would be expected for a bacteria
like Clostridium difficile or an Acinetobacter and not a fungus.

The epidemiological indicators of healthcare-associated transmission are corroborated by the
WGS data. Fewer SNP differences exist between cases who have shared a healthcare facility
as compared to those between cases who have not shared a common facility. In the case of
the seven reported U.S. cases, both the Maryland and New Jersey cases were at one point
admitted to the same New Jersey hospital at the same time. These isolates differed by <10
SNPs, which was comparable to the difference shared between the two Illinois cases that
were admitted to the same hospital (<10 SNPs), to the difference between environmental and
case isolates from the Illinois hospital (<5 SNPs), and also to the difference between
multiple isolates sequenced from a single patient over a 10-day period (<6 SNPs) [10].

Although not as stringent as WGS, amplified fragment length polymorphism (AFLP) typing
also indicates that isolates identified within an institution are more closely related to each
other than isolates from other institutions [2, 3, 7, 12]

Are we going to be able to identify it in our laboratory?

Although the data presented above indicates that it has truly emerged only recently, Candida
auris has likely been under reported due to unreliable identification [4, 18, 19]. Rare
pathogens are often misidentified simply for the fact that they are not included in the
databases of commercially available rapid diagnostic systems, and this has certainly been the
case with C. auris. Unfortunately, this can delay accurate detection by clinical laboratories
and treatment by clinicians, further compounding the underreporting in the field. To allow
laboratories to validate their own internal identification systems and to allow commercial
companies to get it into their databases, the CDC has recently made a panel of C. auris

Standard biochemical identification systems are unreliable for identifying *C. auris* primarily because they do not have *C. auris* in their databases. *C. auris* is misidentified as *Candida haemulonii* by the Vitek-2 (bioMérieux), and as *Rhodotorula glutinis, Candida sake* or *Saccharomyces cerevisiae* by the API 20C AUX (bioMérieux) systems [4, 9, 18]. The CDC panel of ten *C. auris* isolates with representatives from each of the four clades (as described above) were misidentified as *C. haemulonii*, except for one as *C. catenulata*, by BD Phoenix (BD Diagnostics, Sparks, MD) and they were misidentified as *C. parapsilosis, C. famata, C. lusitaniae*, and *C. guillermondii* by MicroScan (Beckman Coulter, Pasadena, CA) [18]. Users of these biochemical identification systems should realize that they may not be able to identify *C. auris* or possibly even rule it out from some of the more common *Candida* species. As these systems update their databases, accurate identifications may become possible. For example, bioMerieux has reported that their most recent software update for the VITEK 2 YST card (with Ver 8.01 software), which is just beginning to be released, allows the closely related species, *C. auris, C. duobushaemulonii*, and *C. haemulonii* to be correctly identified.

Sequencing of the internal transcribed spacer or the D1/D2 region of the ribosomal DNA provides accurate species level identification, but the cost, technical demands, and lengthy turnaround times for those without in-house sequencing capacity makes this less suitable for some laboratories. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a broad spectrum tool capable of faster and more accurate identification of most bacterial and fungal clinical isolates. MALDI-TOF takes a microbial sample input and produces a unique protein profile spectrum capable of discriminating microbes to the genus and species level [20]. The spectral profile is unique to the organisms being analyzed, and an accurate phylogenetic classification of the organism is automatically identified by the instrument using software to compare the spectral profile to reference databases. However, the important limitation to MALDI-TOF MS is that it can only identify organisms that are in the reference databases. A recent study comparing the Vitek-MS (bioMérieux) and the Bruker Biotyper (Billerica, MA) to their respective FDA-approved libraries using the panel of ten CDC *C. auris* isolates found that neither instrument were able to identify *C. auris* because it was not included in the database [18]. Identification is possible after installing the research-use-only Saramis Version 4.14 database and Saccharomycetaceae update for Vitek-MS or Bruker’s 6903 MSP databases for Biotyper [19, 21]. CDC’s MicrobeNet (https://www.cdc.gov/microbenet/index.html) is a unique tool designed to provide subject matter expert-curated information for the most relevant identification methods in today’s laboratories, including MALDI-TOF. In collaboration with Bruker, MicrobeNet recently released the Biotyper Classification Module which now provides MicrobeNet users with access to CDC spectral libraries as well as Bruker’s most up-to-date database. The strains of *C. auris* represented in the MicrobeNet database represent all of the known worldwide clades and accurately classify to the species level on the Biotyper.
The time until the initiation of antimicrobial treatment impacts patient mortality, so rapid and accurate diagnostic methods are a critical component of patient care. Historically we have endured lengthy turnaround times with clinical laboratories running phenotype testing and conventional identification methods. As most rapid candidemia treatment is empiric, the slower time to species identification has not been a problem. However, in the case of C. auris this does pose a problem because of the infection control considerations and the need to implement contact precautions (As will be outlined below). Infection prevention guided by rapid accurate detection in healthcare settings is essential for containment of this pathogen. MALDI-TOF MS has greatly decreased the turnaround, and it has seen rapid adoption worldwide, quickly becoming the standard practice in clinical microbiology [22, 23].

The limitations of the other identification systems, particularly with the available databases, should be closely monitored by lab directors, clinicians, and technologists, especially when it comes to emerging pathogens, because there is often a lag before these organisms are added to the various databases.

What about susceptibility testing, most Candida is antifungal susceptible, right?

The number of antifungal compounds that are available is very limited and essentially consists of only three classes; azoles, echinocandins, and polyenes. With this limitation in place, identifying species capable of developing resistance to those compounds is important in shaping treatment plans. Susceptibility is measured clinically as the reduction in growth in vitro as compared to drug-free control growth. This yields the minimum inhibitory concentration, or MIC, value which is used as a measure of antifungal activity. Broadly, Candida species are quite susceptible to antifungal drugs but exceptions include intrinsic fluconazole resistance in C. krusei and acquired echinocandin resistance in C. glabrata [24]. Approximately 6–8% of all clinical Candida isolates are resistant to fluconazole while 1% are resistant to the echinocandins, the majority in both categories being C. glabrata [25]. Amphotericin B resistance is extremely rare in Candida species with the exception of C. lusitaniae [26].

That being said, susceptibility testing for an emerging fungal species is complicated by the fact that little susceptibility information has been compiled, there are usually no breakpoints, and the correlation, if any, between MIC values and clinical outcome is not yet known. This is the case for C. auris. But by using breakpoints for other Candida species as guidance tentative MIC values defining resistance have been proposed (https://www.cdc.gov/fungal/diseases/candidiasis/recommendations.html). The MIC values for a very large number of isolates have been determined, and the distribution contains isolates on both the higher and lower end of the tested range. The isolates on the lower end of the range are used to set the values for susceptible isolates while those on the higher end help determine the MIC at which an isolate may be considered resistant. While not an official breakpoint, this serves as a guideline to use with such an emerging species. What has been determined regarding C. auris and antifungal susceptibility can be summed up in three words: it is concerning.
This species can be highly resistant to fluconazole (MIC ≥32 µg/mL), and can also show cross resistance to voriconazole (MIC ≥4 µg/mL) [3, 8–10, 17]. This is troubling given that the azoles are a mainstay in treatment of Candida infections and access to antifungals other than fluconazole is often poor in resource-limited countries. Some initial reports had nearly universal fluconazole resistance and the species was initially thought to have intrinsic resistance [3, 19]. However, as more isolates from around the world have been tested, isolates with fluconazole MIC values similar to those of C. glabrata (in the 2–8 µg/mL range) have been collected and fluconazole resistance is now thought to be acquired (CDC, unpublished data). In a recent study, 54 isolates of C. auris were analyzed for their antifungal susceptibilities and 93% and 33% were resistant to fluconazole and voriconazole, respectively [9]. In this study it was determined that the majority of the isolates with elevated MIC values to fluconazole and voriconazole carried mutations in the protein target of the azoles that had been previously shown to impact drug resistance in other Candida species. These mutations were geographically-distinct, with the isolates within a particular region having the same mutation, but different mutations for each region. Those isolates that did not carry such mutations had much lower MIC values to theazole drug class, suggesting that these isolates carried the wildtype version of this protein and were most likely susceptible. All reported MICs for both itraconazole and posaconazole have been relatively low; there are no isolates with elevated MICs which might be considered resistant. Because the chemical structure of itraconazole and posaconazole are somewhat different from fluconazole and voriconazole, it is not known whether this due to the shape of the target molecule or whether the isolates have just never been exposed to those two compounds which are rarely used to treat candidemia.

There are some isolates that exhibit resistance to one or more echinocandin (MIC ≥4 µg/mL for anidulafungin and micafungin, MIC ≥2 µg/mL for caspofungin) and even some with resistance to amphotericin B (MIC >2 µg/mL). An analysis of 102 C. auris isolates from 4 hospitals in India reported isolates to have a 37% resistance rate to caspofungin by broth microdilution [19]. In fact, other reports describe several isolates from various countries with resistance to one or more echinocandins or with resistance to amphotericin B [7, 9, 12] To date, the mechanisms underlying these types of resistance are unclear. Even more concerning are those isolates which show in vitro resistance to all 3 major classes of antifungals [9]. This leaves no further option for treatment and will pose an important clinical challenge if C. auris increases in prevalence.

It is worth reiterating that breakpoints for C. auris are not established and what is considered resistant at this point should be taken as a general rule and not definitive. Clinicians should not necessarily rule out the use of a specific antifungal based on MIC findings; treatment decisions should be made on a case-by-case basis. However, the high occurrence of in vitro antifungal resistance among clinical isolates of C. auris is unlike what has been observed in other species of Candida and is a serious concern.

**Is there anything else we need to consider when dealing with C. auris?**

As the title of the article suggests, C. auris is not the typical Candida species. As outlined above, it seems to be a skin commensal rather than gut flora, development of antifungal
resistance seems to be the norm, and unlike the majority of Candida species, it can extensively contaminate healthcare environments, a scenario more typical of bacteria than fungi. For these considerations, the Centers for Disease Control and Prevention, and Public Health England each released clinical alerts, followed shortly by a Rapid risk assessment released by the European Centers for Disease Control (https://www.cdc.gov/fungal/diseases/candidiasis/candida-auris-alert.html; https://www.gov.uk/government/publications/candida-auris-laboratory-investigation-management-and-infection-prevention-and-control; http://ecdc.europa.eu/en/publications/Publications/Candida-in-healthcare-settings_19-Dec-2016.pdf). Each of these publications provides guidance to both clinicians and clinical laboratories on how to identify and report C. auris as well as infection control practices and environmental cleaning. The CDC recommendations are updated on a continuing basis and can be found here: https://www.cdc.gov/fungal/diseases/candidiasis/recommendations.html. Aside from what has been outlined above, the CDC recommends that patients who are infected or colonized by C. auris be placed under contact precautions to prevent the spread to other patients and to the hospital environment. This has never been a recommendation for any other Candida species. In addition, there is now a recommendation for daily and terminal cleaning with an EPA-registered hospital-grade disinfectant effective against Clostridium difficile spores. C. auris is not a nationally notifiable disease. However, CDC encourages all U.S. laboratory staff who identify C. auris strains to notify their state or local public health authorities and CDC at candidaauris.cdc.gov.

Summary

Candida auris is the fungal equivalent of the new kid on the block. Our knowledge of this emerging organism is at the point where what we know about C. auris is less than what we do not know about C. auris. There are many important questions that remain to be answered. Why has it suddenly emerged? What was its prior natural niche? Will it continue to spread across the world? Can we decolonize people once they are colonized? With the assistance from clinical microbiology laboratories in the US and throughout the world, public health and laboratories, infection control specialists, and clinicians can begin to answer these questions. The first step is awareness of the problem at hand and it is hoped that this short review helped further your awareness and understanding of the emerging threat that is C. auris.

References


Figure 1.
Neighbor-joining dendrogram of whole genome sequences of *Candida auris*. Each color represents a unique country. The isolates fall into four very distinct clusters that differ widely from each other but are internally very clonal.