Antimicrobial Resistance in Perspective: Using New Technology To Help Identify Resistant Fungal Pathogens

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Although the federal COVID-19 Public Health Emergency has ended, the long-term impact on healthcare cannot be determined, yet. One area of major public health concern is antimicrobial resistance. Opportunistic infections were common in critically ill COVID-19 patients who often required long-term invasive devices (eg, endotracheal intubation, central lines, urinary catheters, etc) and the use of immunomodulatory agents for the treatment of severe disease (eg, systemic corticosteroids, baricitinib, tocilizumab [Actemra, Genentech], etc).[1,2] This scenario led to the widespread use of broad-spectrum antimicrobial agents globally. Overcrowded medical facilities and shortages of personal protective equipment due to supply chain issues led to widespread ineffective infection control practices.[2] These factors lead to an increase in rates of many antimicrobial resistance threats—both bacterial and fungal.[2]

Antimicrobial resistance is a major concern in medical mycology where antifungal resistance is being seen in both yeasts (*Candida* species) and molds (*Aspergillus* and *Trichophyton* species).[3] This is due to a combination of factors, such as increased use of broad-spectrum antifungals in hospitalized patients, overuse of topical antifungal ointments/creams, and environment exposure to antifungals due to agricultural practices.[1,2] This article focuses on the diagnostic and treatment options available for emerging antimicrobial-resistant fungal pathogens, namely antifungal-resistant *Candida* species, including *Candida auris*, azole-resistant *Aspergillus* species, and antimicrobial-resistant dermatophytes.[3]

Candida species are a particularly concerning antimicrobial resistance threat. The CDC lists antifungal-resistant *Candida* species as a serious threat and antifungal-resistant *C. auris* as an urgent threat.[1]

During 2020, there was a significant increase in cases of antifungal-resistant *Candida* species reported by the CDC; an estimated 28,100 cases resulted in 14,000 deaths, a 12% overall and 26% hospital-onset increase from 2019.[1] *C. auris* cases have risen exponentially from 328 reported cases in 2018 to 2,377 clinical cases and 5,754 screening cases reported to the CDC in 2022.[4,5]

Antifungal-resistant Fungi: Diagnostic Dilemma

Clinical mycology is a challenging field, as most fungal isolates from nonsterile body sites are often representative of colonization or contamination rather than true pathogenicity. Yeasts are a part of the human microbiome and considered part of the normal flora, especially in the gastrointestinal and genitourinary tracts. Yeast can also be found colonizing the upper respiratory tract but rarely is associated with pneumonia, except for *Cryptococcus* species. Molds are not as commonly associated with the human microbial flora; however, they are ubiquitous in nature and can find their way into clinical specimens in a variety of ways. Fungal spores can be inhaled leading to growth in respiratory cultures. They can also land on specimens during collection or on agar plates during media inoculation. Given this characteristic, susceptibility testing is not routinely performed on fungal isolates from nonsterile sources, as they often represent benign colonization or contamination of the specimen. Susceptibility testing on fungi can be challenging and is often only performed at reference labs and large academic centers with expertise in this area. These factors can make detecting antifungal-resistant fungi challenging.

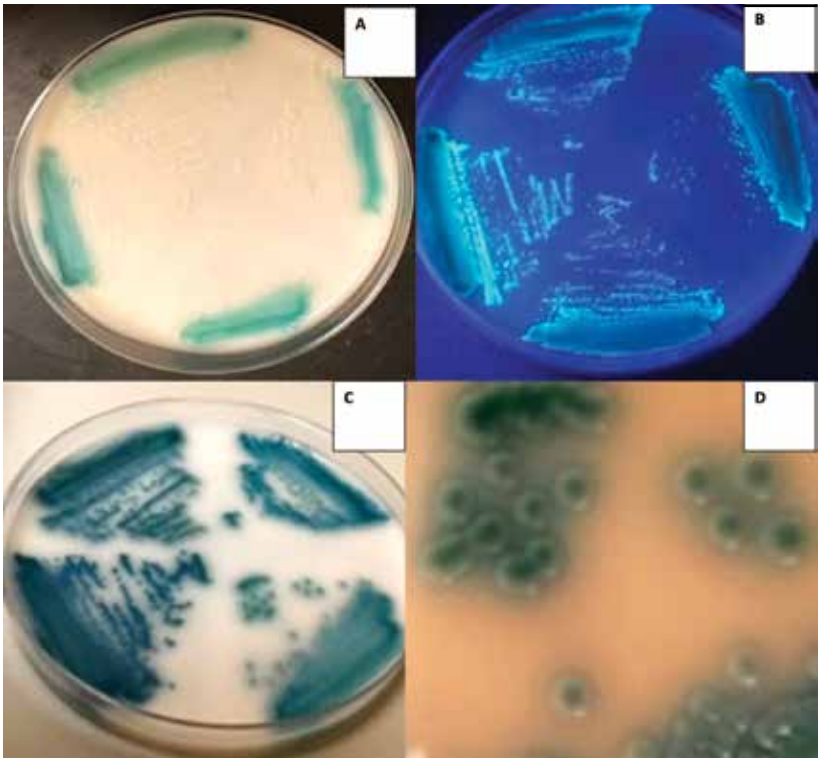

Figure. HardyCHROM Candida+auris. *Candida auris* colonies appear white (light teal in areas of heavy inoculation) at 24 hours under aerobic incubation at 35°C (A). Illumination of these colonies with a 365-nm UV lamp in a dark-room results in light greenish-white fluorescence (B). At 48 hours, *C. auris* appears dark teal in areas of heavy inoculation (C) with isolated white colonies containing a central teal coloration, giving a "bull's-eye" appearance (D).

Although most laboratories can identify the majority of clinically encountered yeasts, *C. auris* can be difficult to identify routinely in many testing facilities. Morphological characteristics and biochemical testing alone cannot be used to identify *C. auris*. Molecular assays and matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF) with an updated reference library are the only reliable definitive testing of clinical isolates currently available.[6] In some circumstances, sequencing can be used for confirmation testing of presumptive isolates.[7] Whole-genome sequencing (WGS) has the added benefit of allowing phylogenetic evaluation of the isolate which may aid in infection control practices.[7]

Invasive infections from *Candida* species are associated with significant morbidity and mortality. In patients with candidemia, rapid and accurate detection of *Candida* species associated with antifungal resistance is of utmost importance. The Biofire FilmArray BCID2 (Blood Culture Identification 2) panel and the GenMark Dx ePlex BCID-FP panel are FDA-approved molecular microarray panels used widely in clinical labs.[6] These panels can rapidly and accurately detect commonly resistant yeasts, such as *C. glabrata*, *C. krusei* and, most importantly, *C. auris* (Table 1). Although no resistance markers are provided by these microarray panels, species level identification is important, as the known resistance patterns of certain species can be applied to treatment decisions (Table 2). This can allow broadening or narrowing of antifungal therapy based on pathogen identification.

The Bruker MBT Sepsityper Kit allows for processing of blood culture bottles to create a microorganism pellet, which can be analyzed by MALDI-TOF for rapid identification.[8,9] The two commercially available MALDI-TOF instruments are the Bruker MALDI Biotyper (MBT) and BioMérieux VITEK MS. Accuracy for this method depends on the quality of protein extraction and robustness of the instrument's reference library. Of note, these tests can identify *Candida* species associated with resistance but do not detect resistance genes or provide antimicrobial susceptibility data. Culture and susceptibility testing are still required.

As cases of *C. auris* have risen in the United States, so has the use of colonization screening in high-risk patients. *C. auris* differs from other yeast species, as it commonly colonizes the skin and mucocutaneous sites of individuals. Due to this phenomenon, swabs collected from the bilateral axilla and groin are the preferred specimen for screening.[10] Although patients are often asymptomatic, colonization with *C. auris* predisposes individuals to superficial cutaneous infections and invasive disease.[11] There are several

Table 1. Fungal Targets on Blood Culture Identification Multiplex Panels

Multiplex BCID Panel	Targets
GenMark Dx ePlex BCID-FP Panel	*Candida abicans*
	C. auris
	C. dubliniensis
	C. famata
	C. glabrata
	C. guilliermondii
	C. kefyr
	C. krusei
	C. lusitaniae
	C. parapsilosis
	C. tropicalis
	Cryptococcus gattii
	Cryptococcus neoformans
	Fusarium
	Rhodotorula
GenMark Dx ePlex BCID-GP Panel	Pan *Candida*
GenMark Dx ePlex BCID-GN Panel	Pan *Candida*
Biofire FilmArray BCID2 Panel	*C. albicans*
	C. auris
	C. glabrata
	C. krusei
	C. parapsilosis
	C. tropicalis
	Cryptococcus (*C. neoformans*/*C. gatti*)

BCID, blood culture identification; **GN,** gram-negative; **GP,** gram-positive.

Table 2. Fungal Intrinsic Resistance

Organism	Intrinsic resistance to:
Yeasts	
Candida krusei	Fluconazole
Cryptococcus species	Echinocandins
Rhodotorula species	Echinocandins and fluconazole
Trichosporon species	Echinocandins
Molds	
Aspergillus species	Fluconazole
Lomentospora prolificans	Amphotericin B
Mucorales	Fluconazole
Purpureocillium lilacinum	Amphotericin B

Organism	Asscociated with high rates of acquired resistance to:
Yeasts	
Candida glabrata	Fluconazole
C. lusitaniae	Amphotericin B
C. auris	Azoles, echinocandins, amphotericin B, flucytosine
C. haemulonii complex[a]	Fluconazole, amphotericin B
Molds	
Aspergillus fumigatus	Triazoles
A. flavus	Triazoles
Trichophyton species	Terbinafine

[a] *Candida haemulonii* complex includes *C. haemulonii*, *C. duobushaemulonii*, C. *pseudohaemulonii*, and *C. vulturna*

Adapted from Table 8.104-5 (*Clinical Microbiology Procedures Handbook,* 4th ed).[30]

options for screening. Molecular testing provides accurate testing results with a quick turnaround time. There are several commercially available reagent kits. However, these are not cleared by the FDA and would need to be created as a laboratory-developed test requiring a comprehensive validation study.[6] Chromogenic agars are available, such as CHROMagar Candida Plus (ChroMagar) and HardyCHROM Candida+auris (Hardy Diagnostics), that are selective and differentiate media for the presumptive identification of *C. auris* and other *Candida* species (Figure).[12,13] The added benefit of chromogenic agar over molecular assays is the ability to identify other potentially antimicrobial-resistant *Candida* species. The downside is the need for prolonged incubation time (48-72 hours) until a final negative result can be determined, as well as the need for confirmatory testing, such as MALDI-TOF.

Laboratory diagnosis of molds still relies heavily on morphological characteristics, especially observation of fruiting bodies. This is a process that can take several weeks depending on the growth characteristics of the organism. The use of MALDI-TOF for identification of filamentous molds can significantly decrease identification time and often provide more detailed information, such as species-level determination, over morphological characterization alone.[14] Diagnosis of invasive mold infections can be challenging, as molds do not often undergo sporulation in blood or infected tissues. *Fusarium* species is an exception, as it undergoes adventitious sporulation. The GenMark Dx ePlex BCID-FP panel contains a target for *Fusarium*, which can provide a rapid diagnosis for disseminated *Fusarium* infections. Outside of *Fusarium*, blood culture collections are low yield for detection of invasive fungal infections. Detection of fungal-specific antigens in blood and body fluids can be helpful in certain clinical scenarios (Table 3).

There has been an increase in the use of metagenomic studies to identify invasive mold infections in at-risk patients.[15,16] Cost, turnaround time, and specificity issues limit the widespread use of these metagenomic studies. Detection of resistance in mold isolates usually is determined from susceptibility testing of invasive isolates or recurrent treatment-resistant infections. The application

Table 3. Examples of assays that detect fungal antigens.

Assay	Antigen	Specimen Source	Diagnostic Aide In:
Fungitell assay (Associates of Cape Cod, Inc., E. Falmouth, MA)	(1->3)-β-D-Glucan	Serum	• Invasive Candidiasis and Aspergillosis • *Pneumocystis jirovecii*
Clarus *Aspergillus* Galactomannan EIA (IMMY, Inc., Norman, OK)	*Aspergillus* galactomannan	Serum, bronchoalveolar lavage (BAL)	Invasive aspergillosis
CrAg LFA (IMMY, Inc., Norman, OK)	Glucuronoxylomannan (GXM)	Serum, CSF	Cryptococcal meningitis
Histoplasma GM EIA (IMMY, Inc., Norman, OK)	*Histoplasma* galactomannan	Urine	Histoplasmosis

of targeted polymerase chain reaction from formalin-fixed paraffin-embedded tissue may be useful for rapid identification of invasive mold infections if tissue sample is obtainable.[17] Common resistance mechanisms can also be targeted with this technique.[17]

Resistant Mechanisms in Fungal Pathogens: Evolving Threat

Multidrug-resistant isolates of *C. auris* have been identified throughout the world.[5,18] These strains are difficult to treat, as they can be resistant to all 3 major classes of antifungal therapy commonly used for systemic treatment: azoles, echinocandins, and polyenes (amphotericin B). Some pan-resistant strains are also resistant to the antimetabolites class (flucytosine).[19] There are 4 major clades of *C. auris*, each with varying resistance patterns.[19] Azole resistance appears to be associated with mutations in the *ERG11* and *CDR1* genes, while echinocandin resistance is seen in mutations of the *FKS* gene.[19] Mutations in the *ERG11* gene, part of the cytochrome P450 family, cause alterations in the structure of lanosterol 14alpha-demethylase, resulting in decreased azolebinding affinity for the enzyme.[19] Other mutations associated with azole resistance include *hapE*, *hmg1*, and multiple *mdr* genes.[18,19] *ERG* gene mutations also result in cross-resistance to amphotericin B due to decreased production of ergosterol.[18,19] Decreased levels of ergosterol lead to ineffective amphotericin B binding across the cell wall, resulting in reduced pore (ion-channel) formation.[18] Overexpression of efflux pumps is associated with mutations in the *CDR* gene.[18] *FSK* gene mutations result in structural changes to 1,3-beta-D-glucan synthase, resulting in reduced enzymatic binding toward echinocandins.[18] Mutations in the *FUR1*, *FCY1*, and *ADE17* genes are seen in isolates with resistance to flucytosine.[19] These genes are key in the conversion of flucytosine into 5-fluorouracil, the active antimetabolite.[18,20] Similar mutations described in *C. auris* are seen in other antimicrobial-resistant *Candida* species.

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The azole drug class is the front-line treatment option for aspergillosis. Azole resistance has emerged, mainly due to mutations in the *cyp51A* gene of molds, the corresponding gene to *ERG11* in yeast.[17,21-23] Non-*cyp51A* mutations resulting in azole resistance have also been described.[24] *Aspergillus fumigatus* and *A. flavus* are the 2 species where azole resistance is most common.[24] Rare reports of azole resistance have been documented in *A. terreus, A. niger*, and *A. tubingensis*.[23]

Susceptibility testing on dermatophytes is rarely performed; therefore, clinical data are scarce and no Clinical & Laboratory Standards Institute clinical breakpoints are available for interpretation. Reports of potentially terbinafine-resistant strains have been reported in *Trichophyton* species.[25] Elevated minimum inhibitory concentration values compared with established epidemiological cutoff values have been observed in treatment-refractory patients—a concerning finding for resistance.[25] Mutations in the *SQLE* gene have been observed in these isolates. The *SQLE* gene encodes for squalene epoxidase, the enzyme target for terbinafine's antifungal activity.[25]

Novel Antifungal Agents; A Brief Introduction

Several novel antifungal agents have been developed and may be useful in the treatment of highly resistant fungi. Ibrexafungerp (Brexafemme, Scynexis) is a triterpenoid with FDA approval for treatment of vulvovaginal candidiasis in adult and post-menarchal pediatric females. It is the first orally available 1,3-beta-D-glucan synthase inhibitor and has activity against most *Candida* species including *C. auris*.[26,27] Rezafungin (Rezzayo, Melinta Therapeutics) is a novel echinocandin with recent FDA approval to treat candidemia and invasive candidiasis in adult patients with limited treatment alternatives.[26,28] Fosmanogepix (Pfizer) is a first-in-class broad-spectrum antifungal agent in clinical trials with the potential to treat highly resistant invasive fungal pathogens.[29] It is the first glycosylphosphatidylinositol inhibitor and functions by disrupting Gwt1 enzyme function.[26] Olorofim (F2G) is another first-in-class antifungal agent currently in clinical trials. It has gained FDA Orphan Drug status and Breakthrough Therapy designation for invasive mold infections and central nervous system coccidioidomycosis when treatment options are limited.[26] It belongs to the novel orotomides class and is an inhibitor of dihydroorotate dehydrogenase (pyrimidine synthesis).[26]

Conclusion

The rise in antimicrobial-resistant fungal pathogens, especially *C. auris*, is a concerning global issue. Rapid and accurate identification of these organisms is paramount for successful treatment and infection control practice. The use of molecular assays and MALDI-TOF has supplanted morphological and biochemical testing as the quickest, most accurate process of identifying clinically significant fungi. Several novel antifungal agents may provide effective treatment options in the future for invasive infections from highly resistant yeast and molds.

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