The Impact of Contemporary Emerging Resistant Fungal Infections in Vulnerable Populations and Methods for Treatment

Medical illustration of *Aspergillus Fumigatus*
Source: https://www.cdc.gov/fungal/antifungal-resistance.html

May 7, 2024
Temple University College of Engineering
ENGR 2996 Honors Technical Communication

Hafsah Ahmad

Abstract

Over the past decade emerging resistant fungal infections have become apparent as the next healthcare threat. These infections are opportunistic and affect immunocompromised or hospitalized populations and with the development of modern medicine the rates of these populations are rising. There are only four classes of antifungals: azoles, polyenes, antimetabolites, and echinocandins. The concern is with multidrug resistant fungi's the mortality rate for these infections has been increasing with a large surge seen during the Covid pandemic. The Candida and Aspergillus species of fungus are considered the biggest threats as they have multidrug resistant strains, and they can cause lethal complications. Resistance is caused by overuse of the antifungals and selective pressure causing the fungi to develop genetic mutations to avoid the drug's therapeutic effects. To tackle this problem new treatments or intervention methods must be introduced. It has been difficult to produce new treatments as fungi are very similar to humans and pharmaceutical companies are struggling in finding unique pathway or targets that won't be toxic to humans. However, there are some known targets that require more testing before they can be developed into an approved drug.

Keywords: Candida auris, azole resistance, Aspergillus, nosocomial infections, antifungal stewardship programs, amphotericin B resistance, therapeutic index

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Overview of Problem and Significance

Fungi are heterotrophic eukaryotes whose structure can either present as molds, yeasts, or as mushrooms. Molds appear as multicellular fungal protrusions called hyphae while yeast are single-celled organisms. Fungi use hydrolytic enzymes to externally digest their food through a process called absorptive nutrition. The characteristics of fungi that separate them from other kingdoms include synthesizing the amino acid lysine through the L-α-adipic acid pathway while most other organisms, including humans, use the meso-α,ε-diaminopimelic acid pathway. Additionally, fungi contain chitin in their cell wall, have rRNA sized at 80S, have beta tubulin microtubules, and have ergosterol in their plasma membranes. A representative model of a fungi can be seen in Figure 1. The fungus kingdom is extremely diverse and contains a multitude of taxa and species including those pathogenic to humans (McGinnis & Tyring, 1996).

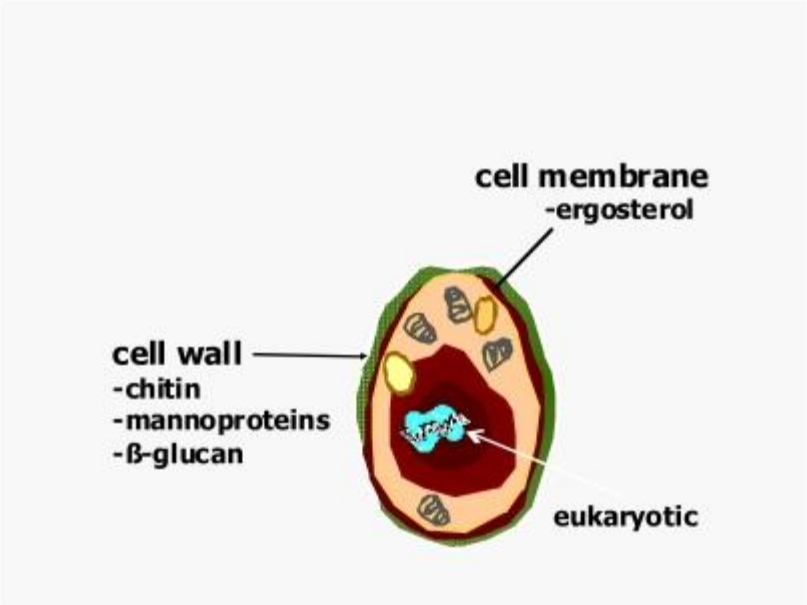

Figure 1. Representative Fungi model

Source: de Pauw, 2011

The majority of fungi are not pathogenic and grow everywhere on earth uninvolved with the mammalian population. However, the few species that are pathogenic to humans have greatly increased their virulency in the past decades. Even among pathogenic fungi most are not virulent enough to infect a host in good health. They are opportunistic and largely infect hosts who are immunocompromised, putting this population at highest risk for infection. Fungal infections

originate either through skin contact from the environment or through ingesting or inhaling fungal spores (de Pauw, 2011).

Emerging fungal infections are the next public health concern. They have largely gone undetected for the majority of modern medicine with public health officials only sounding the alarm in the last four decades. Due to their significant impact on human health bacteria and viruses have a multitude of treatments and public knowledge on preventative tools. Diseases like the black plague, smallpox, influenza, and Coronavirus have shaped human existence while fungal infections have mostly been innocuous or very rare. Fungal infections are in principle opportunistic pathogens and with the exponential growth of modern medicine and the surge of immunocompromised patients they now have a much larger pool of victims. Invasive fungi have a devastating effect on those who live with HIV or AIDS. Fungal infections are among the leading opportunistic infections. Tuberculosis is the leading cause of death in this population, but complications from fungi are the second. Invasive fungi, systemic mycoses, infect 300 million people annually and kill up to 1.5 million globally (Rodrigues & Nosanchuk, 2020).

Fungi are eukaryotes and share many characteristics with human cells, making it very difficult in creating antifungal medications. Pharmaceutical manufacturers must ensure their drugs are toxic only to the fungal cells and not to the similar human cells. Additionally, there have not been many advancements in the creation of antifungal medications. The antifungal drug amphotericin B was discovered in 1955 and seven decades later it is still the first line drug used in treatment. The lack of variability in available antifungal medication is one of the reasons behind the emerging health threat of resistant fungal infections. Among resistant fungal infections, the yeast species Candida auris is an emerging threat as it is resistant to most of the major antifungal drug classes. This fungus lives on the skin and very commonly infects hospitalized patients resulting in large outbreaks (Rodrigues & Nosanchuk, 2020).

In the United States the cost of fungal infections on the healthcare system were over 6.7 billion in 2018 and deaths over 7,000 in 2021. Also, from 2019 to 2021 there were 59,212 hospitalizations with fungal infections and rates of hospitalization had increased 8.5% annually (Gold et al., 2023). The concern for antifungal resistance is largest for the Candida and Aspergillus species. Candida and Aspergillus fungi are both pathogenic and largely resistance to most classes of antifungals. When untreated they have a high mortality rate, especially in immunocompromised groups. To combat this growing health concern more research must be done into novel treatments

and medical professionals should be more educated on the threat their patients face (Vitiello et al., 2023)

How Fungal Infections Affect the Body

Fungal infections can affect different layers of tissue and thus have vastly differing consequences for an individual. Infections can be superficial, subcutaneous, or systemic where severity increases the deeper in the body the fungi infect. Superficial infections are those in the outermost skin, hair, nails, and mucous membranes. Dermatophytes are fungi infecting the epidermis and keratinized tissues, including hair and nails. Dermatophytes are named based on where in the body they infect. Tinea pedis, foot fungus, is one of the most common types of superficial fungal infections (Garber 2001). However, it is better known by its common name, athletes' foot. Superficial fungal infections can easily be treated and diagnosed as they present in physical examinations. With mild benign symptoms, many medical professionals ignore the impact on the patient's life. However, superficial infections may cause patients emotional distress through self-esteem issues or even limit the scope of their abilities. Infections of the mucous membrane include the genitals, mouth, or esophagus. The most common gynecological infection in women is vaginal candidiasis which is caused by the yeast fungus Candida albicans. With fungi being largely opportunistic microbes they are often a complication of immunodeficiencies. Over 90% of people living with HIV develop oropharyngeal candidiasis during their ailment (Garber 2001). This is caused by the same yeast fungus Candida albicans. The current concern is the rising rates of drug resistant strains of Candida yeasts.

Subcutaneous fungal infections infiltrate beyond the epidermis and into the dermis and hypodermis. The origin of infection is usually through lesions in the tissue. Through these trauma sites fungus can enter and disseminate through the lymphatic channels. It can be difficult for healthcare providers to diagnose subcutaneous infections without performing a biopsy of the legion. The most alarming class of fungal infections is those that are systemic. These infections target the organs and can be lethal in immunocompromised patients, who are classified as high-risk. As illustrated in Figure 2, there are a multitude of fungi and fungal diseases that can affect different parts of the body. Due to the nonspecific symptoms of fungal infections, it is difficult for medical professionals to differentiate from bacterial or viral infections. For many patients who die

from fungal infections diagnosis is largely determined postmortem through performing an autopsy (Garber 2001).

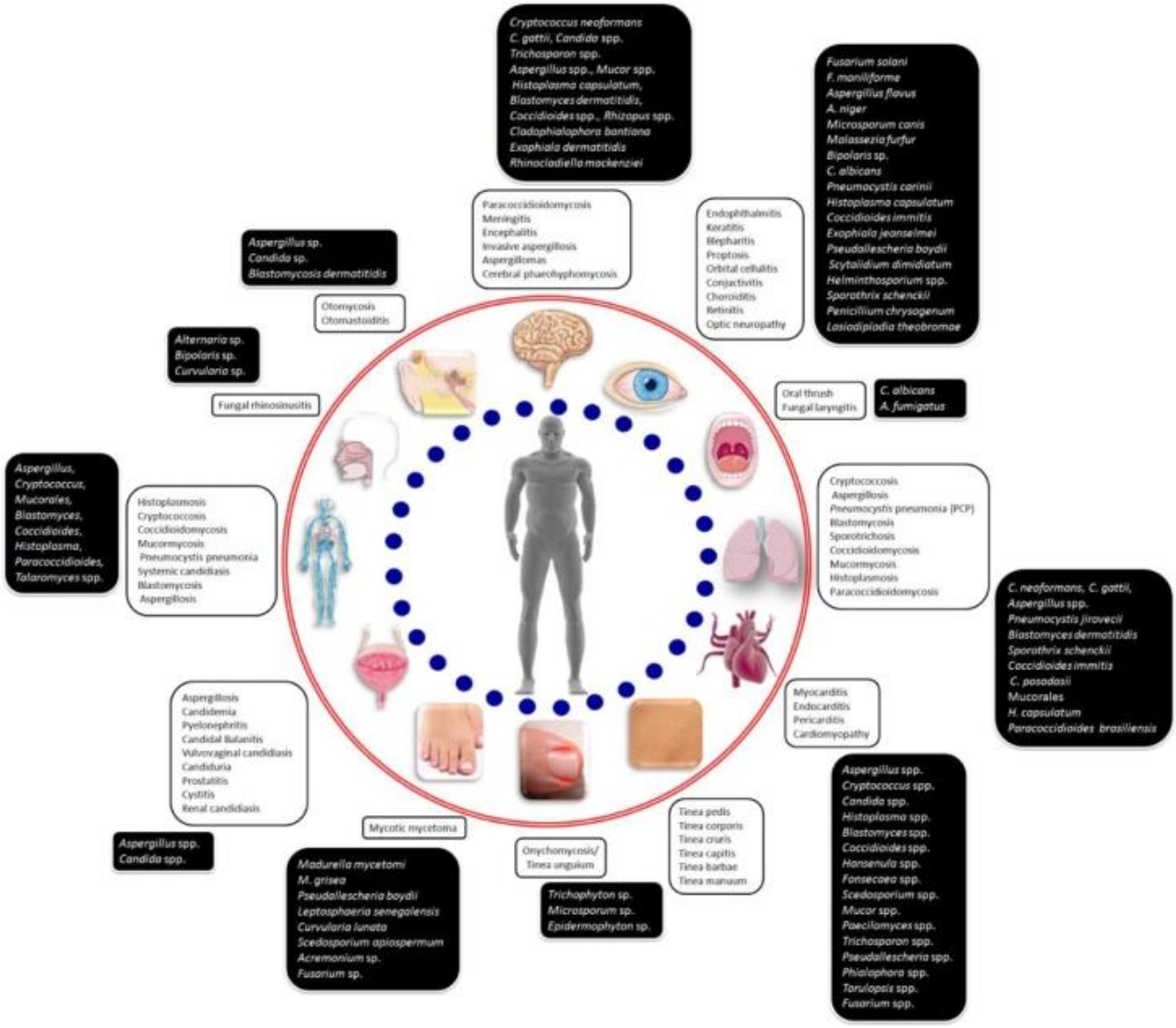

Figure 2. Fungi affecting the different parts of the body

Source: Reddy et al., 2022

Diseases Caused by Fungal Infections

The diseases that are caused by fungal infections can range from benign to severe. Candidiasis is an infection caused from an overgrowth of the yeast fungus Candida. It can grow in the mouth, throat, or vagina. Invasive candidiasis results in a more serious infection in the heart, eyes, blood, or brain. The most common form is Candidemia, a bloodstream infection that

seriously concerns those in a hospital setting. Another widespread disease is cryptococcosis, caused by the cryptococcus fungi. Infections result from breathing in the fungus that is present in soil or bird droppings. It affects the lungs and nervous system. Meningitis caused by the cryptococcal fungus is the predominant cause of death for those living with HIV or AIDS in many parts of Africa (National Institute of Allergy and Infectious Diseases, 2022).

Aspergillosis is an infection affecting the respiratory system caused by the mold Aspergillus. Aspergillus is found everywhere and largely only sickens those with lung issues or immune deficiencies and can have mild to lethal effects. Coccidioidomycosis, otherwise known as Valley Fever, is a disease caused by the Coccidioides fungus which is found in desert soil. Through breathing in the fungus infectious spores can settle in the lungs and cause fever and pneumonia. Histoplasmosis is a similar disease caused by breathing in Histoplasma capsulatum fungus that live in soil with animal droppings. Blastomycosis is caused by inhaling Blastomyces dermatitidis fungus that is in rotting natural material. Finally, pneumocystis pneumonia is caused by the pneumocystis jirovecii fungus that lives in the lungs and spreads through contact. The range of fungal diseases is wide, but overall they tend to have more serious effects on those with compromised immune systems. The variety in fungal diseases can be illustrated in Table 1, alongside the fungus that causes it, the number of people each disease effects, the afflicted region and the genera of fungus involved (National Institute of Allergy and Infectious Diseases, 2022).

Table 1. Human fungal diseases and the related pathogen

Disease	Major / notable pathogens	Region	Burden	Genera involved
Aspergillosis[8]	*A. fumigatus*, *A. flavus*	Worldwide	invasive: >300,000 annually chronic pulmonary: ~3 million global burden allergic bronchopulmonary: ~4.8 million global burden	*Aspergillus*

Blastomycosis[128]	*B. dermatitidis*	Regional (Central and Eastern US)	~3,000 global burden	*Blastomyces*
Candidiasis[129]	*C. albicans*, *C. glabrata*, *C. auris*	Worldwide	Invasive: ~750,000 annually Oral: ~2 million annually Oesophageal: ~1.3 million annually Vulvovaginal: ~134 million global burden	Many genera (*Candida*, *Nakaseomyces*, *Clavispora, Pichia*, *Meyerozyma*)
Coccidioidomycosis / Valley fever[130]	*C. immitis*, *C. posadasii*	Regional (Southwestern US, Central and South America)	~25,000 global burden	*Coccidioides*
Cryptococcosis[131]	*C. neoformans*, *C. gattii*	Worldwide (*C. neoformans*), Worldwide (*C. gattii*: expanding in California and Pacific Northwestern US)	~223,000 annually	*Cryptococcus*
Emergomycosis[132]	*E. pasteurianus*	Regional (Southern Africa)	Tens / hundreds	*Emergomyces*

Fusariosis[133]	*F. solani*, *F. oxysporum*	Regional (South America)	Hundreds	*Fusarium*
Histoplasmosis[13][4]	*H. capsulatum*	Regional (Central and Eastern US, South America, Southern Africa, and Southeastern Asia)	Infections: ~500,000 annually / ~25,000 global burden Disseminated: ~100,000 annually	*Histoplasma*
Microsporidiosis[4][6]	*Entercytozoon bieneusi*, *Encephalitozoon intestinalis*	Worldwide	~10% prevalence[135]	Many genera (*Encephalitozoon*, *Anncaliia*, *Enterocytozoon*, *Microsporidium*)
Mucormycosis[136]	*Rhizopus arrhizus*	Worldwide	>10,000 annually	Many genera (*Mucor*, *Rhizopus*, *Lichtheimia*, *Apophysomyces*, *Rhizomucor*, *Cunninghamella*)
Paracoccidioidomycosis[137]	*Paracoccidioides brasiliensis*	Brazil, Central and South America	~4,000 global burden	*Paracoccidioides*
***Pneumocystis* pneumonia**[98]	*Pneumocystis jirovecii*	Worldwide	~500,000 annually	*Pneumocystis*

Sporotrichosis[65]	*S. brasiliensis*, *S. schenckii*, *S. globosa*	Worldwide, with increased prevalence in Central and South America	>40,000 annually	*Sporothrix*
Talaromycosis[97]	*Talaromyces marneffei*	South and Southeastern Asia	~8,000 annually	*Talaromyces*
Eye infections / fungal keratitis[138]	*Aspergillus* spp., *Fusarium* spp., *Candida* spp.	Worldwide	~1 million global burden	Multiple genera (*Fusarium*, *Aspergillus*, *Candida*)
Skin, hair, nail infections[64]	*Trichophyton rubrum*, *Trichophyton tonsurans*, *Microsporum canis*, *Malassezia globosa*	Worldwide	~1 billion global burden	Multiple genera of dermatophytes (*Trichophyton*, *Arthroderma*, *Microsporum*) and *Malassezia*

Source: Rokas, 2022

Overview of Current Treatments

One of the difficulties in treating fungi is that they are more closely related to human and plant cells than other organisms like bacteria or viruses which have a plethora of treatment options. In fact, fungi are more analogous to humans than to plants. The difficulty in targeting fungal cells in the body is to ensure that similar human cells do not mistakenly get targeted in the process. Traditionally, treatments for fungal diseases have been focused on targeting the characteristics of fungi that make them unique from other kingdoms. One of these targets is in the plasma membrane. One of the major differences between fungal plasma membranes and humans is the presence of the sterol ergosterol instead of cholesterol. Many antifungal agents target ergosterol to

either prevent it from being active in the membrane or to prevent its synthesis. The ergosterol synthesis pathway can be seen in Figure 3. Another class of antifungal agents targets the microtubules in fungal cells as they are made of the protein beta tubulin. Targeting the fungi's unique microtubules can stop the cells from proliferating and spreading in the host (McGinnis & Tyring, 1996).

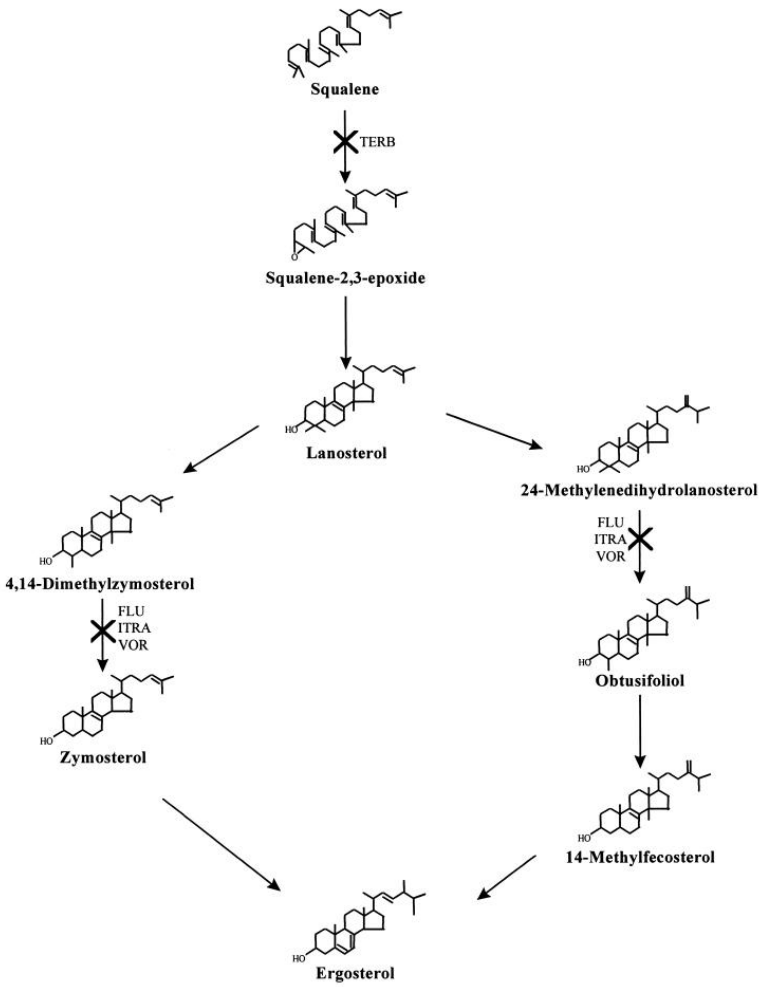

Figure 3. Biosynthetic pathway of the formation of Ergosterol

Source: Ghannoum & Rice, 1999

There are four main classes of antifungal treatment: azoles, polyenes, antimetabolites and echinocandins. Azole based drugs were found in the 1960s. This class of drugs works through targeting the ergosterols in the cell membrane. More specifically they target the heme protein that aids in catalyzing the cytochrome P-450-dependent 14α-demethylation of lanosterol. Through inhibiting this demethylase, the lanosterol cannot be converted to ergosterol.

There is a large reduction of ergosterol in the cell membrane and instead sterol precursors form: 24-methylenedihydrolanosterol, 4,14-dimethylzymosterol, and lanosterol. These sterol precursors modify the structure of the fungal plasma membrane and thus its function resulting in cell death. Examples of azole drugs include Itraconazole, Fluconazole, and Voriconazole (Ghannoum & Rice, 1999).

Polyenes have been the oldest class of antifungals and include amphotericin B. Polyene drugs can be used on any microorganism that has sterols in their plasma membrane. The mechanism for this drug involves the antifungal interacting with sterols in the plasma membrane and producing pores. These pores severely increase the membranes permeability resulting in leakage of the cytoplasmic organelles and eventual cell death. Another class of antifungals is echinocandins. This class of drugs works against the compounds in the cell wall of the fungi that are unique to them. Echinocandins are β-Glucan inhibitors and they obstruct β-(1,3)-glucan synthetase, a major structural protein of the fungal cell wall. This inhibition causes a thicker cell wall, the formation of pseudohyphae, and the inability to reproduce or bud. The last major class of antifungals are those that are antimetabolites. These are drugs that inhibit nucleic acid like DNA or RNA. The major antifungals in this class include 5-Fluorocytosine. The mechanism works by the cytosine deaminase enzyme converting the 5-Fluorocytosine to 5-fluorouracil. Then it is converted to 5-fluorouridylic acid through UMP pyrophosphorylase. 5-fluorouridylic acid is integrated into RNA which interrupts the proteins synthesis process and stopping metabolic activity of the fungus. Additionally, the drug can disrupt DNA synthesis through becoming the inhibitor of thymidylate synthase. A summary of the four classes of antifungals can be seen in Table 2 (Ghannoum & Rice, 1999).

Table 2. Major classes of antifungal drugs

Antifungal Class	**Mechanism of Action**	**Biological Effect**	**Spectrum of Action**
Polyenes	Target ergosterol and extract sterols from fungal cell membranes	Fungicidal	Broad spectrum antifungal in treatment of invasive fungal infections; resistance is rare

Flucytosine	Inhibits DNA and RNA synthesis	Fungicidal against *Cryptococcus* spp.	Almost exclusively used for cryptococcal meningitis, but resistance is extremely common so never used in monotherapy
Azoles	Inhibit 14-α-lanosterol demethylase thereby inhibiting ergosterol synthesis	Mostly fungistatic	As a class they display broad spectrum against yeasts and filamentous fungi, although some species display intrinsic resistance to commonly used derivatives; secondary resistance can often develop during treatment
Echinocandins	Target 1,3-β-d-glucan synthase, thus preventing production of cell wall 1,3-β-d-glucan	Fungicidal against *Candida* spp., but fungistatic against *Aspergillus* spp.	First line of defense for candidiasis and used in aspergillosis when refractory to other treatments; resistance is emerging

Source: Wall & Lopez-Ribot, 2020

Current Detection Methods

With emerging fungal infections, it is important to have current detection methods in place so medical professionals can quickly and accurately provide a diagnosis. This is especially pertinent in hospital settings where delay in detection could cause hospital wide breakouts. Additionally, with many resistant fungal infections having high mortality rates it is important to catch the fungus early. The two factors that are essential in detection tests are being quick and easy to use by professionals. To detect invasive Candida fungus, which usually present as candidemia, a

blood culture test via PCR assay can be performed. This uses a T2Candida nanodiagnostic panel that detects Candida DNA in the blood. It has a sensitivity of 91.1% and specificity of 99.4% with an average result time of 4.2 hours (Sanguinetti et al., 2019). For detecting Aspergillus infections, using a specific lateral-flow device (LFD) is both sensitive and specific enough to get accurate data. The LFD uses a monoclonal antibody through an immunochromatographic test to detect the glycoprotein mannoprotein's antigen that is present in the extracellular matrix of Aspergillus fungi. Additionally, there is a proximity ligation assay that can be used for diagnosis of invasive aspergillus. This test is performed through an immune-PCR assay to also test for mannoprotein. When testing for Cryptococcus a lateral flow assay for the cryptococcal antigen can be performed on body fluids. This test is very rapid, affordable, specific, and sensitive. Finally, detecting Histoplasma capsulatum fungus in samples can be performed through loop-mediated isothermal amplification (LAMP) using urine samples. It can detect fungal DNA and has shown to be quicker and more cost effective than other PCR tests. A summary of the different non culture diagnostic tests and its associated disease is illustrated in Table 3 (Sanguinetti et al., 2019).

Table 3. Nonculture laboratory diagnostic tests

Disease	**Test**	**Comments**
Candidaemia/deep-seated candidaemia	T2 *Candida* panel.	Detects *Candida* DNA directly in whole blood. Detection limit of 1 cfu/mL. Greater than 10-fold decrease in reporting time.
Pulmonary aspergillosis	Immunochromatographic technology – lateral flow devices/dip sticks.	POC or near-POC test for detecting cell wall components in serum, BAL and urine. Impact of antifungal prophylaxis on sensitivity not known.
	Aspergillus proximity ligation assay.	Detects cell wall mannoprotein.
Cryptococcosis	Various lateral flow formats.	Designed to detect cryptococcal polysaccharide antigen in CSF,

		whole blood and saliva. Adopted by WHO on their Essential Diagnostics List.
Histoplasmosis	Various ELISA platforms, lateral flow devices, and a loop-mediated isothermal amplification assay.	Rapid, lateral flow devices are suitable for POC testing, urine is the preferred clinical sample.

Source: Sanguinetti et al., 2019

Epidemiology

The epidemiological study of fungal infections investigates fungal outbreaks and observes trends. The most recent epidemiologic trends and the associated fungal pathogen can be seen in Table 4. Trends have shown a drastic increase in novel resistant infections. It is estimated that annually over 300 million people are afflicted with an acute fungal infection and 1.5 million people die due to these infections (Seagle et al., 2021). Candida infections in the bloodstream, candidemia, are the most frequent form of Candida infections which as a genus are one of the most widespread healthcare infections. It has the highest occurrence among senior populations. Other fungal epidemiologic trends can be seen in Table 2 (Seagle et al., 2021).

Table 4. Recent fungal epidemiologic trends and the associated pathogen

Fungal Pathogen	Top Epidemiologic Trends
Candida	Injection drug use has emerged as a risk factor and is becoming more common in the context of the opioid epidemic. Although *C albicans* remains the leading cause of invasive candidiasis, a growing proportion of diagnoses have been attributed *C glabrata* and *C parapsilosis*. *C auris*, a multidrug-resistant organism shown to cause outbreaks in clinical settings, has rapidly emerged since first identified in 2009.

Mold (*Aspergillus* and non-*Aspergillus*)	Influenza-associated pulmonary aspergillosis and coronavirus disease 2019–associated pulmonary aspergillosis are emerging coinfections. Azole-resistant strains of *A fumigatus* are increasing in prevalence. Reports point to a potential increase in non-*Aspergillus* invasive mold infections among patients with hematologic malignancies and transplant recipients.
Blastomyces	The US annual incidence remains consistent at 1–2 cases per 100,000 population. Recent cases have been reported in areas not known to be endemic.
Coccidioides	US cases have increased in recent years, reaching >15,000 in 2018. Recently reported cases beyond the traditional geographic areas indicate a northward expansion into Northern California, Utah, and Washington.
Histoplasma	Evidence suggests that the occurrence of histoplasmosis may extend beyond the already broadly defined historical region. Histoplasmosis-associated hospitalizations nearly doubled from 2001 to 2012.
Cryptococcus	As the rate of disease among people living with HIV has decreased, the rate among those living without HIV has not. Approximately 20% of non-HIV patients who develop *Cryptococcus* disease have no known immune impairments. Understanding of *C gattii's* geographic distribution expanded during an outbreak in Canada and a recent uptick in cases in the US Pacific Northwest.
Pneumocystis jirovecii	Among people living with HIV in the United States and Canada, *Pneumocystis* pneumonia is the most common opportunistic infection. Populations at risk have shifted toward HIV-uninfected

	immunocompromised groups owing to immunosuppressive regimens that weaken the immune system.
Sporothrix	The US incidence is estimated to be 2 cases per 1 million people, with the highest incidence in southern and south-central states. *S brasiliensis* recently emerged in South America and is associated with zoonotic transmission, spreading via animal scratches and bites.

Source: Seagle et al., 2021

Emerging Antifungal Resistance

The mechanisms for antifungal resistance to current antifungal drugs can occur due to a combination of many factors. However, it is the adaptive nature of fungi that allows them to become resistant. This is as they can quickly mutate and change their physiology. Fungi exposed to concentrations of antifungals that are below the lethal dose have shown to develop resistance. This is as natural selection selects for fungi that have survived the treatment and the inherent resistance in these fungi will proliferate onwards. Fungal resistance to the major classes of antifungals was presented as early as 1983 where Azole resistance was discovered for the Candida albicans species. Some different mechanisms for antifungal resistance are illustrated in Figure 4. One of the methods of resistance is from fungal biofilms forming on the human tissue or on medical implants; these biofilms have a higher resistance to antifungal drugs. Biofilms of fungus are a network of fungal cells that make a protective extracellular matrix. The extracellular matrix prevents the antifungal drugs from reaching the fungus. Another mechanism is mutations in the resistant fungus's genes where the target for the drugs is located. Through single nucleotide polymorphisms and the resultant amino acid change, the fungus is less likely to be targeted by antifungal medications (Hokken et al., 2019).

Another mechanism for resistance is formed through the overuse of azole drugs. In response to these drugs, fungi have become resistant through a variety of mutations, but the most common is a mutation in the cyp51A gene. A non-synonymous point mutation occurs in this gene resulting in a change in the shape of the channel that the azole drug binds to in order to get access to the heme molecule. With the azole drug no longer having access to the protein, it cannot inhibit the formation of ergosterol and destroy the fungi's cell membrane (Meis et al., 2016). Polyene

antifungals have a lesser level of resistance. However, resistance to this drug forms through mutations in the fungi decreasing the ergosterol in the plasma membrane and lowering the ability for the antifungals to target them (Reddy et al., 2022).

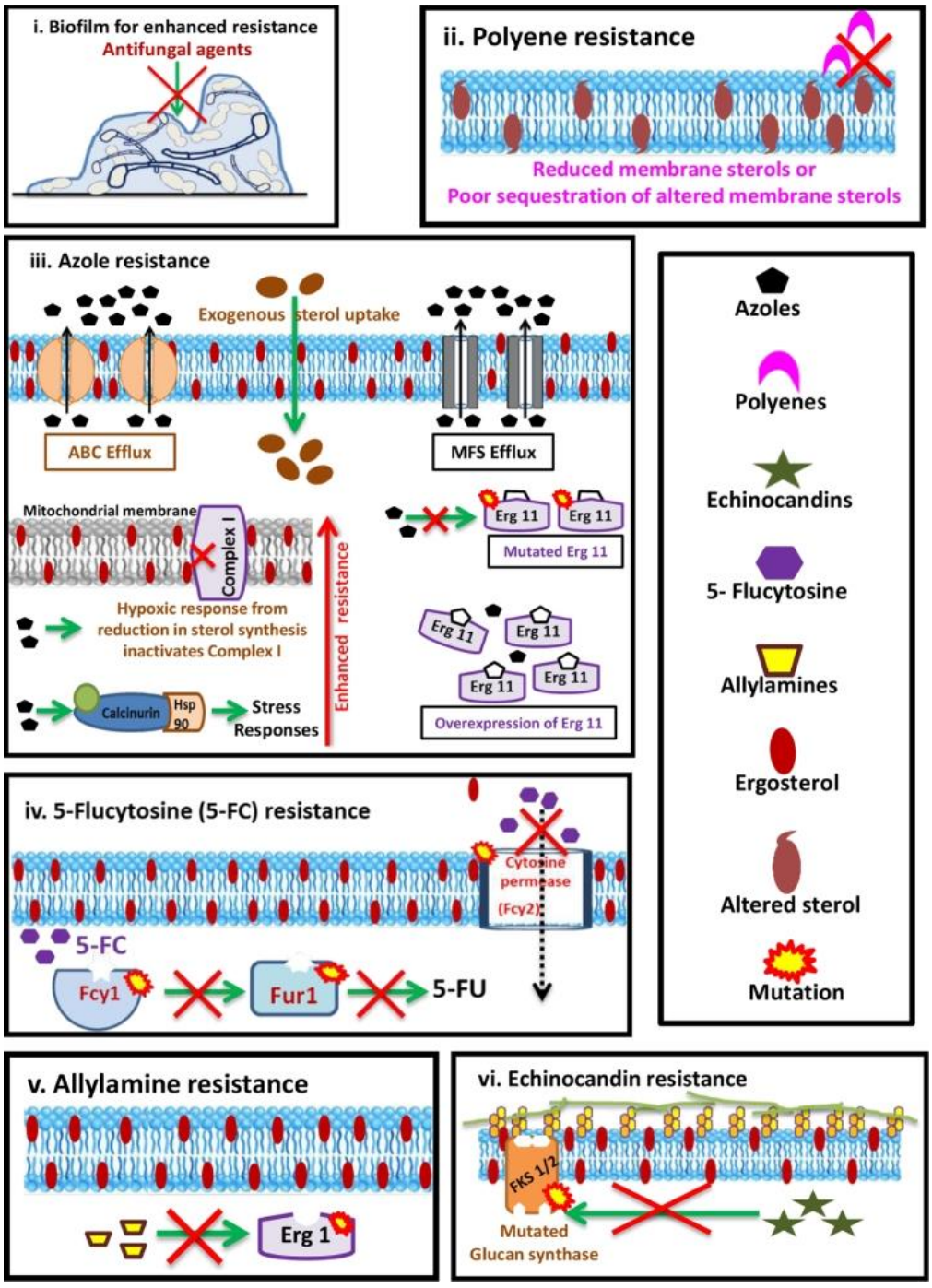

Figure 4. Mechanisms for antifungal resistance

Source: Reddy et al., 2022

Resistance to the class of echinocandin antifungals is still low, with resistance being less than 3% of most of the Candida family. However, the concern with echinocandin resistance is that 36% of the resistant fungi are also resistant to the azole class of drugs (Perlin, 2015). Multidrug resistance is very concerning as there are already a limited number of treatments. Echinocandin resistance happens through mutations in the fungi's Fks glucan synthase subunits. An amino acid substitution that can occur in two specific spots on the FKS1 gene can decrease the sensitivity of the resultant enzyme to the antifungal. This resistance could occur through the overuse of echinocandin drugs (Perlin, 2015).

Antibiotic Resistance

When comparing antifungal resistance to antibiotic resistance the latter is recognized as a much bigger threat. According to the CDC in regards to bacterial infections that are resistant to antibiotics there are over 2.8 million cases in the United States and 35,000 deaths per year (Uddin et al., 2021). The most resistant bacteria of concern are E. coli, S. aureus, S. pneumoniae, and K. pneumoniae. Bacteria have developed antibiotic resistance because of the overuse of prescription by medical professionals, use in agriculture, lack of personal hygiene, or from self-medication of over-the-counter drugs. Due to the prevalence of bacteria worldwide resistance to new antibiotics is inevitable and has been shown to occur on average five years after first use (Uddin et al., 2021). Due to the unavoidable resistance many large pharmaceutical companies find the creation of new antibiotics to be not profitable and thus the majority of antibiotics are produced by smaller laboratories or companies. The last discovery of an antibiotic class was in 1987 and instead many are looking towards antibiotic alternatives like bacteriophages. However, it is still important to address bacterial diseases and allocate more resources towards research (Uddin et al., 2021).

Contemporary Resistant Fungal Infections

Of the genera of fungi that are pathogenic, the most relevant are among the Candida, Aspergillus, Cryptococcus, and Pneumocystis species. These fungi largely cause invasive fungal infections that are devastating to immune compromised populations. Resistant fungal infections include those resistant to azole antifungals: Candida tropicalis, Aspergillus fumigatus, and Candida parapsilosis. More cornering are pathogenic fungi resistant to multiple drug which include Candida auris and Candida glabrata. When specifically looking at the Candida genus, five species make up

over 90% of cases: C. glabrata, C. parapsilosis, C. albicans, C. tropicalis, and C. krusei. As seen in Table 5, Candida albicans is the most common species followed by Candida glabrata (Arastehfar et al., 2020).

In 2022 the World Health Organization (WHO) released a fungal priority pathogens list, the first of its kind. It listed the top 19 fungal pathogens to help guide research. The most critical pathogens were Cryptococcus neoformans, Candida auris, Aspergillus fumigatus, and Candida albicans. Cryptococcus neoformans has been listed at such a high priority as it has high mortality rates even after treatment. Cryptococcus disease caused by this fungus has mortality rates of 41% to 61% in populations with HIV (World Health Organization, 2022). There are concerns of growing resistance to fluconazole, the current antifungal treatment. For Candida auris it is resistant to all current antifungal classes. It can cause invasive candidiasis and has a mortality rate of 25% to 53% (World Health Organization, 2022). Candida auris shows the highest resistance to azoles then polyenes and the lowest resistance to echinocandins. However, this fungus is very concerning due to its high outbreak potential. Candida auris is extremely difficult to detect and hard to prevent against. Aspergillus fumigatus is a mold found in the environment that can cause invasive aspergillosis. It has mortality rates of 47% to 88% (World Health Organization, 2022). This fungus is resistant to azoles. Finally, the last critical fungi on the WHO's priority list is Candida albicans. This fungus can cause invasive candidiasis which has a mortality rate of 20% to 50%. Resistance for this fungus to echinocandins and azoles has been growing over the last ten years (World Health Organization, 2022).

Table 5. Resistance rates of Candida and Aspergillus fungi

Species	Prevalence (%)	Azole Resistance Rate (%)	Echinocandin Resistance Rate (%)
C. albicans	>20.9–70	0–7.8	0–7
C. glabrata	<15	0–21	0–23.1
	15–38	0–76	0–100
C. tropicalis	<10	0.6–31.5	0–8

Species	Prevalence (%)	Azole Resistance Rate (%)	Echinocandin Resistance Rate (%)
	10–49	0–66.6	0–10
C. parapsilosis	<15	0–8.3	0–10.8
	15–37	0–53	0–1.6
C. auris	0–14	>90–100	0–40
A. fumigatus	33.2–92	<2–30	0

Source: Arastehfar et al., 2020

Covid -19's Impact

During the COVID-19 pandemic, hospitals faced many challenges in the treatment of patients. There is a drastic lack of data from testing and diagnosis of microbial diseases from this time. In fact, there was a 23% decrease in CDC testing from previous years (National Center for Emerging and Zoonotic Infectious Diseases, 2022). Many patients were never diagnosed or treated for fungal infections as hospitals barred patients with milder symptoms. However, the available data shows significant increases in resistant fungal infections that occurred during hospitalization, above all those with COVID-19. From 2019 to 2020 there was a staggering overall 60% increase in resistant Candida auris infections (National Center for Emerging and Zoonotic Infectious Diseases, 2022). Due to decreased testing this number is a conservative estimate, but the danger of this fungus is that it is becoming resistant to the major classes of antifungals. Candida auris has long since been a threat for those in long term care, but the pandemic saw an increase in infection of those in acute care. The CDC names this fungus as an urgent threat of antimicrobial resistance and is encouraging hospitals to continue to surveil and enact stronger infection prevention and control protocols.

The increase of fungal infections during the COVID pandemic could be due to supply shortages of personal protective equipment, an increase in ill patients, or a shortage in staff. Another possible increase in healthcare-associated infections could have been the frequent use of ventilators or catheters. While the specific Candida auris fungus is a major concern, the entire

species as a whole saw a significant increase over the pandemic. The resistance of the Candida species is extremely concerning as they can commonly cause lethal blood infections and are difficult to test for resistance. The number of these cases were on a decline before the pandemic, but they saw a 26% increase from 2019 to 2020 in cases of those in the hospital. Additionally, a 14% increase of Vancomycin resistant Enterococcus was seen (National Center for Emerging and Zoonotic Infectious Diseases, 2022).

Why Resistance is Currently on the Rise

The recent increase in fungal infections should come as a shock to no one. With the advancement of modern medicine and a much more widespread use of immunosuppressants in the overall population it has created the perfect breeding ground for pathogenic fungi. Fungi, being mainly opportunistic pathogens, target hosts who have weakened immune systems. In addition, the overuse of antibiotics has also aided invasive fungal infections by giving them a less competitive environment to live in. Furthermore, until recently fungal infections were not recognized as a serious health threat. As seen in Table 6, there was very little research and limited production of antifungals as compared to antibiotics (McCormick & Ghannoum, 2024).

Table 6. History of antifungal development

Decade	Tradename	Generic name	Approval Date	Manufacturer's Name
1950s	Nystatin	mycostatin	Discovery (1950) FDA approval date (1971)	Division of Laboratories and Research; New York State Department of Health
	Amphotericin B	Amphotericin B	Discovery Date (1955)	Squibb Institute for Medical Research
	Ancobon	5-fluorocytosine	Discovery (1957) FDA	Roche

			approval (1971)	
1960s	Miconazole	imidazole	Discovery (1969) FDA approval (1974)	Insight Pharmaceuticals
1970s	Allylamines Naftifine	Allylamines	Discovery (1977)	Sandoz Research Institute
1980s	Grifulvin v	Griseofulvin microcrystalline	06/02/1980	OrthoNeutrogena
1990s	Diflucan	Fluconazole	01/29/1990	Pfizer
	Lamisil	terbinafine hydrochloride tablets	05/01/1996	Novartis
	Menatax	butenafine hcl cream	01/01/1997	Viatris
	Sporanox	Itraconazole	03/01/1997	Janssen
	Nizoral	ketoconazole	10/10/1997	Johnson & Johnson
	Ambisome	Amphotericin B liposomal Preparation	08/11/1997	Astellas
	Nystatin	nystatin oral suspension usp	06/25/1998	DL laboratories, Inc
2000s	Totrisone	clotrimazole/betamethasone dipropionate cream	12/01/2000	Merck
	Cancidas	caspofungin acetate	01/01/2001	Merck
	Vfend	voriconazole	05/01/2002	Pfizer

	Loprox	Ciclopirox Topical Suspension	08/06/2004	Altana
	Mycamine	Micafungin	03/16/2005	Fujisawa healthcare
	Noxafil	posaconazole	09/15/2006	Merck
	Eraxis	anidulafungin	02/01/2006	Pfizer
2010s	Jublia	efinaconazole	06/01/2014	Ortho Dermatologist
	Kerydin	tavaborole	07/01/2014	Anacor
	Cresemba	isavuconazonium sulfate	03/01/2015	Astellas
	Luzu	luliconazole cream	02/22/2018	Valeant
2020s	Brexafemme	Ibrexafungerp	06/01/2021	Scynexis
	Vivjoa	Oteseconazole	04/01/2022	Mycovia
	Rezzayo	Rezafungin	03/01/2023	Melinta therapeutics

Source: McCormick & Ghannoum, 2024

With there being only four classes of antifungals and limited drugs within those classes there are very few options for medical professionals to treat with once a fungus has become resistant. One reason for the source of Aspergillus resistance is the overuse of azole antifungals in industrial agriculture. Azole antifungals are used as fungicide on crops. The Aspergillus fungus lives in soil and when the antifungals lands on the soil the fungus becomes resistant. People breathe in the resistant Aspergillus and are unable to be treated with azoles. While the source of resistance of the Candida species is from the human body itself. The Candida species lives inside and on the human body and when prescribed antifungals they can develop resistance (McDermott, 2022).

Who These Fungal Infections are Afflicting

One of the reasons for the severe uptick in fungal infections that were observed in the past decade is the HIV epidemic and the increase in immunosuppressed or compromised patients. Those at risk for fungal infections are those who are transplant patients on immunosuppressants,

cancer patients undergoing cytotoxic chemotherapy, patients in the intensive care unit or post-surgery, those on broad-spectrum antibiotics, patients with catheters, people with diabetes, the elderly, or very young. The major fungal infections these patients face is of the Candida, Aspergillus, or Cryptococcus family. The risk factors for differing fungal infections can be seen in Table 7 (Wall & Lopez-Ribot, 2020).

Specifically, when looking at those at risk for invasive Aspergillosis infections are those who have received a solid organ transplant, have hematological malignancies, or those who face immunosuppression. The largest risk factor of this fungi is having low neutrophil cell counts, neutropenia, which most severely affects those undergoing chemotherapy for leukemia or hematopoietic stem cell transplantation. When untreated this group can have mortality of 100% and even after treatment mortality ranges from 40% to 60% (Rüping et al., 2008). Patients who have received an organ transplant are on immunosuppressives which makes them more at risk of Aspergillosis infections. Their risk correlates to the organ being transplanted where heart or lung has a risk of 14% while renal transplants have less than a 1% incidence rate (Rüping et al., 2008).

Those at risk for invasive Candida infections and thus developing candidiasis are those who have recently had a gastrointestinal surgery, have a hematological malignancy, or those who are very young or very old. Their risk can be compounded by other factors including if they are on broad spectrum antibiotics, receiving intravenous nutrition, using a central venous catheter, have renal failure, or are in the ICU for a lengthy time. Knowing who is more at risk for developing deadly fungal infections is important for medical professionals as they can continue to monitor and catch the infection early (Rüping et al., 2008).

Table 7. Risk factors of fungal infections

Candida	Aspergillus	Mucorales	Fusarium	Scedosporium
Acute necrotizing pancreatitis	Alemtuzumab	CMV disease	Corticosteroid	Corticosteroid
Abdominal surgery; anastomotic leak; or repeat laparotomies	Allogeneic HSCT	Corticosteroids	Myeloma	Neutropenia

Broad-spectrum antibiotics	Anastomotic complications in lung transplantation	Diabetes mellitus	Severe GVHD	Severe GVHD
Central venous catheters	*Aspergillus* colonization	Echinocandin use		
Hemodialysis	CMV disease	Iron overload		
HSCT	Corticosteroids	Malnutrition		
Immunosuppression including corticosteroids, chemotherapy	Infliximab	Myelodysplasia		
Malignancy	Neutropenia	Neutropenia		
Mechanical ventilation >3 d	Older age	Older age		
Multifocal candida colonization	Prolonged ICU stay	Renal failure		
Neutropenia	Renal failure requiring dialysis	Severe GVHD		
Prolonged ICU stay	Retransplantation	Voriconazole use		
Prolonged hospitalization	Severe GVHD			
SOT (kidney and liver)	T-cell depleting agents			
Total parenteral nutrition				

Source: Suleyman & Alangaden, 2021

Nosocomial Infections

Nosocomial infections are infections that are obtained from the hospital. They are an unfortunate side effect of receiving medical care in a designated facility. Fungal health care associated infections have been on the rise. One of the increasing means of infection is through an

invasive device like central venous catheter. These cause central line-associated bloodstream infections (CLABSIs). CLABSIs are usually of the Candida genus and more specifically Candida parapsilosis fungi as they can create biofilms on the catheters. 25% of all CLABSIs in an ICU setting are a result of Candida fungal infections (Suleyman & Alangaden, 2021). Candida fungi have been found on all types of surfaces in hospitals including countertops, floors, or even in the food. In North America this fungus makes up 11% of infections in an ICU (Suleyman & Alangaden, 2021). Nosocomial outbreaks are particularly concerning for healthcare professionals, especially those of resistant fungi. The current most concerning nosocomial outbreak is Candida aruis. This fungus spreads very quickly in a hospital setting and is very difficult to diagnose. There have been multiple outbreaks seen in COVID-19 units and there is concern with this fungi's growing resistance to fluconazole and amphotericin B. Nosocomial Aspergillus infections are usually a result of mold contamination in the air, but they have also been found in the water and plumbing. It is believed that many patients breathe in aerosolized spores from their showers. Those most at risk of nosocomial Aspergillus infections are patients in the ICU markedly those with influenza, chronic obstructive pulmonary disease, liver cirrhosis, or are being treated with steroids (Suleyman & Alangaden, 2021).

Implementation of a New Standard of Care and Prevention

With there being only four classes of antifungals and a rising resistance to these classes of drugs new antifungals are heavily needed in the market. There have not been many new drugs created; in the past decade only one new triazole drug was approved by the FDA. The concern of the new multidrug resistant Candida auris fungi should inspire pharmaceutical companies to allocate more money towards antifungal research before it is too late. However, discovering new antifungal drugs is not the only path towards treating antifungal resistance. Reusing or improving upon current antifungals, finding novel pathways in fungi, or creating new methods for intervention are all possible routes of implementing a new gold standard for tackling resistance (Arastehfar et al., 2020).

Improvements in Existing Antifungal Classes

Current Antifungal drugs do not have to be totally omitted from use; they can be improved to better combat the resistant pathogens of the modern age. Polyene's therapeutic index has always been a weakness of this class of drug. To reduce its toxicity a different formula can be used. By adding a lipid complex to these drugs, the size will increase and result in a faster uptake by macrophages in the body. This will ensure less polyene particles to be circulating in the body and accumulating in the kidneys causing the toxic effects. Specifically for amphotericin B two lipids can be added: l-α-dimyristoyl phosphatidylcholine and l-α-dimyristoyl phosphatidylglycerol. This amphotericin B lipid complex will reduce the previously hazardous nephrotoxicity and inflammation. Through making this class of drug less toxic a higher concentration can be prescribed to the patient thus reducing the chances for resistant fungi formation (Robbins & Cowen, 2016).

Novel azole drugs include Isavuconazole, a prodrug that can be orally or intravenously absorbed to treat invasive Aspergillus or mucormycosis. BAL-8557 is the prodrug in the formulation and becomes active in the plasma membrane of human cells. It has shown high efficacy in clinical trials in vulnerable populations. For Echinocandins they are largely limited by their short half-life. New treatments of this class include aminocandin, which is a product of the Aspergillus sydowii fungus. This drug has a longer half-life and thus can be dosed less frequently in the treatment of Candida and Aspergillus fungi. Additionally, enfumafungin is an offshoot of the echinocandin class in that it also targets the glucan synthase enzyme. It is a natural product that targets the same fungus (Robbins & Cowen, 2016).

Novel Treatments

The market for new antifungals is currently wide open, but the significant barrier to many savvy pharmaceutical companies is the high difficulty in developing new antifungal drugs. Using current synthetic compound libraries to screen for new drugs is complicated as many of these compounds will also target human cells in addition to fungal. However, despite the challenges there are new targets for novel antifungals to pursue beyond the existing four classes of treatment.

One new promising target is fungal sphingolipids. These are parts of the fungal plasma membrane that aid in the regulation of the fungal cell, virulence, and signal transduction. Through targeting the processes that form the sphingolipids fungal virulence can be prevented.

Glucosylceramides (GlcCer) are a type of sphingolipid that only fungi have. Two compounds were found to inhibit this lipid: *N*'-(3-bromo-4-hydroxybenzylidene)-2-methylbenzohydrazide (BHBM) and 3-bromo-*N*'-(3-bromo- 4-hydroxybenzylidene) benzohydrazide (D0). GlcCer can also be targeted through using monoclonal antibodies which can reduce inflammation. Additionally, using enzymes that metabolize GlcCer, like Cerezyme, can break it down in the fungal cells resulting in an obliteration of the fungal plasma membrane and cell death (Robbins & Cowen, 2016).

Another process that novel antifungals could target is the synthesis of the GPI anchor. Glycosylphosphatidylinositol (GPI) post translationally modifies protein on the cellular surface through the addition of a lipid, phosphoethanolamine, myoinositol, mannose residues, and glucosamine. This modification aids the transport of proteins from the endoplasmic reticulum to the cell wall and plasma membrane. Studies have shown a mutation in the GPI anchor synthesis gene results in decreased virulence and a defected cell wall ensuing in cell death. Possible molecules that could be used as treatment were identified. These molecules inhibit the synthesis of the GPI anchor. 1-[4-butylbenzyl] isoquinolone and gepinacin were found to inhibit Gwt1, the acyltransferase used in the pathway (Robbins & Cowen, 2016).

Farnesyltransferases are enzymes that aid in post translationally modifying signaling proteins through lipidation. This signal performs similar actions as GPI where it aids in moving proteins from the endoplasmic reticulum to the plasma membrane. They modify Ras proteins, which modulate signaling cascades and specifically in fungi they aid in the regulation of morphogenesis, virulence, and the cell cycle. An inhibitor of this enzyme is manumycin A which has been shown to work as an antifungal against Candida neoformans, Aspergillus fumigatus, and Candida albicans. Farnesyltransferases were found as possible targets for antifungals as when being compared to the human version significant structural differences were observed allowing for fungal specific targeting (Robbins & Cowen, 2016).

Calcineurin is a protein that regulates the stress response through being activated via Ca2+-calmodulin. In fungi, this protein is heavily involved in regulating cellular responses: cation homeostasis, virulence, cell cycle, morphogenesis, and drug response to antifungals. Through inhibiting this protein fungal virulence and response could be reduced. Cyclosporin A and tacrolimus are two inhibitors of this protein and they have been used as immunosuppressants for transplant patients. Their use was shown to reduce the activity of the transcriptional regulator of T cells. When used in fungi they aid in making antifungals more effective and reduce the fungus's

ability to grow at human body temperature. They are also especially valuable against fungi resistant to azoles and echinocandin (Robbins & Cowen, 2016).

An alternative target for novel antifungals is Hsp90, the chaperone protein that aids in regulating other proteins including vital signal transducers. Through this modulation this protein can manipulate the implementation of genetic mutations. When targeted in fungi the inhibition of the Hsp90 protein results in the decreased ability to further develop resistance to azoles and echinocandins. It also improves antifungal activity. Additionally, Hsp90 aids in stabilizing calcineurin, so through inhibition of Hsp90 it also affects the Calcineurin pathway. Hsp90 also can affect fungal morphogenesis which largely affects virulence. For example, inhibition of Hsp90 causes the filamentation of fungi, or the formation of hyphae, which largely reduces virulence (Robbins & Cowen, 2016).

Acetyltransferases and deacetylases, specifically lysine deacetylases (KDACs) and lysine acetyltransferases (KATs), in pathogenic fungi can be another novel treatment for pharmaceutical companies to pursue. KDACs and KATs are enzymes that respectively remove and add the acetyl group from ε-amino group of lysine. This alters gene expression and the structure of chromatin through histone changes. This results in a multifaced impact on the stress response and cell signaling pathways. Through inhibiting KDAC with trichostatin A antifungals like azoles can be more effective. KDACs are also involved in deacetylating other proteins including Hsp90, and thus Calcineurin, which also improves azole resistance (Robbins & Cowen, 2016).

Novel Intervention Methods

While creating novel antifungal treatments will take some years, in the meantime other interventions can still occur to reduce the effects of emerging antifungal resistance. To constrain the further evolution of fungal pathogens it is important to better detect and surveille these pathogens in the population. Antifungal stewardship programs are programs hospitals can put into place that optimize antifungal use to better manage resistance and protect the existing antifungal drugs. There is a centralized team in the institution who are heavily educated on current fungal infections and are providing recommendations to clinicians on the antifungal drugs being prescribed, the dose, and in monitoring drug use. Under this program when prescribing antifungals medical professionals first accurately diagnose the fungal infection in their patients. Then they go to the antifungal stewardship program to gain recommendations for antifungal therapy. Through

first getting an accurate diagnosis of the infection the antifungal stewardship program can prescribe specific doses of antifungals to target the infection as compared to a broad-spectrum antifungal. Having a centralized force providing recommendations can curtail emerging resistance in hospitals as a whole. Additionally, a large part of this program involves early detection of invasive antifungals to quell any spread, especially when the infections are very transmissible (Hamdy et al., 2017).

Combination therapies, i.e. treating a patient with two different classes of antifungals, have also been shown effective in preventing resistance. This is as they are more effective treatments when paired together which prevents treatment failure and the resultant resistance that may form. Studies have shown using fluconazole and flucytosine in tandem can prevent fluconazole resistance especially in populations that have cryptococcal meningitis (Fisher et al., 2022). An alternative approach is to fight resistance inside the patient. This can happen through fungal vaccines, immunotherapy, and having antibodies for fungal targets. Fungal vaccines have been difficult to make as with the difficulties in targeting fungal cells, but success has been shown in clinical trials for a Candida albicans recombinant Als3 protein vaccine. Immunotherapy involves using monoclonal antibodies, recombinant cytokines, and fungal specific T cells. This therapy is very effective for immunocompromised patients who are already most at risk for fungal infections. Antibodies that can specifically target antifungal agents can work hand in hand with other antifungal medications and can reduce their toxicity (Fisher et al., 2022).

Outcome and Impacts

The outcome of releasing new antifungal treatments or even starting to begin research and development on new methods to treat them will have widespread effects. As of right now there is limited funding and research on fungi, with bacteria and viruses taking precedence. Just for tuberculosis and malaria alone there were 8,827 and 5,687 scientific papers released in 2017 respectively while there were less than 500 papers on fungal diseases (Rodrigues & Nosanchuk, 2020). In addition, in the last ten years there was less than 0.5 billion dollars applied for invasive fungi as compared to the 8 billion for bacterial infections globally (Vitiello et al., 2023). Putting more money into this field will help better drug treatment and diagnostics. Furthermore, by involving clinicians in this process more surveillance on emerging infections can be performed and thus a better response to outbreaks. Public health officials can work on both local and national levels to

keep fungal infections from becoming resistant through monitoring epidemiological data. This is an important issue to address now as rates of immunocompromised and critically ill individuals rise each year and with it the rates of mortality from fungal infections. The burden of these infections is high, while awareness is still low (Vitiello et al., 2023).

However, a multitude of challenges still exist even after novel treatments for resistant fungal infections can be created. One of the challenges is still not having the best understanding of the fungal species as a whole. Of the estimated millions of species existing in the world only around 150,000 species have been discovered. Additionally, there is a large knowledge gap in fungal biodiversity even among the few species that have been studied. There needs to be a better understanding of the ecological niches and natural distributions of fungi to treat the pathogenic ones before they can become resistant. A further challenge in treating resistant fungi is predicting where the new pathogenic fungi will emerge from. There are a multitude of fungi lineages, and any may evolve to become pathogenic to humans and thus resistant to antifungals. Evolutions may include evolving to be able to grow at human body temperature which was observed in the pathogenic Candida neoformans species (Rokas, 2022).

The rise in resistant fungal infections should sound the alarm for health care officials everywhere. The future will be bleak if changes are not made. There is a need for new classes of treatment, quicker diagnostic protocols of infection, and more clinician education on this growing threat. More pharmaceutical companies need to invest money and time into research and development for new antifungal drugs. Doctors need to be more active in the diagnosis of these infections in their patients and respond quickly with effective drugs and dosages. In order to quell this imminent threat everyone needs to work together as a united front to protect the most vulnerable populations. The medical community was able to unify for the COVID pandemic, so they know it can be done.

Glossary

Antigen "Any substance that causes the body to make an immune response against that substance. Antigens include toxins, chemicals, bacteria, viruses, or other substances that come from outside the body. Body tissues and cells, including cancer cells, also have antigens on them that can cause an immune response. These antigens can also be used as markers in laboratory tests to identify those tissues or cells."[1]

Bacteriophages A virus that targets bacteria through injecting its genetic material into the host bacterial cell and killing it. They can be used in place of antibiotics to combat rising bacterial antibiotic resistance[2]

Cytochrome P-450 A hemoprotein involved in drug metabolism that is commonly targeted by drugs. The antifungal class of azoles works by inhibiting this protein's steroid metabolism in fungi[3]

Eukaryote "Organisms whose cells contain a nucleus and other membrane-bound organelles. There is a wide range of eukaryotic organisms, including all animals, plants, fungi, and protists, as well as most algae. Eukaryotes may be either single-celled or multicellular. Eukaryotes are differentiated from another class of organisms called prokaryotes by way of the presence of internal membranes that separate parts of the eukaryotic cell from the rest of the cytoplasm. These membrane-bound structures are called organelles."[4]

Glycoprotein Proteins with sugar molecules. Large part of a fungus's cell wall and can be targeted to determine the presence of fungi[5]

Loop-mediated isothermal amplification Clinical assay that uses a bacterial polymerase to form a template strand of target DNA to detect specific diseases. Can be used as a diagnostic test to discover the presence of fungal DNA in urine samples[6]

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[1] *National Cancer Institute,* https://www.cancer.gov/publications/dictionaries/cancer-terms/def/antigen
[2] Uddin et al., 2021
[3] *National Library of Medicine,* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4093435/
[4] *Scritable*, https://www.nature.com/scitable/definition/eukaryote-eucariote-294/
[5] *Essentials of Glycobiology*, https://www.ncbi.nlm.nih.gov/books/NBK579942/#:~:text=Glycoproteins%20are%20a%20major%20component,as%20well%20as%20GPI%20anchors.
[6] Sanguinetti et al., 2019

Macrophages "A type of white blood cell that surrounds and kills microorganisms, removes dead cells, and stimulates the action of other immune system cells"[7]

Mannoprotein A glycoprotein found in a fungus's cell wall. Used for detection by fungal infections diagnostic tools for presence of Aspergillus fungi[8]

Monoclonal antibodies "A type of protein that is made in the laboratory and can bind to certain targets in the body, such as antigens on the surface of cancer cells. There are many kinds of monoclonal antibodies, and each monoclonal antibody is made so that it binds to only one antigen. Monoclonal antibodies are being used in the diagnosis and treatment of many diseases"[9]

Non-Synonymous Point Mutation Change in a single nucleotide in the organisms DNA that results in a change in the amino acid and protein[10]

PCR assay Analytic test that amplifies small pieces of DNA through synthesizing copies. Can be used to determine identity of fungi from small sample[11]

Prodrug A drug that is originally inactive, but activates in the body usually through enzyme activity. [12]

Therapeutic index The ratio for a drug at which concentration it is toxic to which concentration it is effective. Amphotericin B has a very low therapeutic index meaning it is highly toxic when most effective[13]

Virulence For a "specific fungal strain is often measured as the minimum dose of fungal spores required to kill 50% of individuals from a given host model of fungal disease" [14]

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[7] *National Cancer Institute*, https://www.cancer.gov/publications/dictionaries/cancer-terms/def/macrophage
[8] Sanguinetti et al., 2019
[9] *National Cancer Institute*, https://www.cancer.gov/publications/dictionaries/cancer-terms/def/monoclonal-antibody
[10] Author, based on Redd et al., 2022
[11] *National Human Genome Research Institute,* https://www.genome.gov/about-genomics/fact-sheets/Polymerase-Chain-Reaction-Fact-Sheet
[12] Robbins & Cowen, 2016
[13] Robbins & Cowen, 2016
[14] Rokas, 2022

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