PNAS NEWS FEATURE

Drug-resistant fungi on the rise

It's a dangerous and underappreciated trend. New drug approaches could soon offer relief.

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Superbug fungi, such as the drug-resistant *Aspergillus fumigatus* depicted in this illustration, pose an urgent and growing threat. Image credit: CDC/ Antibiotic Resistance Coordination and Strategy Unit, and Stephanie Rossow (artist).

Seven years ago, a woman came into the intensive care unit of Radboud University Medical Center in the Netherlands with symptoms of a bad flu. She was a new mom, 38 years old, with no prior medical history, but she'd been coughing and feverish for a week. Doctors confirmed she had the flu, and a CT scan of her lungs revealed a fungal infection with a microbe called *Aspergillus*, which was quickly spreading. She could have inhaled it anywhere—*Aspergillus* lives in soils worldwide and can infect people—although it rarely gets a foothold in healthy bodies.

"Immediately when we saw lesions we started treatment," says Frank van de Veerdonk, the clinician and infectious disease specialist who handled her case. He prescribed a course of antifungal drugs, but the woman's lesions kept growing. Subsequent tests found that she had a drug-resistant strain of ***Aspergillus***. First-line antifungal medicines couldn't kill it. In such cases, doctors throw everything they have at the infection, van de Veerdonk says. He tried every antifungal therapy on the market. But sadly, none of it worked. Sixteen days after her admission to the ICU, the young woman died. "She had little children, she just got the flu," van de Veerdonk remembers. "It's very fast."

Today, he recalls her tragic story as an early example of the growing reality of antifungal drug resistance. Thirteen other young and healthy patients died the same way in the Netherlands that winter (1).

Since then, many more have died worldwide, although a lack of surveillance, including diagnostic testing for resistant infections, makes the numbers hard to

Published November 21, 2022.

pin down. What is clear from the clinical data, including hospital patient records, is that cases of serious invasive disease are on the rise. The numbers, and the rising costs to treat the afflicted, are a cause for concern, researchers say.

A limited number of treatments is part of the problem. In the last several decades, only three classes of antifungal drugs have received clinical approval. Fortunately, a range of new drugs is in development—including one recently approved by the FDA that belongs to a fourth drug class. And there's some early promise with antifungal vaccines (See **An elusive fungus vaccine**). These and other treatments could offer relief.

Troubling Trend

In 2019, the U.S. Centers for Disease Control and Prevention estimated that 18 superbugs—both drug-resistant fungi and bacteria—cause at least 2.8 million drug-resistant infections every year in the United States, resulting in more than 35,000 deaths (2). Of the 18 microbes, three are fungi: *Candida auris*, according to the CDC, is an "urgent threat," drug-resistant *Candida* species are "serious threats," and azole-resistant *Aspergillus fumigatus* is on the "watch list."

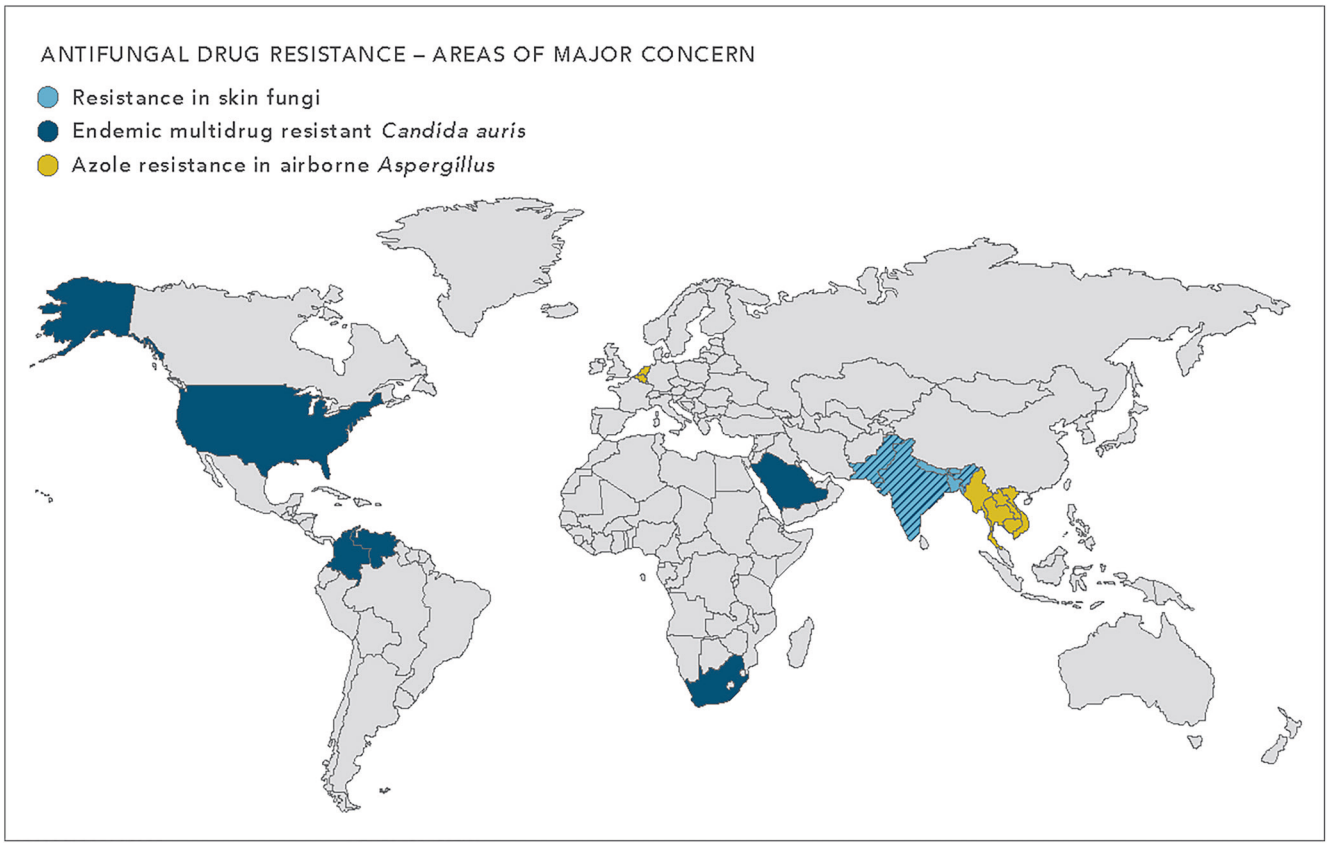
The number of fungal infections reported as the cause of death steadily increased in the United States between 2013 and 2018, from 4,000 to about 5,000, according to one longitudinal analysis (3). It's a trend that has likely continued in recent years, says Emily Rayens, a postdoctoral infectious disease researcher at the University of Georgia in Athens. "One of our biggest concerns is a rise in antifungal resistance," she says. Although antibacterial drug resistance has received the lion's share of public attention, antifungal drug resistance is a growing problem as well.

Early indications suggest that drug resistance stems both from treating an increasing number of immunocompromised patients and the extensive use of some antifungal drug classes in agriculture. Plus, some fungi have a natural resistance to some existing drug classes. When these fungi adapt to evade even one or two of the drugs that once worked, that spells trouble for patients. "It really limits our options," Rayens says.

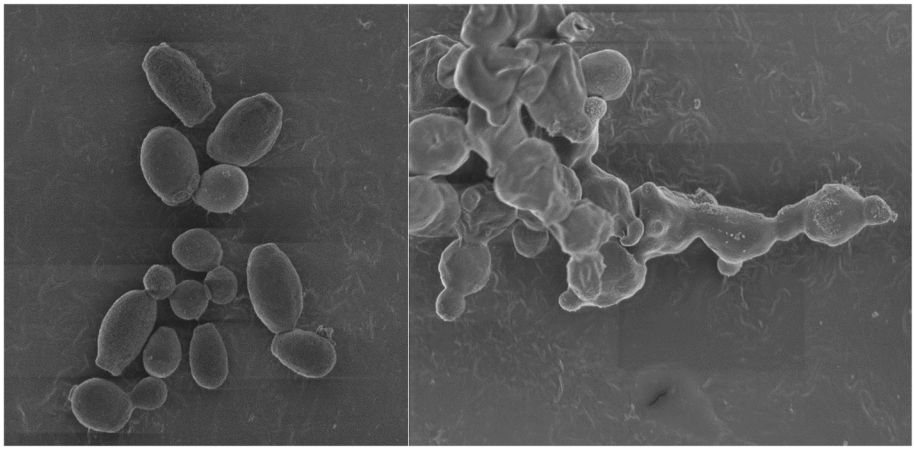
The Unlucky Many

Most fungal infections are superficial, perhaps a little painful, but not life-threatening—yellow toenails, yeast infections, athlete's foot.

Fungal infections turn deadly when they get into deeper tissues, such as the blood or lungs, explains Jatin M. Vyas, a physician-scientist at Massachusetts General Hospital and Harvard Medical School in Boston. The unsettling reality is that millions of fungi swirl around us all the time, drifting through the air, water, and soil. We eat them, drink them, and breathe them in. Some take up residence in our mouths and guts as a normal part of the human flora. For the most part, the immune system keeps these myriad fungi in check.



Countries worldwide, including the United States, are now contending with major concerns over drug-resistant fungi. Image credit: David Denning (The University of Manchester, United Kingdom), and Lucy Reading-Ikkanda (artist).



Prior to treatment with the antifungal ibrexafungerp, *Candida auris* cells exhibited normal morphology and ultrastructure (Left), whereas *C. auris* cells treated with ibrexafungerp were swollen, and lost the classic yeast elliptical appearance (Right). Image credit: SCYNEXIS

But occasionally invaders break through. The same fungal species that once lived quietly on the skin or in the gut penetrate a wound and infect the blood or create lesions in the lungs. More than 2 million people die every year of fungal infections (4), and some 13 million people worldwide are at risk, says clinician David Denning, a leading infectious disease researcher at the University of Manchester in the United Kingdom. That huge number largely consists of people who are immunocompromised—for instance after receiving an organ transplant or chemotherapy—those with severe asthma, and those with breathing problems from chronic obstructive pulmonary disease, Denning says.

But even healthy people like the young woman in the Netherlands occasionally catch an invasive fungal disease. Early evidence suggests cases like hers may occur as a complication of certain viral infections, such as the flu (5). The flu virus, and now SARS-CoV-2, can dysregulate the immune system, van de Veerdonk says, enabling *Aspergillus* to infect otherwise healthy lungs. Complicating matters, different fungal pathogens are developing resistance at different rates and in different parts of the world. Drug-resistant strains of *Aspergillus*, for example, are still uncommon in the United States (for reasons not well understood) but are a larger problem in parts of Europe and Asia (6).

In the Netherlands, resistant strains account for about 20% of all *Aspergillus* in the environment; In Southeast Asia, some 80% to 95% of *Aspergillus* strains are now drug-resistant, Denning notes, referring to a 2021 article he coauthored (7). Other pathogens, such as certain fungal skin infections, are developing hotspots of drug resistance in India and Pakistan. In the United States, multi-drug resistant strains of *C. auris*, a yeast that invades the bloodstream, have emerged in hospitals and nursing homes since 2009. *C. auris* now kills 30% to 60% of patients with bloodstream infection (8).

The origins of the resistance problem are similarly hard to pin down, but industrial agriculture is one likely source for fungi such as *Aspergillus* (9). In the vast tulip fields of the Netherlands and the endless rural highways of California and Iowa, farm workers are out, carrying long-nozzled jugs of fungicide to spray on their crops. Sloshing in those farmers' hands are azole antifungal drugs: molecules similar to the ones doctors use to treat their patients. Tiny droplets of these fungicides land on the soil, where *Aspergillus* lives, and farms become Petri dishes of selection for drug-resistant strains. Northern Europe is a hotspot because its climate allows the fungus to grow for prolonged periods and because fungicides are widely used there. *Aspergillus* tends to thrive in compost piles; peelings from bulbs are an ideal incubator (6).

Azole-class antifungal medicines are the gold standard to treat *Aspergillus* lung infections, but drug-resistant outbreaks are becoming more common in agricultural areas and in cities where compost is used in flowerbeds. Recalcitrant lung infections also pop up in hospitals near construction sites, Rayens notes, where drug-resistant *Aspergillus* is lurking in the dust as it lifts into the air.

The other likely source of resistance, especially for *Candida*, is within patients themselves. After a transplant, for instance, immunosuppression can be lifelong. Doctors may prescribe years of antifungal therapy, which "significantly increases selection pressure," Rayens says. "It's a daunting length of time to try to prevent these infections with prophylactic antifungals."

Regardless of where exactly the problem comes from, it's clearly getting more difficult and more expensive to treat. In Rayens' 2022 analysis of patient records, she found that the cost of treating hospital fungal infections was $6.7 billion in the United States in 2018, compared with $4 billion in 2014 (10, 11). The average hospital bill for fungal infections has gone up by $27,000.

Rising costs may be a proxy for a disturbing trend: more infections are progressing to severe invasive disease, Rayens says. Invasive aspergillosis or *Candida* in the blood are the two most common invasive infections. They can require intravenous antifungals, a longer hospital stay if first-line drugs fail, and surgery if the infection causes necrosis. Patients with preexisting risk factors, including not only a history of organ transplant or cancer but also more common issues such as autoimmune disease or diabetes, walk into the hospital with an average baseline cost of $30,000. In her analysis, Rayens found that "If you added fungal infection on top, that average baseline went up to $60,000."

Fighting Fungi

All this means that researchers and clinicians are eager to develop new drugs. The three existing classes, the azoles, the echinocandins, and the polyenes, all work by attacking the fungal cell wall or cell membrane. Some new approaches attack fungi via entirely novel pathways, whereas others target the cell in tried and true ways. "Our understanding of fungal disease is rapidly evolving," Rayens says, and so are treatments. Mycologists must now attempt to answer two big questions, she says: "Can we develop new formulations within existing drug classes? And can we develop new drug classes?"

The FDA recently approved one new drug in 2021, with the memorable name "ibrexafungerp" (I-brex-ah-fun-gerp), to treat vaginal yeast infections. It's the first of a new class called the triterpenoids, which have the same mechanism of action as the existing echinocandin class of molecules. Both inhibit a glucan synthase enzyme, which makes the building blocks of the fungal cell wall. Disabling this enzyme kills the cell (12). What sets the triterpenoids apart, says physician Nkechi Azie, vice president and head of clinical development at Scynexis in Jersey City, NJ, is that they are much smaller, about half the molecular weight of the echinocandins, and are therefore available in a pill form, whereas the echinocandins can only be taken intravenously. With a pill, patients can leave the hospital during treatment. "We're bringing the potency of the echinocandins to a tablet," Azie says. The same drug is now in phase-three clinical trials to treat life threatening infections.

Despite ibrexafungerp's shared mechanism of action with the existing echinocandins, Azie doesn't anticipate a rapid emergence of resistance. So far, fungi have only found one way to get around the echinocandins, and resistance typically occurs after a prolonged or repeated course of drugs. The adaptation involves amino acid changes, which decrease the sensitivity of a key target enzyme to the drug in the cell wall.

In contrast, fungi have evolved a variety of mutations to evade other drug classes, such as the azoles, which clinicians often choose first when seeking a broad-spectrum antifungal (13). And although echinocandins treat *Candida* infections well, as in yeast infections, they haven't been as effective with *Aspergillus*.

Last June, Scynexis released a one-day oral ibrexafungerp pill called Brexafemme to treat yeast infections. Azie says the company is aiming for approval to treat a range of invasive hospital infections by 2024. In addition to fighting *Candida*, ibrexafungerp may eventually be paired with an azole pill to fight *Aspergillus*. Animal models suggest that the two pills may be a more effective treatment for invasive *Aspergillus* than an azole alone, Azie says.

Meanwhile, a small brown tablet called olorofim could battle fungi in a whole new way. Now enrolling for phase-three clinical trials, and fast-tracked by the FDA with two Breakthrough Therapy designations in 2019 and 2020, olorofim doesn't target the cell membrane or cell wall as other antifungals do. Instead, it binds to a mitochondrial enzyme in molds (14). By binding and inhibiting this enzyme, the drug interrupts DNA synthesis, RNA synthesis, and protein production and ultimately causes rupture of the fungal cell wall and cell death. The beauty of any pill that kills is that patients may not need a functioning immune system to finish off the infection, says pharmaceutical physician Emma Harvey of F2G Ltd, a biotech company which is developing the drug. And because olorofim has a new mechanism of action, it could offer relief from drug-resistant bugs. If approved, this would be the first member of a new antifungal drug class, called the orotomides, with broad spectrum activity against a range of molds. Olorofim is active against *Aspergillus*, including species that are resistant to azoles and polyenes. But it, too, is limited: olorofim lacks activity against yeasts such as *Candida*.

An elegant solution: The hunt for a fungus vaccine

Vaccines may one day become a key weapon against dangerous fungi, especially for high-risk patients. Importantly, a vaccine would sidestep the drug resistance issue entirely.

That's the aim for immunologist Karen Norris' lab at the University of Georgia in Athens. "We have a candidate," she reports, and so far preclinical testing suggests that it's effective in animal models. Phase one clinical trials could begin within the next two years.

Vaccines in general work by triggering an immune response to an "antigen" of interest—the invader molecule that the immune system is being primed to recognize and attack. And the vaccines may contain an "adjuvant" partner molecule, often aluminum, which gets the attention of the immune system to stoke a stronger inflammatory reaction. The body remembers the invader and mounts a strong response if and when it's exposed again. Immunologists have kicked around the idea of a fungal vaccine for 25 years now. But most people dying of fungal infections are immunocompromised, and researchers haven't been able to design a vaccine potent enough to protect them when the immune system can't mount a strong response, Norris explains.

Norris' strategy began with the ubiquitous fungus *Pneumocystis*, which drifts through the air. Most of us have been exposed to *Pneumocystis*, Norris says. "We breathe it in." The fungus has a tiny surface peptide that's also found on many other fungal species. Norris explains that she and collaborators "constructed a consensus peptide based on the common amino acids of this peptide that are shared by other organisms" as the antigen in her vaccine. At least in animals, she's found that the immune system remembers the peptide well enough to fight off a range of fungi, even during immunosuppression. The reason, Norris suspects, is because the body has seen that peptide so often that the immune system can remember it *really* well, once primed to attack it by a vaccine. "When you've seen something immunologically and then you see it again, that second sighting induces a really strong immune response," Norris says.

One challenge to new antifungal drug development has been finding novel targets, Harvey says, in large part because fungi are eukaryotes with similar metabolic pathways to humans. "It's hard to find a druggable target that won't cause toxicity in humans," she says. The mitochondrial enzyme that olorofim attacks is also found in human cells. But for still-unknown reasons, likely related to the chemical scaffold of the enzyme itself, olorofim is about 2,000 times less able to inhibit the human enzyme than the fungal one. Because of its fast-track designation with the FDA, olorofim could come to market as soon as one or two years from now.

Better Predictors

Fungal infections are not only dangerous, they're also tough to predict and diagnose. Vyas at Massachusetts General remembers a patient from 25 years ago, a lawyer, who'd been through chemotherapy and was redeveloping his immune system when he caught an invasive fungal bug. "He asked a lot of questions. A question he asked me was 'why?'" Vyas remembers. His patient said, "You've told me I have no immune system and I'm developing an infection from an organism that's ubiquitous, and yet down the hall to the left and right, all these patients are in the same circumstance. Yet as far as I can tell, I'm the only one with this infection, so why me?" A few days later, he was moved to the ICU, where he died of that infection.

In the intervening years, Vyas has dedicated his research to understanding why common fungi such as *Aspergillus* and *Candida* infect some fragile patients but not others. We've learned "frighteningly, not enough," he says. Mutations in certain genes, for example carbohydrate lectin receptors, seem to portend higher risk. Defects in these genes and others can prevent the immune system from even recognizing fungi, according to one 2018 review coauthored by van de Veerdonk (15). The mutations inhibit various receptors on the surface of immune cells, which are necessary to mount even the earliest stages of defense.

Most hospitals still don't routinely test for genetic mutations though, in part because the "actual causative alleles and their functional consequences" have yet to be pinpointed, according to the review. Doctors have a pocketful of genes of interest but don't yet know the precise biological mechanisms by which these genes influence risk, and clinical data translating these observations into medical protocols are also lacking.

Vyas hopes that predicting who is at risk could soon buy a little more time—"a small window," he says, to give a vaccine or prophylactic new antifungal, to interrupt the infection process and prevent it. "I think," he says, "that's where the future of our field is moving."

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