# Confronting diagnostic gaps in fungal infection

## Karen Titus

April 2024—The rise in fungal infections in recent years troubles Sean Zhang, MD, PhD, for reasons near and far.

It's readily apparent in the patient populations at Johns Hopkins Hospital, where he is director of the mycology laboratory. Especially concerning is the increase in *Candida auris* following the height of the COVID-19 pandemic, both in terms of colonization and infection cases, says Dr. Zhang, who is also associate professor of pathology, Division of Medical Microbiology, Department of Pathology, Johns Hopkins University School of Medicine. "Since 2022, we suddenly saw an uptick in *Candida auris* cases across the Johns Hopkins Health System."

But the situation isn't unique to Johns Hopkins. Pointing to CDC figures, he notes that the tide is rising more broadly as well. The agency reports that in 2020, there were 757 clinical cases and 1,310 screening cases of *C. auris* in the United States. In 2022, there were 2,377 clinical cases and 5,754 screening cases. Says Dr. Zhang, "It has been causing outbreaks in hospital settings and is multidrug resistant."

Similarly concerning are recent reports of drug-resistant *Trichophyton indotineae*, Dr. Zhang says. Though initially reported in India, this novel dermatophyte species is now being reported in the United States, with the first reported cases appearing in New York City between 2021 and 2023. "Based on the data from India, it has almost a 70 percent resistance rate to terbinafine," says Dr. Zhang. "So that is also concerning." Even more recently, he adds, an azole-resistant *Candida parapsilosis* has emerged.

Though bacterial and viral infections outpace fungal infections in terms of sheer numbers, that's hardly reassuring to Dr. Zhang, who was a driving force behind the Fungal Diagnostics Laboratory Consortium, made up of 29 U.S. and Canadian laboratories (https://labs.pathology.jhu.edu/fdlc) that provide fungal diagnostic services to patient populations. "We've come together as a joint force to improve laboratory diagnosis of fungal diseases," says Dr. Zhang, who is a member of the CAP Microbiology Committee.

These infections are a threat to immunocompromised patients as well as nonimmunocompromised patients with various medical conditions, with transplant and hematology-oncology patients at especially high risk. But it's not always clear-cut—sometimes a less common fungal organism lies behind an infection. "That can be challenging, and we have to do it case by case," Dr. Zhang says. "We cannot rely on just one kind of test. We have to look at the overall picture and other laboratory findings."

Moreover, the potential for even wider spread of fungal infections is a troubling possibility, he says. To get a sense of the laboratory community's capacity to respond to the growing threat, the Fungal Diagnostics Laboratory Consortium surveyed its members and noted a number of gaps in fungal diagnostics specific to disease as well as methods and approaches (Zhang SX, et al. *J Clin Microbiol.* 2021;59[7]:e0178420).

It's a sobering list:

- Lack of molecular detection of mucormycosis.
- Lack of an optimal diagnostic algorithm that uses fungal biomarkers and molecular tools to diagnose, early and accurately, *Pneumocystis* pneumonia, aspergillosis, candidemia, and endemic mycoses.
- Lack of a standardized molecular approach to identify fungal pathogens directly in formalin-fixed, paraffin-embedded tissues.
- Lack of robust databases to enhance mold identification with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

- Suboptimal diagnostic approaches for mold blood cultures, tissue culture processing for Mucorales, and fungal respiratory cultures for patients with cystic fibrosis.
- Inadequate capacity for point-of-care testing aimed at new, emerging or underrecognized, rare, or uncommon fungal pathogens.
- Performance of antifungal susceptibility testing.

The consortium has also noted the need to develop a more robust workforce and is making education a priority. It is collaborating with the Netherlands-based ATLAS of Clinical Fungi Foundation ("They have been teaching medical mycology courses for decades," Dr. Zhang says) to provide courses in clinical fungi. The first was held in Chicago in 2022; the second will be taught onsite at Mayo Clinic in Rochester, Minn., in September (https://ce.mayo.edu/fungi2024). The morning will be devoted to lectures, while the afternoon sessions will cover practical laboratory training, says Dr. Zhang, who is a course director.

Such education is essential, he says, given that fungal infections have typically been an under-resourced field in medicine, particularly compared with bacterial infections. "For laboratory bench technologists, to gain the skill sets for fungal infection, they usually need more time for training," Dr. Zhang says. "It also depends on their years of experience, and some of the very experienced technologists are at retirement stage." Very few recently trained technologists have the necessary knowledge, he adds, "so there is a huge gap." While other groups, including the CDC, also provide education courses, he favors the in-person approach the consortium is using. "We really want to teach people the laboratory skill sets."

New fungal threats are emerging, and the Fungal Diagnostics Laboratory Consortium came together to address them and to educate. "We really want to teach people the laboratory skill sets," says Dr. Sean Zhang, consortium co-chair. [Photo by Shan

Gordon]

## The consortium further prioritized its findings in its *Journal of Clinical Microbiology* commentary, including a discussion of how to approach six disease-specific gaps.

*Pneumocystis* pneumonia is caused by the yeast-like fungus *Pneumocystis jirovecii.* In recent years, the authors note, laboratories have been able to use nucleic acid amplification tests of respiratory samples to directly detect *P. jirovecii* DNA, a method that offers more sensitivity than direct microscopic methods that have long been the gold standard. However, the approach lacks standardization, both for nucleic acid amplification tests and quantitative NAAT. β-d-glucan, which is used as a diagnostic adjunct for *Pneumocystis* pneumonia, has its own limitations, including lack of specificity and questions about its optimal cutoff value and negative predictive value.

Consortium members call for reevaluating optimal tissue processing for isolating Mucorales. Though it has long been standard practice to process tissue specimens by mincing, rather than homogenizing, before inoculating onto culture plates, no studies support this practice, Dr. Zhang says. “We need to look at this.” It would also be useful to develop Mucorales NAAT to diagnose mucormycosis early, to improve patient survival rates, the authors note.

NAAT might also be potentially useful, in conjunction with galactomannan antigen assays, to diagnose invasive aspergillosis. Potential advantages of *Aspergillus* NAAT might include rapid turnaround times, increased clinical specificity versus galactomannan alone, the ability to differentiate between *Aspergillus* species, and detecting antifungal resistance markers.

Consortium members would like to see a rapid test algorithm for candidemia, which, they note, is one of the most common bloodstream infections in health care settings and the predominant severe fungal infection in critically ill ICU patients. Others at high risk include those with malignancy, transplantation, immunosuppression, abdominal surgery, prolonged broad antibacterial use, and injection drug use. Faster detection and identification of *Candida* spp. could reduce mortality rates, they note.

Diagnostics for endemic mycoses (histoplasmosis, blastomycosis, and coccidioidomycosis) need to perform better and become more widely available, say the authors. Culture, histologic/cytologic studies, antigen testing, serology, and molecular studies may all be part of improved approaches to detect and identify these fungal pathogens.

Among the trickiest gaps to bridge will be addressing emerging and underrecognized fungal pathogens, such as the by-now-familiar *C. auris*. They note that other rare or uncommon yeasts causing bloodstream infection include uncommon *Candida* species, *Cryptococcus* spp. (other than *C. neoformans* and *C. gattii*), *Trichosporon, Rhodotorula, Malassezia, Geotrichum,* and *Saprochaete*. Most of these infections are associated with catheter lines or due to breakthrough on antifungal treatments.

## Members of the consortium are primarily large academic medical centers and reference laboratories and perform high-volume fungal diagnostic testing.

Smaller hospitals lack those resources, “so those are also gaps we need to take into consideration,” Dr. Zhang says. Those institutions will require simple-to-perform, cost-effective methods for diagnosing fungal infections in their patient populations. “It’s a need that at this point is not very well addressed,” he says.


The fungal threat to humans exists and is growing, Dr. Zhang says: “We need to be more proactive, understanding from basic research science how these fungal organisms adapt to high temperatures and how any of them could be turning virulent to humans. And on the laboratory side we need to have better tools to increase our sensitivities and improve our speed.” [Photo by Shan Gordon]

It’s possible that point-of-care devices could be deployed in such settings. He points to the cryptococcal antigen test, which is a lateral flow device. “It’s very easy to perform, and its cost is not very high,” Dr. Zhang says. “But we need to have more such tests available to those settings.”

In the past, he continues, manufacturers were less committed to developing tests for the fungal diagnostics market, in part, he suggests, because they felt the return on investment was low, given the low volumes and low prevalence of fungal infections. “But this has changed as of recently,” he says. “We’re seeing more companies interested in bringing kits to the market for improving accuracy and speed of fungal diagnoses. The consortium is trying to work closely with those companies to bring their products into clinical validation and eventually deploy [them] to patient care testing. So there is progress.”

At Johns Hopkins, Dr. Zhang and colleagues use MALDI-TOF mass spectrometry to identify the rising number of *C. auris* cases. It’s an invaluable tool, he says. “But in more limited settings, if you don’t have MALDI-TOF, and if you’re only doing a morphological or biochemical test, you may find it difficult to identify the organism.”

Laboratories that don’t have access to MALDI-TOF will need to send out their testing or rely on their existing knowledge and available testing to make the diagnosis. “Which is not ideal,” Dr. Zhang says. “For *Candida auris*, you really have to use the MALDI or PCR sequencing-based methods,” because other methods cannot reliably differentiate *Candida auris* from other *Candida species*.

Consortium members also addressed problems with direct identification of fungal pathogens in formalin-fixed, paraffin-embedded tissue. “It is very subjective,” says Dr. Zhang. The consortium’s survey, which asked surgical pathologists to review cases where fungus was present in the tissue, showed a nearly 20 percent error rate, he says.

Again, he says, using more advanced molecular tools, such as FFPE tissue sequencing, could provide more accurate answers on such specimens. The need is great. “When you see those fungal organisms in the tissue, often those are associated with tissue damage already,” he says. “So this is a proven diagnosis of fungal infections, and we need to know which organism is causing the infection. It’s very important to get to the species level to manage patients.” As with other advances, however, lack of standardization remains an issue. Moreover, fungal databases are more limited than their bacterial counterparts. Finally, the authors note, FFPE blocks are prone to contamination.

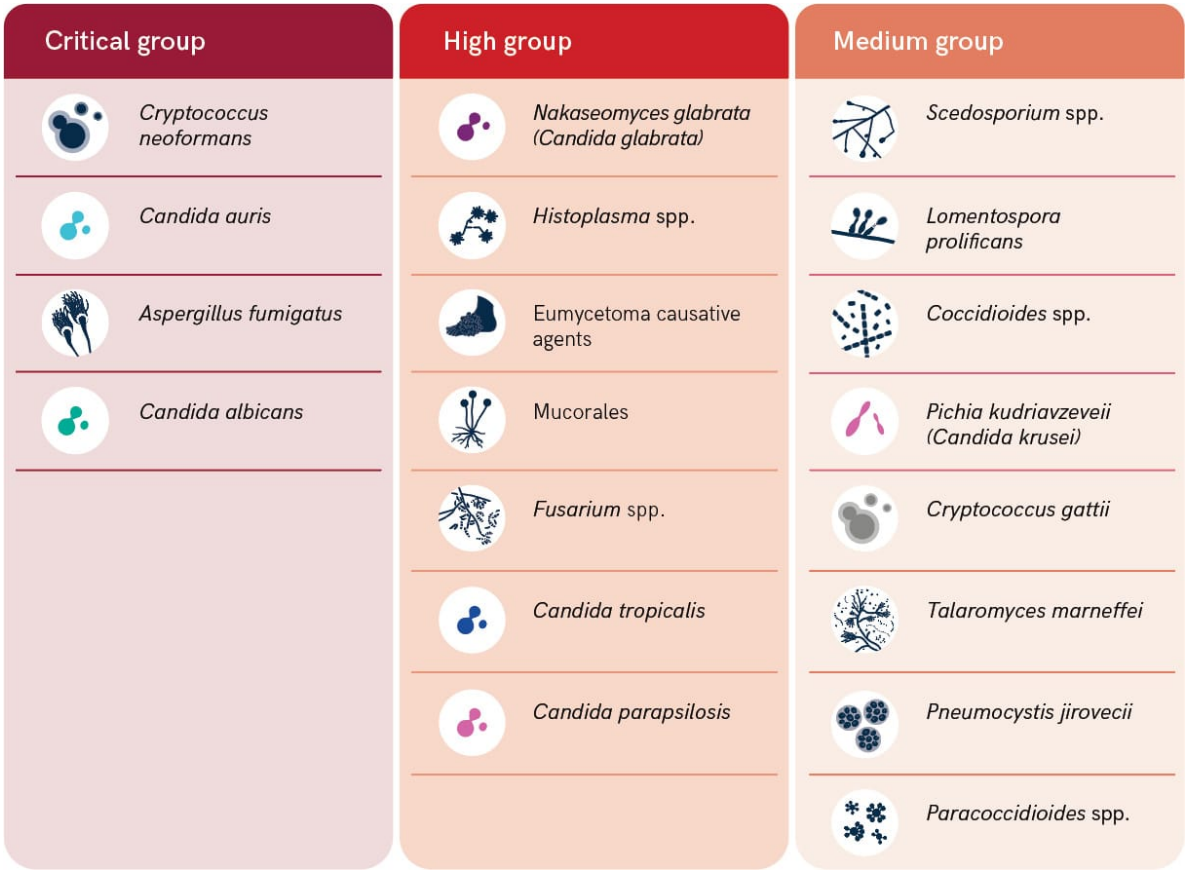
Dr. Zhang peers even further into the future with his hope that artificial intelligence will become a valuable tool. Noting recent advances in using AI-based early-warning systems to identify patients at risk for sepsis—“That’s very encouraging,” he says—he asks whether something similar might work for fungal infections. The potential is there, and AI coupled with more advanced technology might be a powerful way to improve diagnosis, he says.

## If fungal diagnostics have lagged their bacterial counterparts until recently, the same can be said of fungal therapeutics, Dr. Zhang says.

That’s starting to change, but the challenges are considerable, and many organisms are pan-resistant to current treatments.

Antifungal susceptibility testing is also behind the curve, compared with bacterial susceptibility testing. “It is challenging to develop antifungal breakpoints,” Dr. Zhang concedes, as the clinical data to support them are scarce. “You need to have pharmacodynamic/pharmacokinetic data. You also need to have clinical outcome data,” which are in short supply as well. But progress is not at a complete standstill, he says, noting that a breakpoint is now available for *Aspergillus fumigatus*.

Another approach—using the epidemiological cutoff values—can be useful in the interim. The ECV is not a clinical breakpoint, Dr. Zhang notes, because it’s based purely on the minimum inhibitory concentration distributions. Instead, its value lies in the fact that it can tell whether an organism is wild-type versus nonwild-type. This can provide clinicians with useful information about possible resistance and guide their selection of antifungal drugs.




**WHO fungal priority pathogens list**
Reproduced from *WHO Fungal Priority Pathogens List to Guide Research, Development and Public Health Action.* Geneva: World Health Organization; 2022.

## Given his expertise in the field, few things surprise Dr. Zhang about fungal infections.

The rise in *C. auris* was certainly notable in the wake of COVID-19, he says. But as he considers trends he's seeing at Johns Hopkins, throughout the country, and then across the globe, Dr. Zhang is hearing the word "pandemic" being whispered again.

No matter what, "We need to be more proactive," he says, "understanding from basic research science how these fungal organisms adapt to high temperatures and how any of them could be turning virulent to humans. And on the laboratory side we need to have better tools to increase our sensitivities and improve our speed."

Expanding the view of the problem is crucial, he continues, noting that the World Health Organization in 2022 recognized the problem as well, listing 19 fungal pathogens (see list, above) as having the greatest threat to public health and noting that new groups at risk of invasive fungal disease are constantly being identified (https://bit.ly/WHO-fungal). This is a key step, Dr. Zhang suggests. "I think it's a very important document that actually further calls attention to fungal infections."

There are a number of speculations about the changing fungal landscape, Dr. Zhang says. "Global warming is probably a driving force. Fungal organisms don't usually live on the human body temperature, but now they have adapted to live on humans," he says, with the potential to cause infections.

Is it reasonable to think about fungal infections in terms of another pandemic? "I don't know," Dr. Zhang says. It might have value in spurring experts and leaders to act more quickly. "Certainly the fungal threat to humans exists and is growing." It is a stark fact, he says—and one that, for now, remains largely unacknowledged.

*Karen Titus is* CAP TODAY *contributing editor and co-managing editor.*