The Emerging Fungal Threat

Once considered a nuisance, fungi are becoming a serious public-health hazard, particularly for the growing population of immunocompromised patients in hospitals

In the mid-1980s, when epidemiologist Richard Wenzel, then at the University of Virginia, began a study of infections acquired in the hospital, he expected to find the usual array of bacterial and viral diseases. Instead, he found something unexpected and potentially more alarming: Nearly 40% of all deaths from hospital-acquired infections in his study were due not to bacteria or viruses but to fungi. In most cases, the culprit turned out to be *Candida*, a virulent relative of baker's yeast. Wenzel's study, published in 1988, provided some of the first evidence that fungi had begun preying on the growing population of patients with impaired immune systems due to AIDS, cancer chemotherapy, or drugs designed to prevent rejection of transplanted organs.

Wenzel's study is part of a growing body of literature that is changing how researchers view fungi. Says Mitchell Cohen, director of the division of bacterial and mycotic diseases at the Centers for Disease Control and Prevention (CDC) in Atlanta: "When you think of diseases of the immune-compromised host, you now think of *Cryptococcus*, *Candida*, *Histoplasma*, *Coccidioides*, and all sorts of fungal agents that were almost unrecognized as important causes of disease."

And the threat isn't limited to patients with compromised immune systems. In January 1994, tremors from the Northridge earthquake near Los Angeles stirred fungi from the soil of Ventura County, propelling infectious spores into the atmosphere and causing 170 cases of disease. The outbreak capped a 3-year, statewide epidemic of fungal infections with case tolls that have spiraled from 400 to more than 4500 a year, according to CDC statistics.

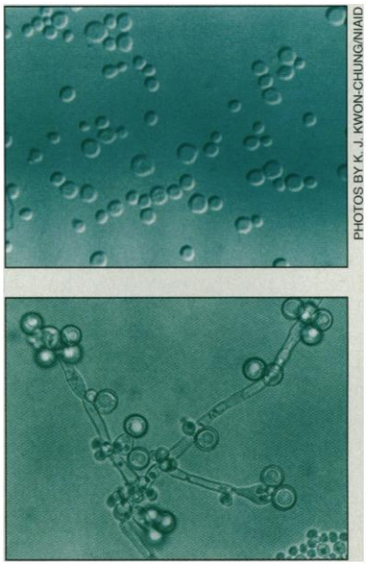
Indeed, fungal infections, once dismissed as a nuisance, have begun to spread so widely that they are becoming a major concern in hospitals, health departments, research laboratories, and pharmaceutical companies. As the case list mounts, public health officials say several factors make them uneasy about the nation's capacity to mount an effective defense. Their concerns include:

■ *Lack of drugs*. A drug known as Amphotericin B has become the mainstay of therapy for fungal infection despite side effects so severe that the drug is known as "amphoterrible" by patients. Only a few second-tier drugs exist, and until recently drug companies had invested relatively little in research because the market was so small.

■ *Increasing resistance*. Long-term treatment of oral candidiasis in AIDS patients has begun to breed species resistant to older anti-fungal drugs. Several other species of fungi have also begun to exhibit resistance.

■ *A growing list of pathogens*. Species of fungi that once posed no threat to humans are now being detected as a cause of disease in immune-deficient people. Even low-virulence baker's yeast, found in the human mouth, has been found to cause infection in susceptible burn patients.

■ *Lagging research*. Because pathogenic fungi are difficult to culture, and because many of them do not reproduce sexually, microbiological and genetic research into the disease-causing organisms has lagged far behind research into other organisms.



PHOTOS BY K. J. KWON-CHUNG/NIAID

Candid camera. *Candida albicans* enters the body as a ball-shaped yeast *(upper)*; in the body it develops threadlike hyphae and spherical chlamydospores *(lower)*.

As fungi begin to be taken seriously by the biomedical community, the pace of research has quickened, spawning innovative efforts—including attempts to engineer genes from virulent species into species that are easier to work with, providing better laboratory models (see Report by Liu on p. 1723). But whether these renewed efforts can keep pace with the resourceful fungi is an open question.

Radical departure

This turn of events is a radical departure for the fungi, which were once classified as plants but have since been reclassified as a separate kingdom of unmoving organisms that lack chlorophyll. Mycologists estimate that there are 100,000 species of fungi, ranging from baker's yeast to dermatophytes (fungi that cause ringworm and athlete's foot) to potentially invasive species such as *Candida albicans* and *Aspergillus*. As many as 150 of these organisms have now been linked to animal or human diseases.

Because fungi are relatively new as a serious threat to human health, the prevalence of fungal disease is a mystery. Indeed, although state health departments report surveillance data to CDC for 49 diseases, not one fungal disease is on the list. CDC's National Nosocomial Infections Surveillance System (NNISS), however, does collect data on two million nosocomial (hospital-acquired) fungal infections a year through a network of "sentinel" hospitals, and these provide data on the scale of the problem.

A CDC review of NNISS data from 115 hospitals that were in the network from 1980 to 1990 has found that over that decade the percentage of fungal infections in hospitals nearly doubled, increasing from 6% of patients to more than 11%. Like Wenzel, the CDC found that "most deaths in patients with fungemia were believed to be related to the [fungal] infection" rather than to the condition that brought them to the hospital.

The largest threat in hospitals, the CDC found, are species of *Candida*: Nearly 80% of the organisms that caused hospital-acquired infections were from that group. These fungi are ordinarily harmless denizens of the gastrointestinal and genitourinary tracts. But when a person's bodily defenses are diminished, the fungi can spread into the bloodstream, which carries them into the brain, heart, kidneys, eyes, and other tissues. *Candida* and other species of fungi can be transmitted through catheters and other invasive equipment.

The symptoms and severity of fungal illnesses depend on the site of infection. Oral candidiasis, which produces curdlike white patches on the tongue or mucus membranes, can be readily treated with drugs. Candidiasis of the eye is less readily treated, often rupturing the retina, clouding the aqueous humor, and causing blindness. In many cases of systemic infection, fever is the only initial symptom, but the effects of fungal infections can be widespread, including heart failure,

shock, and disseminated intravascular coagulation—sudden, catastrophic clotting of much of the blood.

Although fungal infections appear in organ-transplant patients, burn patients, and older patients (because immune function declines with age), they are often most devastating in AIDS patients. According to Thomas J. Walsh of the National Cancer Institute (NCI), the most common AIDS-associated fungal infections are oral and esophageal candidiasis, which occur in more than 70% of patients over the course of the disease. Even if science could eliminate the risk of *Candida*, however, other species of fungus would probably emerge to take its place. Among the other leading contenders are *Aspergillus*, *Histoplasma*, and *Cryptococcus*; *Cryptococcus* causes potentially fatal meningitis in from 7% to 10% of AIDS patients.

The invasion of hospitals by fungal infections happened so surreptitiously that most clinicians weren't aware of it. CDC's Robert Gaynes, head of NNISS, says the increasing death toll from fungal infections went unnoticed until investigators began trying to dissect the causes of hospital mortality. "It was not an abrupt change," he says. "But when you look at it after 10 years, there is a dramatic difference."

Among the healthy

Some types of fungal infections—those by the *Coccidioides* that emerged after the Northridge earthquake, for example—are found not only in hospitals but among healthy residents of regions where a particular species of fungus has become endemic. Such endemic fungi, primarily *Coccidioides* and *Histoplasma*, reside in the soil and are usually harmless to humans. But disruption of the soil releases them into the atmosphere, where their spores infect anyone who breathes the air.

The California epidemic shows just how widespread these fungal infections can become. Skin-test studies of nearly 300,000 military recruits between 1958 and 1965 found that *Coccidioides* were endemic in the San Joaquin Valley. According to a recent CDC analysis, the current epidemic was triggered by a cycle of torrential rains and droughts, which allowed the fungus to proliferate. The Northridge earthquake exacerbated the problem by stirring the soil, disseminating the fungi widely. Symptoms among those infected range from none to short-lived bouts of flulike illness to deadly extreme cases in which the fungi spread through the patient's entire body. These disseminated forms are now found with some frequency among immunocompromised patients in endemic areas.

Although fungal infections do not yet approach the magnitude of bacterial and viral epidemics, their cost can be high. In Kern County, where 70% of the California cases are concentrated, a review of medical records by the CDC found that the 3-year epidemic has rung up medical bills totaling $45 million for hospitalization and outpatient care.

A similar pattern of endemic fungi breaking out in epidemics is seen with the soil fungus *Histoplasma*. The study that localized *Coccidioides* to the San Joaquin Valley found that the endemic area of *Histoplasma* extends through the Midwest into Texas. In this endemic zone, the organism has been responsible for repeated outbreaks of infection, known as histoplasmosis. Indianapolis, for instance, has experienced at least three since 1978, but neither the source of infection nor the conditions promoting its spread have been determined. Although those outbreaks have dwindled, the fungus has emerged as the leading opportunistic infection in AIDS patients in Indianapolis, according to L. Joseph Wheat of the Indiana University School of Medicine.

Stories like these have greatly sensitized biomedical researchers to the need to control fungal pathogens. After decades of relative neglect, emerging epidemics have renewed interest in new ways to prevent and treat fungal infections. Wenzel, now at the University of Iowa, who pinpointed the toll from hospital-acquired fungal infections, is turning his research into ways to prevent hospital patients from falling prey to fungi.

In a 1991 study of hospital-acquired pneumonia in bone-marrow transplant patients, Wenzel found that more than one third of 55 cases were caused by the fungus *Aspergillus*; 85% of the cases were fatal. The study demonstrated, says Wenzel, that even though bone-marrow transplants are intrinsically risky, many fewer patients would have died of their transplant or complications had they not developed *Aspergillus* pneumonia.

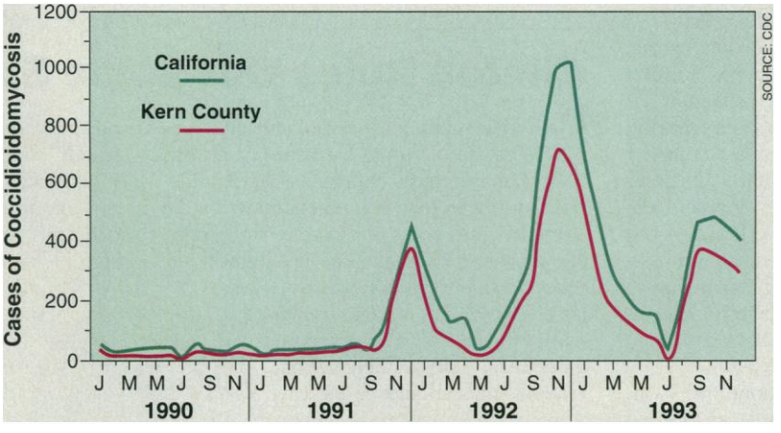
Alarmed by Wenzel's data, hospital administrators at the University of Iowa agreed to turn the rooms for bone-marrow transplant patients into "laminar-flow rooms," which draw in contaminated air before filtering it and expelling it outside the hospital. That move had "a profound affect on our *Aspergillus* rate," says Wenzel. "Before the renovation *Aspergillus* accounted for 36% of all pneumonias. Now it's about 10%. Overall mortality before was 75%. We had hoped we would be able to reduce that to 45% overall. We're pretty close to that."

Search for new drugs

Although this strategy for prevention was apparently successful, treatments for fungal infections have lagged far behind new treatments for bacterial infections. Says John Edwards, an infectious-disease specialist and mycoses researcher at the Harbor–University of California, Los Angeles, medical center, "Fungi have a big head start on the pharmaceutical industry." One reason the industry is off to a slow start has been the comparatively small market; another is the difficulty of developing effective anti-fungals.

One factor in the success of antibiotics is that bacteria are prokaryotes. Prokaryotic cells have distinctly different properties from the eukaryotic cells of mammals, which have nuclei surrounded by membranes. For this reason researchers have been quite successful at finding drugs that target bacteria without harming human beings. Fungal cells, on the other hand, are eukaryotic, like human cells. Because the main structures of the two cell types overlap, drugs that target the main fungal-cell structures have proven to be highly toxic to patients.

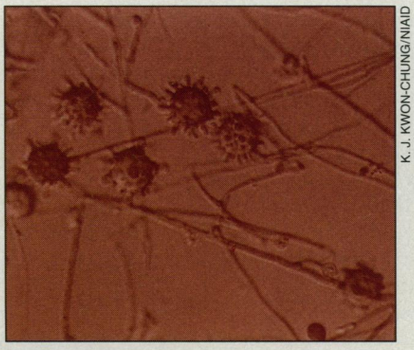
Indeed, all three main groups of anti-fungal compounds now in use can be toxic. Chief among them are the polyene derivatives, which include Amphotericin B and its kin, nystatin and pimaricin. These are the broad-spectrum anti-fungals that attack ergosterol, lipidlike solid alcohols that compose fungal cell membranes. Because human cell membranes contain cholesterol rather than ergosterol, the drug does not readily break them down. Still, the drug is highly toxic—among its side effects are fever, chills, low blood pressure, headache, nausea, vomiting, inflammation of blood vessels, and kidney damage—and it is recom-



Boom and bust. In Kern County, California, an epidemic of infections with soil fungi known as *Coccidioides* may be due to a cycle of droughts and heavy rains.

mended only in severe cases.

But Amphotericin B remains the most effective anti-fungal drug on the market, and several companies are experimenting with liposomal formulations of this and other polyenes, which researchers hope will reduce the drugs' toxicity and perhaps even improve their efficacy, says NCI's Walsh. Fujisawa-USA Inc. of Chicago and Vestar Inc. of San Dimas, California (in a collaboration); the Liposome Co. of Princeton, New Jersey; and Liposome Technology Inc. of Menlo Park, California, are working with Amphotericin B; Argus Pharmaceuticals of Houston is working with nystatin.



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Dirty business. Spores and hyphae of the soil fungus *Histoplasma capsulatum*.

The other two classes of anti-fungals are less toxic than amphotericin B, but they have their own drawbacks. The azole derivatives, including ketoconazole, fluconazole, and itraconazole, are synthetic compounds that interrupt synthesis of ergosterol. Although not as difficult to tolerate as the polyenes, they do have side effects. The third class are the allylamines-thiocarbamates, used mostly for treating skin infections.

The growing market for these drugs, as illustrated by the success of fluconazole, which suppresses *Candida* and has found a large market among AIDS patients, has prompted drug companies to begin making up for lost time. One approach is to target a fungal-cell structure that human cells don't share: the cell wall. Two types of potent experimental agents that attack cell walls—the echinocandins and the pneumocandins—appear particularly promising, according to Walsh; these drugs, which are being developed by Eli Lilly and Co. and Merck & Co., Inc., are almost ready for clinical trials.

Such efforts are coming none too soon, because new approaches are urgently needed to outpace drug resistance (which is now cropping up against some azole derivatives) and to keep pace with once-benign fungi that are being seen in clinics. "Microbial resistance has become a particular problem in AIDS patients who've been treated repeatedly for thrush [oral candidiasis] with small doses of the azoles, usually fluconazole, itraconazole, and ketoconazole," says UCLA's Edwards. "It is common in most centers where AIDS patients are being treated deep into the course of their disease."

The pattern of new, or newly resistant, species emerging in response to widespread and prolonged drug treatment, particularly with the azoles, is not limited to AIDS patients, adds Gaynes of CDC. Several new species of *Candida* have emerged as tormentors in the last decade, he says. Among them are *Candida krusei*, which generally afflicts patients with solid tumors or leukemias; *Candida lusitanae*, a new cause of infection in people who have had cytotoxic or long-term anti-microbial treatment and who have had in-dwelling catheters; and *Candida lypolytica*, a mildly virulent organism that has so far been isolated from just six patients.

While *Candida* has long been known as a pathogen, some other species of yeasts that were once considered benign are now being seen in destructive form in immunocompromised patients. According to a report by Bertrand Dupont of the Institut Pasteur in Paris, *Trichosporon*, *Rhodotorula*, *Malassezia*, and even *Saccharomyces cerevisiae* (baker's yeast) have been observed as causes of systemic infection. "As we suppress susceptible species," says Nafsika Georgopapadakou of the Roche Research Center in Nutley, New Jersey, "others begin to take over."

If the new drugs aren't sufficient to address these emerging killers, there is another hope: vaccines. John Robbins of the National Institute of Child Health and Human Development and John Bennett of the National Institute of Allergy and Infectious Diseases are conducting clinical trials of a vaccine designed to prevent *Cryptococcus* infection in AIDS patients. Several groups of investigators have begun looking at candidate vaccines against *Coccidioides* and *Histoplasma*. The antifungal vaccine effort is "gaining momentum" and has "much promise," says Walsh.

The recent CDC report on the outbreak in California says a vaccine is probably the best hope for preventing future epidemics in endemic areas. But the report comes to stark conclusions about the nation's defenses against fungal infections. CDC acknowledges that surveillance of fungal infections is "generally inadequate" and that vast gaps remain in medicine's understanding of the "environmental, behavioral, and host risk factors for acquiring infection and developing [fungal] disease." As researchers search for ways to fill those gaps, medicine may begin to catch up with the opportunistic and once-benign fungi, in spite of the running start those organisms have on science.

–Steve Sternberg

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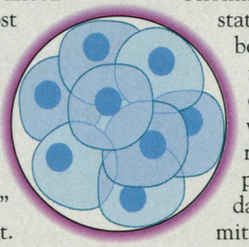
HUMAN EMBRYO RESEARCH

Clinton Rules Out Some Studies

On 2 December, a top-level advisory committee at the National Institutes of Health (NIH) unanimously endorsed a set of guidelines for research on human embryos, crafted over the past year by a commission of researchers, ethicists, and lawyers. Eight hours later, President Bill Clinton announced that he couldn't accept the most controversial of the proposed guidelines. "I do not believe that federal funds should be used to support the creation of human embryos for research purposes, and I have directed that NIH not allocate any resources for such research," Clinton said in a public statement.



Clinton's announcement—widely perceived as an attempt to fend off conservative critics—represents "the president's position" on "a very controversial subject," says M.R.C. Greenwood, associate director for science at the White House Office of Science and Technology Policy. Greenwood noted, however, that the statement should be "very narrowly construed." It explicitly forbids only the use of NIH funds for the creation of embryos for research, such as the study of processes of egg maturation and fertilization. The president presumably has not ruled out other areas of human embryo research covered by the guidelines, which would end a 13-year ban on such research.

Among those apparently surprised by Clinton's announcement was NIH Director Harold Varmus. NIH spokesperson Anne Thomas says Varmus saw the text of the statement just half an hour before it became public. Varmus didn't mention the impending presidential announcement last week when the NIH Director's Advisory Committee considered the proposed new guidelines during a 2-day meeting. The advisory committee accepted the guidelines, which will pave the way for Varmus to implement those not covered by Clinton's statement. The guidelines permit studies on in vitro fertilization (IVF) techniques and basic biological research on "spare embryos" from IVF clinics up to the 14th day of development (*Science*, 19 August, p. 1024).

The president's edict banning the creation of embryos for research purposes leaves some questions hanging, however. For example, it's not clear whether NIH will be allowed to fund research on human parthenotes (eggs that have been artificially stimu-