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TITLE: Diagnosis of Dimorphic Fungi Endemic to the United States

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Slide 1:

Hello, my name is Kristen Smith. I am a Clinical Microbiology Fellow at the University of Rochester in Rochester, New York. Welcome to this Pearl of Laboratory Medicine on "Diagnosis of Dimorphic Fungi"

Slide 2: Mycology Terminology

Mycology is the study of fungi, which are eukaryotic organisms that contain a cell wall, have a true nucleus, and undergo cellular division. Dimorphic fungi are those microorganisms that are able to exist in the environment as a mold and as a yeast when inside a host. Molds are multicellular organisms that are composed of basic units termed hyphae. In general, this is the phase that the dimorphic fungi exist in the environment or at temperatures less than 35C. When the temperatures rise to greater than 35C, the fungi are able to transition to yeast, which are unicellular. The ability to become dimorphic, or exist in two distinct forms, is a virulence factor of these organisms. Within the host, the yeast are engulfed by macrophages but are not destroyed, which allows for the organism to become intracellular and evade the host's immune system.

Slide 3 Dimorphic Fungi

There are three major dimorphic fungi that are endemic to the United States: *Histoplasma capsulatum*, *Blastomyces dermatitidis,* and *Coccidioides immitis*. They all most typically present as pulmonary infections that can be described as 'flu-like', which may include fevers, chills, night sweats, cough, or fatigue. If the host is unable to control the infection, dissemination from the lungs to virtually any other location in the body can occur. This is why those that are immunocompromised, such as the elderly,

pregnant woman, HIV/AIDS and transplant patients, and individuals on anti-inflammatory medication are at particular risk. Additionally, those of African or Filipino descent are more at risk of coccidioidomycosis as well for reasons that are not well described. It is important to note that these infections are not communicable, or capable of being spread from person to person.

Slide 4 Histoplasma capsulatum

Histoplasma capsulatum, the causative agent of Histoplasmosis, was first described in 1906 by Samuel Darling while working in the Canal Zone in Panama. Since this time, two variants of *Histoplasma capsulatum* that cause human disease have been described. *H. capsulatum* var. *capsulatum* is found in north and south America while *H. capsulatum* var. *duboisii* is endemic in Africa and has been observed in Europe when patients seek treatment there. *Histoplasma* is found in soil, particularly when the ground is fertilized with nitrogen rich bird droppings or bat guano. In the United States, the pathogen is found in the Ohio and Mississippi River Valleys and the region around the Great Lakes. Currently, only Arkansas, Delaware, Illinois, Indiana, Kentucky, Michigan, Minnesota, Nebraska, Pennsylvania, Puerto Rico, and Wisconsin require reporting of Histoplasmosis.

Slide 5 Identification of H. capsulatum

Identification of molds relies heavily upon observations of known growth characteristics. The specimen, most often from a respiratory source such as sputum, bronchoalveolar lavage, or even a section of the lung is inoculated onto a specialized media, such as Sabourand's Dextrose Agar, that inhibits the growth of commensal organisms. The media are placed in an incubator between 25 and 30C and is checked for fungal growth. *H. capsulatum* can take several days to weeks to become mature. The colony will be either white or tan in appearance and can be described as 'fluffy, The image on the left of the slide is a typical example. Next, part of the colony is stained with lactophenol cotton blue and a tease prep is performed, which will allow for visualization of the microscopic structures and also kill the organism. The figure on the top right of the slide shows the mycelium, or connected fungal cells, along with conidia or fungal spores. *H. capsulatum* has two different types of conidia – microconidia which are the smaller structures coming off of the mycelium and the tuberculate or macroconidia. The infectious component of this microorganism is the microconidia. The photograph on the bottom right shows a lactophenol cotton blue stain of *H. capsulatum.* Note the large tuberculate macroconidia, which are a hallmark of this mold. In the yeast phase, *H. capsulatum* is approximately 2 to 4 um and is oval. In histopathology images, the yeast can either be present intracellular or extracellularly and it will appear encapsulated, hence the name *H. capsulatum.* This is however a misnomer, as no capsule is present on the yeast.

Slide 6 Blastomyces dermatitidis

Blastomyces dermatitidis was first identified by Thomas Gilchrist in Baltimore, Maryland in 1894. This fungus causes blastomycosis, which has also been referred to as both Gilchrist's mycosis and Chicago's Disease. The organism is typically thought to be endemic in the Ohio and Mississippi River Valleys, similar to *H. capsulatum*. However, this is based on cases and not on serology, as there is not a sensitive and specific serological or skin test for *B. dermatitidis.* Based on incidence rate, the majority of cases are found in Wisconsin with a rate of approximately 10 – 40 incidences/ 100,000. Following Wisconsin, cases are more frequent in Arkansas, Illinois, Kentucky, Louisiana, Mississippi, North Carolina, and Tennessee. It is also interesting to note that this organism is ten times more likely to infect dogs than humans. This is likely due to the fact that dogs spend more time outdoors and in a closer proximity to the soil, where the spores originate. Blastomycosis is similar to Histoplasmosis in that it typically presents as a pulmonary disease via inhalation of conidia with a slightly longer incubation period of about 4 to 6 weeks. While uncommon, the disease can also cause primary cutaneous infection after traumatic injury. Additionally, the skin, bones, and genitourinary tract are common sites of disseminated disease. Up to 50% of cases are asymptomatic and many times this disease presents with common symptoms such as fever and myalgias. At this time, blastomycosis is reportable in Arkansas, Louisiana, Michigan, Minnesota, and Wisconsin.

Slide 7 Identification of B. dermatitidis

Identification of the mold again begins with growth on selective media. *B. dermatitidis* will appear white and fluffy, very similar to Histoplasma, and may turn yellow or tan as the colony matures. On the top right you will see a sketch of the microscopic mold structures. Again, note the septate hyphae of the mycelium. The conidia are oval or pear-shaped with a diameter in the range of 2 to 10 uM, and there are no macroconidia. On the bottom right, you will see the yeast form of *B. dermatitidis.* The cells are thick walled and are 5 – 15 uM in diameter. The hallmark of this organism is the broad base of the dividing yeast cell. When inside a host, the yeast can be inside or outside of the macrophages. When comparing to *H. capsulatum*, this yeast is much larger in size. *B. dermatitidis* is typically larger than a red blood cell and comparable in diameter to a white blood cell.

Slide 8 Coccidioides

The final dimorphic fungi that will be discussed is the most commonly occurring in the United States, *Coccidioides.* This fungi was discovered in 1892. There are only two species within the genus, *Coccidioides immitis* and *Coccidioides posadasii*. Isolates from California are *C. immitis*, whereas isolates from other areas in the southwestern United States are *C. posadasii.* Unlike the other two dimorphic fungi discussed, *Coccidioides* are more prevalent in arid regions. There are many names for the same

disease: Coccidioidomycosis, Valley Fever, San Joaquin Valley Fever, and Desert Rheumatism. The mold is endemic in the desert Southwest, which is evidenced by positive skin tests. Similar to the tuberculin skin test for tuberculosis, antigenic material from *Coccidioides* can be used to test for prior exposure. The incubation period can range from one to three weeks and once again, the primary presentation is that of a flu-like illness with fever, chest pain, cough, chills, and night sweats. One important difference to note is that infection can confer immunity and prevent future re-infections. In general, illness caused by *Coccidioides* is mild, with a majority of cases thought to be sub-clinical. Approximately, 3% of infections are more serious and can lead to disseminated disease, typically in the immune-compromised population. Valley fever is reportable in Arizona, Arkansas, California, Delaware, Louisiana, Maryland, Michigan, Minnesota, Missouri, Montana, Nebraska, Nevada, New Hampshire, New Mexico, North Dakota, Ohio, Oregon, Rhode Island, South Dakota, Utah, Washington, and Wyoming.

Slide 9 Identification of Coccidioides

The plate on the left shows a large glabrous, or smooth, and tan colony of *C. immitis.* Unfortunately, there is a glare because of the lid of the plate. Also notice the yellow tape that is sealing the plate. All molds should be contained in a similar fashion, as spores are easily disturbed and can spread throughout the environment, posing a health threat to laboratorians and anyone else nearby. Molds should be investigated within a biosafety cabinet and by trained staff only. On the right is an image taken by Eileen Rojas. Note the arrow pointing to the infectious particles of the microorganism. The barrel shaped conidia, or arthroconidia, are the hallmark of *Coccidioides*. The arthroconidia are 2 to 8 by 3 to 5 um. This is the structure that would be found in the soil or on media in the lab. However, like the other dimorphs, *Coccidioides* exists in a different form inside the host. The arthroconidia enlarge, forming spherules, and form internal septations that become endospores, which can be released to infect additional cells. These structures are drawn in the bottom right side of the slide. Once an arthroconidia infects a susceptible host it will transform into the immature spherule, which will further mature until it contains a large number of endospores (on the right). Spherules are large in comparison to the arthroconidia, reaching close to 100 um in diameter and containing anywhere from 100 to 300 endospores. The endospores will be released and are then able to become spherules themselves. This cycle will continue until the host's immune system generates a response or proper treatment is given.

Slide 10 – Diagnosis

When considering the pulmonary clinical picture, dimorphic fungi would be on the same differential as other infectious diseases such as tuberculosis, aspergillosis, and bacterial pneumonia, as well as other etiologies such as lung cancer. It is also important to remember that the dimorphic fungi can cause disseminated disease, especially in immunocompromised hosts. There are several available tests to diagnose the dimorphs described here, but the availability of the tests may vary between institutions.

For those with expertise in mycology, pulmonary specimen such as sputum, BAL and lung biopsies can be submitted for slide staining with calcufluor white and for culture of the organisms. Organisms may also be visible in samples submitted for pathology, especially when stained using GMS. Using a fungal isolator for blood samples is useful for the detection of disseminated *Histoplasmosis*, as is urine, skin lesions, lymph nodes, and bone marrow. CSF culture, however, has decreased sensitivity. Culturing of skin biopsies from lesions in patients with *Blastomyces* can also result in recovery of organism. While culture is the gold standard, it can take several weeks for results due to the slow growth of the organisms. An alternative method of detection of the organisms would be to utilize molecular methods such as PCR and sequencing. Further, antigen detection and serology testing can be performed in specialized reference labs and in some cases, reagent kits are available and may be used in some clinical labs. The sensitivity and specificity varies for these rapid methodologies and there can be cross-reactivity between the dimorphic fungi as well as other fungi. In general, sensitivity is better in disseminated disease. For *Histoplasma*, the *Histoplasma* polysaccharide antigen can be detected in urine, serum, and CSF with a sensitivity of approximately 95%. The antigen tests for *Blastomyces* (urine and serum) and *Coccidioides* (urine) are not as sensitive, which are ~89 and 71%, respectively. Serological tests may be less useful clinically, as many weeks are required for antibodies to be generated in a primary infection. Additionally, immunocompromised patients may not mount significant responses, which could give falsely negative results. If one of these dimorphic fungi is suspected, a multifaceted approach is best – obtain the correct specimen for culture, alert the pathologists so that they can use appropriate stains, and test for antigen.

Slide 11 – IDSA Treatment Guidelines

Here I've summarized IDSA treatment guidelines. For both histoplasmosis and coccidioidomycosis, treatment is not recommended until disease is severe or persistent. For histoplasmosis, Itraconazole is the azole of choice as it has been shown to be more efficacious than fluconazole. The duration of treatment is 6 to 12 weeks in mild to moderate cases and in severe disease amphotericin B is included for a shorter duration prior to azole treatment. For coccidiomycosis, there is no distinction made for itraconazole over fluconazole, so either can be utilized. On the other hand, Blastomycosis should be treated regardless of the classification of disease at initial presentation so that dissemination of the disease can be avoided. Itraconazole is recommended once again, as is amphotericin B for severe disease prior to azole treatment. The duration of treatment is increased for this pathogen and is recommended for 6 to 12 months.

Slide 12 – Summary

In summary, the dimorphic fungi are important pathogens that are capable of causing serious disseminated disease. Those described here are the most commonly seen that are endemic to the

United States. Location can help distinguish Coccidioides, but Histoplasma and Blastomyces have an overlapping region of endemicity. Incubation periods can range drastically, but are typically weeks after the exposure. They all primarily present as a pulmonary infection with generalized symptoms similar to flu or pneumonia. Diagnoses can be made if the hallmark structures are observed in pathology slides or from growth in the microbiology laboratory, which are more specific than antigen or serology testing. It is possible for reactivation of these organisms if they are initially controlled by the immune system, however re-infections may be more likely as immunity is not conferred after infection with Histoplasma or Blastomyces. There is evidence to suggest that a primary infection with Coccidioides generates an immune response that will protect against reinfections.

Slide 13: References

Slide 14: Disclosures

Slide 15: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “Diagnosis of Dimorphic Fungi Endemic to the United States”