Mycology at a Distance

ELMER KONEMAN, WAYNE GADE

GermWare Mycology is an image-rich, CD-ROM-based instruction divided into tutorial and reference programs. The tutorial program, designed for new students, provides only for sequential progress through each of the subject modules, so that each page of information is seen. In contrast, the reference program allows the more experienced learner with random and direct access to each facet of information. The aspergilli, the agents of chromomycosis, the dermatophytes, the dimorphic fungi, the hyaline molds, the dematiaceous molds, the yeasts, and the zygomyces are divided into separate modules. The tutorial program also includes an opening 'isolation procedures' module, in which details of specimen collection, culture media, and microscopic techniques are presented. The random access program includes system maps separating out each of the fungal species, and flow diagrams allowing an algorithm approach to species identifications. A global map is also included through which each fungal species can be directly accessed by the simple click of the mouse. Random access to information on the ecology, clinical presentations, pathology, and therapy of the various mycotic diseases is also a feature of the reference program. A series of self-assessment exercises is included at the end of each module, with immediate 'pop-up' feedback to both correct and incorrect answers. The entire program includes over 2500 screens and over 700 color images and diagrams. GermWare Mycology is available through the Colorado Association for Continuing Medical Laboratory Education (CACMLE), who also can provide continuing education credits for individuals who complete a separate examination. For more information contact CACMLE at (303) 321-1734 or info@cacmle.org.

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CONSTRUCT OF THE DISTANCE LEARNING COURSE

This paper describes a distance-learning course in medical mycology presented to the clinical laboratory science (CLS) students enrolled in the Medical Technology Program at the University of Wyoming. The course was eight weeks in duration, and individual modules within the *GermWare Tutorial and Reference Program* by Elmer Koneman served as the basic study materials. The book, Fundamentals of Diagnostic Mycology by Fran Fisher and Norma Cook, provided supplementary material for optional study. Except for an introductory, on-site lecture covering the fundamental techniques and procedures required to recover and identify the fungi of medical importance, no additional formal presentations were made.

Each week, study modules from the *GermWare* program were assigned for preliminary study and a case study related to the organisms being studied was assigned to each student. This format was selected to provide students with 'real life' applications of the didactic and illustrative material included in the *GermWare* program. Results of the discussions and the final exam were evaluated to determine the relative value of these self-study exercises compared to the more conventional lectures.

Each of the five students was assigned a case history to present and discuss during the weekly one-hour teleconference session. A printout from a PowerPoint presentation was given to each student a few days before the teleconference. In addition to relevant case information, each printout contained a series of questions related to each informational slide. These questions helped generate discussion and focused the student toward establishing the final diagnosis. During the teleconference sessions, laptop computers at both sites were used to project case information via PowerPoint images, enabling Dr Koneman in Denver, Colorado and Dr Gade and the students in Laramie, Wyoming to view the materials simultaneously.

Also as part of the course, the students were required to set up a series of three or four unknown specimens related to each of the study modules. The results of these unknown specimens were also discussed during the teleconferences.

To demonstrate the dynamics of these teleconference sessions, the following is an account of one of these case study interactions:

CASE STUDY

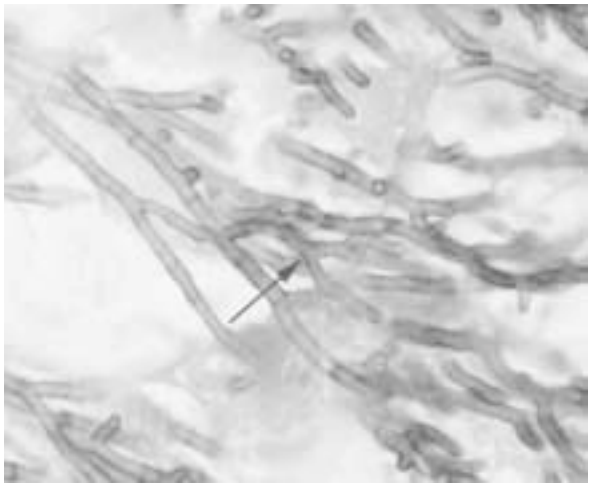
A 30-year-old bone marrow transplant recipient post-operatively developed a feeling of fullness and pain under the right cheek, accompanied by myalgia and malaise. These symptoms lasted for two weeks, after which a physical examination revealed that the overlying skin appeared red, was warm to touch, and pain could be elicited by applying gentle pressure over the right maxillary sinus. A sinus x-ray revealed opacity of the right maxillary sinus. This clinical picture remained essentially unchanged after one week of empiric antibiotic therapy.

DISCUSSION

Teleconference instructor: "Realizing that you, as a student in CLS, are not involved in establishing a clinical diagnosis, nevertheless, what features in this patient's history might suggest a fungal infection?"

Student response: "Perhaps the prolonged infection, despite antibiotic therapy. If the infection were due to a bacterium, the infection would probably be resolved during the course of antibiotic therapy. Of course if the wrong antibiotic were prescribed, a bacterial infection could go on also."

Figure 1. GMS section of sinus material including dichotomously branching hyphae



"Illustrated are regular sized (4µ to 5µm in diameter) hyphae with parallel walls and occasional cross septations. Note the 45 degree branching, which at the tip of the black arrow is 'dichotomous'; that is, a branch on an already branched hypha, much as the growth of tree limbs. This is characteristic of *Aspergillus species*."

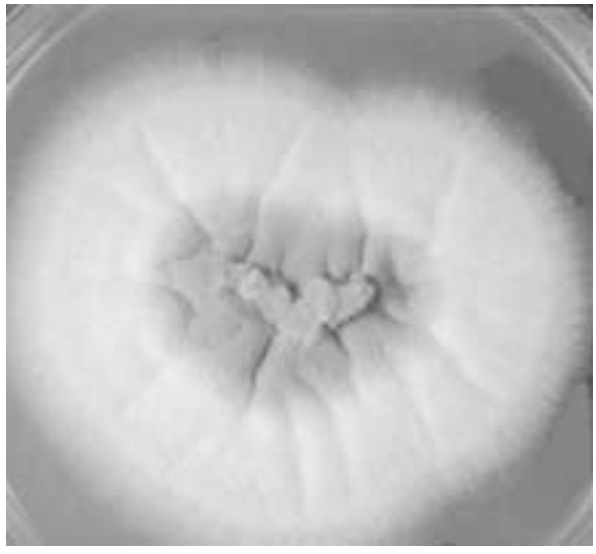
Tissue was aspirated from the infected sinus. Figure 1 is a photomicrograph of a Gomori methenamine-silver (GMS) stain of a tissue section of the aspirated material.

Teleconference instructor: "Again realizing that CLS students are not required to read stained tissue sections, nevertheless, you may be asked to render an opinion by a pathologist. Since fungi do not appear all that differently in tissue sections than in a lactophenol mount, what observations can you make concerning this fungus? Can you determine what group of fungi may be ruled out from the photomicrograph shown on the next slide?"

Student response: "Well, it appears that the hyphae seen in this picture are regular in width and are definitely septate. The Zygomycetes have broad, irregular cell walls that are aseptate. I think that these fungi can be ruled out.

Teleconference instructor: "Good for you. As you have not yet studied the yeasts in this course, I will just relate that the regularity of the cell walls in this isolate, without evidence of 'pinching', pretty well rules out the pseudohyphae of *Candida* species. Do you remember any fungus group that is characterized by the 45° angle

Figure 2. Colony of *Aspergillus terreus*



The colony shown in this photograph grew on Sabouraud's dextrose agar after four days incubation at 30 °C. The margin is entire. The surface is suede to granular in consistency. The older central portion has a brown pigmentation; the remaining colony is buff in color. A few shallow radial rugae are seen centrally. This colony is consistent with *Aspergillus terreus*.

branching seen on the screen you are viewing, or what groups of fungi that can perhaps be ruled out?"

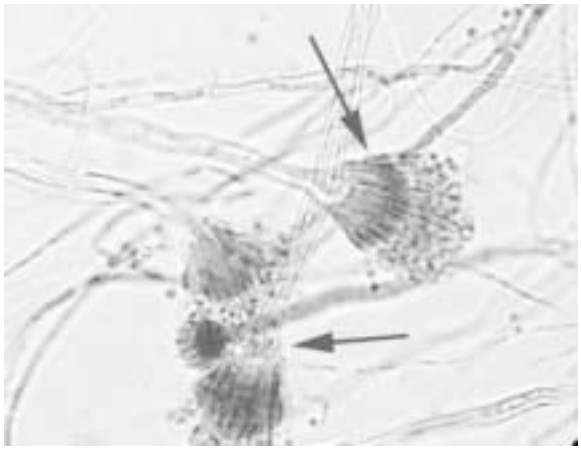
Student response: "A number of hyaline molds may have regular walls and septate hyphae that look like these. However, as I remember, only *Aspergillus* species shows this type of 45° angle branching."

Teleconference instructor: "All right, let us see where that takes us. Move to the next screen (Figure 2). This colony was recovered on Sabouraud's dextrose agar after a five-day incubation at 35 °C. Describe what you see and what you might be able to rule out."

Student response: "The plate certainly doesn't look like a yeast. I see a mold that has a yellow-brown color and a rough surface. I guess it also doesn't look like any *Penicillium* I've ever seen."

Teleconference instructor: "OK. What do you think about the appearance of this mature growth after only five days on a nutritionally poor medium such as Sabouraud's agar? Does this rule out any group of fungi?"

Figure 3. Lacto aniline blue mount of material taken from the colony shown in Figure 2.



"In this photomicrograph are illustrated the fruiting heads of *Aspergillus terreus*. The conidiophores end in a bulbous vesicle (lower black arrow), from the top one-half of which are derived phialides (upper black arrow), giving rise to short chains of conidia. Although sporulation from the top half of the vesicle is suggestive of *Aspergillus fumigatus*, note that the phialides are much too long. The phialides of *A. terreus* are in two rows (biseriate), although careful focusing of the objective is necessary to find the line of separation.

Student response: "Again, the Zygomycetes. They grow more rapidly than this and produce colonies that don't have distinct borders like we see on this screen."

Teleconference instructor: "And what fungi usually grow much slower than this in primary isolation? Do you remember?"

Student response: "I believe the dimorphic fungi grow pretty slowly, but we haven't studied these in our course yet."

Teleconference instructor: "Ah, so. Unfair question! But you are right on target. In most instances, at least a week or more are required for the dimorphic fungi to grow, if even that soon on Sabouraud's dextrose agar. Also, some of the dermatophytes grow slowly. Do you think the colony is consistent with *Aspergillus* species?"

Student response: "No. I read that *Aspergillus* have rough or granular surfaces like this picture. And the book said their distinctive characteristics are distinct margins and white aprons."

Teleconference instructor: "Assuming that we are in the right genus, does the colony morphology tend to rule out any particular *Aspergillus* species?"

Student response: "Sure. *Aspergillus niger* would be black and look peppery. Also, I think most strains of *Aspergillus fumigates* are green or blue green. I'll bet that this isolate is *Aspergillus flavus*. Doesn't 'flavus' mean yellow?"

Teleconference instructor: "Yes it does. Good work. Now, I believe we have gone about as far as we can without a microscopic examination. The next screen (Figure 3) is a lactophenol analine mount prepared from the surface of the colony just seen. Tell me what you see and where your observations might lead you."

Student #1 response: "The CD said that four molds commonly seen in the clinical laboratory produce conidia in chains, as we see here—*Penicillium* and *Aspergillus*."

Student #2 response: *"Paecilomyces* and *Scopulariopsis* are the other two common species that produce conidia in chains."

Teleconference instructor: "That's right, good."

Student #1 response: "In this picture I see a distinct, round vesicle. I think *Penicillium* or *Paecilomyces* produce fruiting heads that branch like trees, don't they?"

Teleconference instructor: "Right again. The term for the branching elements is the phialides — p-h-i-a-l-i-d-e-s. *Scopulariopsis* also does not produce a vesicle, and the conidia are much larger than seen here. So what is your guess?"

Student response: "Again, *Aspergillus*. Both the culture plate and fruiting head looked like an *Aspergillus*."

Teleconference instructor: "I am most impressed with your understanding of the microscopic features of this group of hyaline molds. You said before, however, that you thought the colony looked more like *Aspergillus flavus*. Do you still believe this?"

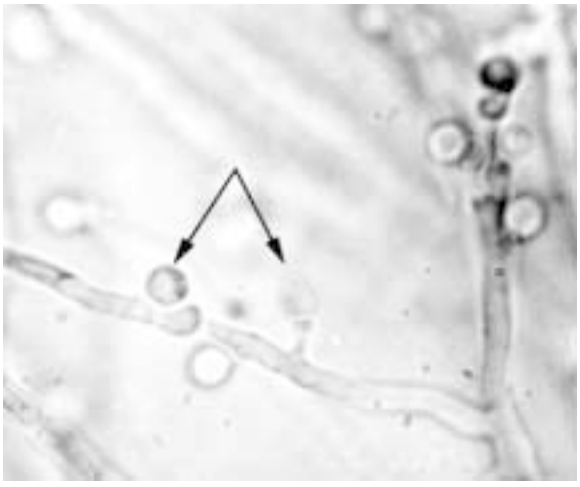
Student response: "Well, I can see you are boxing me into a corner. The fruiting head here shows some long phialides only on the top half or so of the vesicle. Don't the spores of *Aspergillus flavus* go all the way around the head?"

Teleconference instructor: "Yes, so what else comes to mind."

Student response: "I guess this microscopic picture looks more like '*fumigatus*'. Do you ever get brown colonies like this with *fumigatus*? Could be strike two on me!"

Teleconference instructor: "Yes, the culture plate argues against *fumigatus*. But take a closer look. Notice how long the phialides are in this picture. The phialides for *fumigatus* are typically quite short. Anytime you see phialides this long; you probably are looking at *Aspergillus terreus*. They are supposed to be in two rows, which we call biseriate; however, it often is difficult to see a line of separation because of the way they interdigitate. But the long length is the clue. Do you remember a more direct way to confirm the identification of *Aspergillus terreus*?"

Figure 4. Subsurface hyphae of *Aspergillus terreus* illustrating microconidia



Illustrated in this photomicrograph is a portion of the subsurface mycelium obtained from the colony shown in Figure 2. Characteristic is the production of spherical microconidia borne from the side of the hypha. The microconidium shown by the right-pointing arrow is attached to a short condidiophore; the one at the tip of the left-pointing arrow is detached. The production of subsurface microconidia is characteristic of *Aspergillus terreus*.

Student response: "OK. Now I am back on track. I remember a picture in the tutorial of some hyphae with a bunch of microconidia. You make a direct mount of some of the submerged hyphae and look for these microconidia under the microscope. I think this is the answer you are looking for!"

Teleconference instructor: "Wow! You really did your homework. Indeed, on the next screen (Figure 4), you see exactly what you are describing. Remember that several *Aspergillus* species, although not commonly seen in laboratory culture, nevertheless can produce yellow-brown, granular colonies much like we saw before. But the ability to produce these microconidia in the submerged vegetative hyphae is characteristic of *Aspergillus terreus*. Therefore, we can finally conclude that the sinusitis in this patient was caused by *terreus*. Good show! Are there any questions?"

Other student: "There was one question you forgot to answer. It has to do with how biopsy specimens should be handled in the laboratory. I'm not sure I know what you were getting at."

Teleconference instructor: "Thank you for reminding me. You may find it stated in some textbooks that pieces of tissue should be 'ground' before plating. My advice is not to use a tissue grinder. This treatment really tears up any hyphae that may be present, making it more difficult to recover anything in the culture. Remember that fungi rarely produce spores in the tissues, and we must rely on intact hyphae to get anything to grow. The aseptate hyphae of the Zygomycetes are particularly vulnerable to destruction. Therefore, you are better off dicing the biopsy material into small bits with a sharp scalpel and implanting these small bits just under the agar surface. Dr Gade, do you have any questions or comments?"

Dr Gade: "No, that was very impressive. Good job. Do any of the rest of you have any questions before we go on to the next case?"

Silence (except for the nervous gnashing of teeth by the next…student victim).

CONCLUSION

The purpose of presenting this course as a combination of case-based, distance learning in association with a few rudimentary laboratory exercises was to demonstrate that a basic medical mycology course could be taught without an on-campus specialist in mycology. Mycology is a specialty area that is frequently taught by an instructor who has very little clinical experience, even if he or she

has a degree in microbiology. Because bacteriology is the primary emphasis in virtually all microbiology laboratories, mycology is frequently covered as a 'required afterthought', by someone with minimal interest or background.

Although Dr Gade had previously taught the medical mycology course at UW, his role during this course was intentionally limited to organizing the laboratory exercises and setting up the teleconferences. This was done to demonstrate that the entire course could be taught without the direction of an on-site mycologist.

Distance learning can help fill a void in various specialty areas such as mycology, parasitology, and body fluids. Traditional self-study courses are usually based on a textbook or CD-ROM tutorial that are specifically written for self-instruction. While these formats can effectively cover basic topics, they lack the interactive qualities that help students actively 'process' information. Furthermore, without the 'hands-on' aspects of a student laboratory, students have difficulty relating the intellectual concepts learned from a book to practical experience and application.

We developed this course to enhance the information learned from the *GermWare* CD-ROM tutorial with highly interactive discussions of case histories and practical laboratory experiences.

Laboratory exercises illustrated a few of the most basic techniques and materials such as the 'tease' mount, the 'scotch tape' prep, and the 'agar slide' mount. Fungal growth patterns were illustrated by a set of 25 stock cultures inoculated on Sabouraud's agar and sent to the student laboratory by Dr Koneman. Student experience in culture techniques was gained by the inoculation and observation of unknown organisms. Since most fungal cultures are incubated at room temperatures, no special equipment is required. The only materials required for the labs were Sabouraud's or potato dextrose agar plates; microscopes, slides and coverslips; lactophenol stain; teasing needles, and scotch tape.

Student evaluations of this mycology course were excellent. The mean rating for the overall course was 4.60, based on a 1 to 5 scale, and the overall instructor rating was 4.80. An added benefit of this style of teaching is clearly illustrated by the highest rated category by the students. A perfect 5.0 rating was given by each student in the category, 'Stimulates interest in subject.'