Observations on Microbiology Laboratory Instruction for Allied Health Students

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The Problem

Allied health careers are expected to undergo a major growth and expansion in future years. Training programs are commonly found in community colleges, junior colleges and many four-year colleges. A medical microbiology course with a laboratory is a requirement for nursing, physician assistant and other allied health career training programs.

Faculty and students involved in these programs have questioned whether the available laboratory manuals are meeting their needs. For example, many microbiology manuals do not adequately emphasize the medical applications that can be learned from the exercises taught in the laboratory. Frequently, microbiology laboratory manuals presumably designed for allied health students are no more than a literal selection of some of the exercises from a manual designed for students majoring in biology. These exercises are not modified to reflect a medical emphasis for the benefit of the population of allied health students. A microbiology course in health career programs, such as nursing or physician assistant training, is frequently placed in the curriculum as a corequisite or prerequisite to medical surgical nursing or fundamentals of medicine, respectively. These students are actively involved in both a classroom and clinical experience in medical facilities. The microbiology experience must correlate with their career training experience and also enrich their understanding of asepsis; microbial infections; the immune response; the universal precautions of handwashing and wearing gloves, gowns and masks; as well as common pathogenic microorganisms that cause community and hospital acquired infections. Unfortunately, many allied health students have repeatedly challenged the value of this course in their curriculum. Many fail to see the relevance of the microbiology manual and laboratory experience to their career goals. The essential associations between the laboratory experience, and the previously mentioned issues as well as problems encountered by the professional in the areas of asepsis, transmission of disease organisms, control of infection, differential diagnosis of the cause of disease and antimicrobial therapy for patients are not made by the presentations in currently available laboratory manuals.

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The Solution

The two purposes of this paper are 1) to demonstrate that medical microbiology laboratory exercises should be presented with a focus on medical applications, not just traditional microbiology, and 2) that exercises devoted to differential diagnostic decision-making can be used to enhance the problem-solving skills of students.

Selected samplings of typical manual and course exercises are evaluated to illustrate the purposes of this paper. The first portion of this paper focuses on medical applications in introductory topics on microorganism surveys, staining, culturing and handwashing. The second portion focuses on problem-solving and differential decision-making studies. The goal in both sections is to present medical microbiology that correlates with and enriches the allied health training experience of the student.

First Section

Survey of Microorganisms

Exercises that survey bacteria, fungi and protozoa usually are presented as culture plates and commercially prepared slides for the student to examine. Frequently, a medical focus for these is lacking. Bacterial slides can include slides of *Streptococcus pyogenes*, which causes strep throat, impetigo and scarlatina, common streptococcal infections in young children; and *Treponema pallidum*, which causes syphilis. Protozoan slides can include slides of *Trichomonas vaginalis*, which causes a sexually transmitted urethritis and vaginitis; *Toxoplasma gondii*, which causes encephalitis in AIDS patients and serious birth defects; *Giardia lamblia*, which causes gastroenteritis; and stages in the erythrocyte infection cycle of *Plasmodium vivax*, which causes an infection of red blood cells in patients with malaria (Creager, Black & Davison 1990). Fungal slides should emphasize, in addition to the common molds which can cause opportunistic infections in immunocompromised hosts, the yeast *Candida*, a cause of vulvovaginitis and thrush, as well as esophagitis in HIV-infected patients (Laurence 1987).

Gram Stains

Gram stains are commonly used to demonstrate the morphology and Gram reaction of bacteria. Bacteria in a stained smear appear coccus, bacillus or spirillum shaped and are gram-positive and purple or gram-negative and pink. Typically, many manuals emphasize the theory of the Gram stain and differences between gram-positive and gram-negative bacteria, but fail to stress the diagnostic value of this differential stain procedure. However, the Gram stain can be used to illustrate the importance of staining in making a presumptive diagnosis of the etiology of disease in patients. In the clinical setting, Gram stains can be prepared from cerebrospinal fluid, sputum, urine or fluid released from abscesses. Some examples follow. Stratton (1991) has shown that the Gram stain can give accurate results for diagnosis of bacterial meningitis in 80 percent of the cases. Feleke and Khan (1989) report that the Gram stain is a key step in the diagnosis of *Moraxella* (*Branhamella*) *catarrhalis* as a cause of lower respiratory tract infections.

Typically, gram-negative diplococci are seen in sputum samples from these patients. On the basis of the Gram stain results, empiric therapy with erythromycin, tetracycline, cefaclor or amoxicillin-clavulanate has proven satisfactory. Markowitz (1990) reports that the Gram stain allows one to make a presumptive diagnosis for *Streptococcus pneumoniae* from sputum smears as the cause of pneumonia. These appear as gram-positive cocci in pairs or short chains. Most strains respond to penicillin therapy. The American Society of Microbiology recommends the Gram stain of an uncentrifuged sample of urine as a tool to indicate a urinary tract infection. The presence of 1–2 bacteria and one or more leucocytes in a field strongly suggests greater than 100,000 bacteria per ml of urine sample and a urinary tract infection (Clarridge, Pezzlo & Vosti 1987). In anaerobic infections, one finds necrotic tissue, foul smelling discharges and the presence of organisms on the Gram stain that fail to grow on culturing in air. Empiric treatment with antibiotics effective against anaerobes is routinely practiced (Feleke & Forlenza 1991).

Acid-Fast Staining

In the Acid-Fast stain procedure, typically the smear of mycobacteria is flooded with the dye carbol fuchsin and steamed for 10 minutes, followed by decolorization with acid alcohol and counterstaining with methylene blue. Acid-fast bacteria retain the pink color of the carbol fuchsin dye despite treatment with acid alcohol, while non acid-fast cells are decolorized and then stained blue by the counterstain. All manuals emphasize that this stain is important for the identification of *Mycobacterium tuberculosis* in sputum samples. The need for steaming to penetrate the waxy lipids in the walls of these bacteria is usually also discussed. However, the Acid-Fast stain has other advantages. Pollard (1991) has reported that this staining procedure can be used to monitor the effectiveness of therapy against tuberculosis. Specifically, with ongoing therapy, acid-fast smears made on sputum samples should show a decrease in numbers of mycobacteria. If this decrease is not observed, then drug resistance should be suspected.

Culturing Studies

Frequently, introductory exercises on culturing of microorganisms are used to demonstrate the presence of microorganisms on environmental and body surfaces—the physical factors that affect microbial growth and handwashing. In contrast, culturing of swabbings from environmental surfaces and the body can be used to emphasize the universal presence of microorganisms, the need for asepsis and handwashing, and the potential for transmission of microorganisms by carelessness in the laboratory or in one's career.

Studies on the influence of physical factors such as temperature, pH and salt concentration can be used not only to demonstrate the different sensitivities of microorganisms to these particular growth conditions, but also to emphasize models for disease production by specific organisms. For example, the skin flora microbe *Staphylococcus aureus* is salt tolerant and can grow in a pH of five. These are conditions found on human skin. This organism frequently causes skin, burn, wound and surgical infections. The hands of medical staff provide a major route of transmission to pediatric patients. Staphylococci can spread from local lesions to the blood and then to organs such as bones, joints and kidneys (Youmans, Patterson & Sommers 1985).

Simple culturing experiments that study microbial relationships can be constructed to simulate the development of anaerobic infections from initially mixed cultures consisting of aerobes or facultatives and anaerobes as well as synergistic interactions among bacteria to produce more severe infections than the individual organisms alone could produce (Benathen 1993). These types of experiments are simple to do, show the presence and characteristics of anaerobes and demonstrate that mixed cultures can simulate the development of anaerobic as well as synergistic bacterial infections.

Handwashing

The importance of handwashing, as one of the universal precautions for the spread of disease organisms, must be vigorously stressed in the laboratory. Bradley and Norris (1989) note that bacterial pneumonias are responsible for 10 to 25 percent of all nosocomial (hospital-acquired) infections and are commonly caused by gram-negative bacilli such as *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*. Transmission of these gram-negative bacilli from patient-to-patient frequently occurs by the hands of hospital personnel. Handwashing is a critical procedure that can prevent the spread of these bacteria and nosocomial infections. Brown and Williams (1990) demonstrate, by culturing, the importance of antisepsis in handwashing after using different percent solutions of Clorox to reduce the number of microorganisms on the skin. In many medical facilities and college microbiology laboratories povidone-iodine surgical scrub (Betadine) or chlorhexidine-gluconate surgical scrub (Hibiclens) is routinely used for handwashing.

Second Section

Making Differential Diagnostic Decisions

The unknown is a traditional exercise in most medical microbiology courses. It involves an array of fermentation, respiratory behavior, amino acid metabolism and macromolecule digestion test media that must be inoculated by the student. This exercise is usually completed in the second portion of a microbiology course. By this time, the student has perfected sterile inoculation techniques, knows how to contain and disinfect accidental spills in the laboratory, knows the Gram stain reactions and cultural characteristics of a variety of bacteria, and appreciates that any of the commonly studied microorganisms can be pathogenic. For example, *S. aureus* is considered pathogenic. *S. epidermidis* is also pathogenic since this organism can cause infection of implanted artificial devices, wound and urinary infections (Youmans, Patterson & Sommers 1985).

However, for most students this project, found in many laboratory manuals, presents an overwhelming problem to solve that shares no context with the real clinical world. The organisms used in this study should clearly include those that are commonly found in nosocomial and community-acquired infections. Most of these infections are caused by gram-negative bacilli, and a lesser number are caused by gram-positive cocci. They include members of the genera *Kleibsiella*, *Escherichia*, *Enterobacter*, *Pseudomonas* and *Staphylococcus*. These are the microbes the students will be exposed to in their professional careers. I have frequently been informed by my students that they have noted these organisms in microbiology reports as the causes of infections in patients assigned to them during the clinical phase of their training. The four sites of such infections in the human body include the urinary tract, the surgical wound, the respiratory tract and the bloodstream (Harris, Levin & Trenholme 1984).

These unknowns should be presented in the context of nosocomial infections and clinical problems that demand a differential diagnostic solution. The student is presented with a culture tube that simulates an isolate from an ill patient that must be promptly identified by Gram staining and culture tests so that proper antibiotic therapy can be instituted. The student is required to submit a written report listing the Gram reaction; the identity of their simulated clinical sample, which must be based on their laboratory results; and the reasons why specific laboratory tests support that decision. The unknown now represents a realistic clinical problem to solve that requires logical thinking and development (Benathen 1993). My students have enthusiastically embraced the approach of ruling out unwanted organisms by using specific tests of the clinical unknown to make a differential diagnostic decision for the cause of a simulated nosocomial or community-acquired infection. This project, conducted in this fashion, is a very successful exercise in problem solving. In addition, I use the results of bacterial digestion of starch, protein and lipid macromolecules contained in agar plates to present a simulation of bacterial virulence factor enzyme destruction of cells or tissues, abscess formation and gangrene (Benathen 1993).

As a follow-up to this project, the students swab a fresh culture of their identified unknown to Mueller-Hinton agar plates for antibiotic sensitivity testing to determine the appropriate antibiotic therapy for the simulated patient infection. They not only identify the organism but must determine those antibiotics for which the microbe is classified as resistant (R), intermediate (I) and sensitive (S). In this fashion, the students see the progression from identification of the cause of the problem to a decision for proper therapy. They also compare the responses to antibiotics for their unknown to those for other gram-positive and gram-negative bacteria and discuss the ongoing problem of antibiotic resistance in bacteria. In doing so, they have a clearer understanding of the importance of microbiology reports in charts and of the interdependent roles of the microbiology laboratory staff and other future health practitioners like themselves in the decision-making process for the treatment of the patient.

In a similar fashion, all respiratory, gastrointestinal and urinary tract medical microbiology studies should be viewed as simulated clinical unknown samples that demand accurate and prompt diagnostic decisions. I introduce the rapid miniaturized multitest identification test systems at this time as an alternative to the traditional use of tube and plate culture media to show how one can rapidly identify unknown cultures in medical microbiology. Having completed the time consuming inoculations, incubations and interpretations of results on various broth and agar media, my students appreciate the speed, accuracy and reliability of the miniaturized multitest in making differential diagnostic decisions.

Conclusions

The purpose of this paper has been to suggest alternative emphases to the typical presentation of topics in laboratory manuals designed for introductory medical microbiology for the health careers student. Selected areas common to most microbiology laboratory manuals are critiqued in this paper. Medical applications of the experiments are stressed to emphasize the necessary relevance of the laboratory experience to the student's career training and clinical experience. Many microbiology manuals today simply present the material without providing an adequate or proper medical background for the student to appreciate why they are completing an exercise and what it can teach them for their professional career. This paper suggests that some rethinking of approach and format is desirable in microbiology manuals geared for the allied health student. This should lead to a greater appreciation of the contribution of medical microbiology by individuals studying for careers in the allied health professions.

References

Benathen, I.A. (1993). *Microbiology with health care applications*. Belmont: Star Publishing Company.

Bradley, O. & Norris, J. (1989). Bacterial nosocomial pneumonia. *Physician Assistant, 13*(6), 35–52.

Brown, W.E., Jr. & Williams, R.P. (1990). Ignaz Semmelweis & the importance of washing your hands. *The American Biology Teacher, 52*(5), 291–294.

Clarridge, J., Pezzlo, M. & Vosti, K. (1987). Laboratory diagnosis of urinary tract infections. *Cumitech 2A*. Washington, DC: The American Society of Microbiology.

Creager, J., Black, J. & Davison, V. (1990). *Microbiology principles and applications*. Englewood Cliffs, NJ: Prentice Hall Inc.

Feleke, G. & Forlenza, S. (1991). Anaerobic infections. The basis for primary care physicians. *Postgraduate Medicine, 89*(8), 221–234.

Feleke, G. & Khan, F. (1989). Branhamella infections. An increasingly common respiratory illness. *Postgraduate Medicine, 85*(4), 379–386.

Harris, A., Levin, S. & Trenholme, G. (1984). Selected aspects of nosocomial infections in the 1980s. *The American Journal of Medicine, 77*(1B), 3–10.

Laurence, J. (1987). Gastrointestinal infections in AIDS patients. *PA 87 Physician Assistants in Primary & Hospital Care, 4*(1), 9–13.

Markowitz, S. (1990). Pneumococcal pneumonia. Recognizing and treating this persistent disease. *Postgraduate Medicine, 88*(7), 33–47.

Pollard, C. (1991). Diagnostic microbiology in the office setting. *Physician Assistant, 15*(6), 17–27.

Stratton, C. (1991). The laboratory diagnosis of meningitis. *Infectious Diseases Newsletter, 10*(2), 12–14.

Youmans, G., Patterson, P. & Sommers, H. (1985). *The biologic and clinical basis of infectious diseases*. Philadelphia: W. B. Saunders Company.