EDUCATIONAL COMMENTARY – UPDATE ON ANAEROBES

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LEARNING OUTCOMES

On completion of this exercise, the participant should be able to:

- discuss recent epidemiologic trends in infections caused by anaerobic bacteria.
- discuss general principles for selecting, collecting, and transporting samples suitable for anaerobic culture.
- develop a system for identifying anaerobic isolates.

Anaerobic bacteria comprise a substantial portion of the normal flora in humans and animals. They live on most body surfaces and mucous membranes, in the gastrointestinal tract, and in the female genitourinary tract. Anaerobes benefit their host by helping digest food, producing vitamins and cofactors such as vitamin K, and preventing proliferation of pathogenic bacteria. However, anaerobes can cause infections if they multiply excessively in an area where they are normal flora or if they contaminate an area where they are not. For example, *Clostridium difficile* can cause diarrhea when antibiotic use suppresses the intestinal flora that would normally limit its growth, or a penetrating wound can introduce anaerobic bacteria into a normally sterile site.

Before antibiotics were routinely administered prior to abdominal and genitourinary tract surgery, anaerobic infections developed in as many as 20% of patients. Today, the incidence of anaerobic infections in these types of surgeries is 0.5% to 9.0%. However, other types of anaerobic infections are increasing in frequency. *C. difficile*, once predominantly a hospital-acquired infection, now causes diarrhea in outpatients worldwide, and many strains are highly virulent. *Propionibacterium acnes* is often implicated in infections of prosthetic joints, and *Fusobacterium necrophorum* and other *Fusobacterium* species are now recognized as an important cause of chronic sore throat. In particular, *F. necrophorum* has been linked to an increase in Lemierre syndrome, a disease characterized by peritonsillar abscesses and thrombophlebitis of the jugular veins after a bout of acute sore throat. On the other hand, anaerobes rarely cause meningitis or urinary tract infections.

Sample Collection and Transport

Determining whether an anaerobic isolate is the cause of an infection can be difficult, because most anaerobic infections are caused by normal anaerobic flora and because many anaerobic cultures grow multiple species of bacteria. Consequently, detection of anaerobic pathogens begins with the collection
of a suitable sample. In general, the best specimens for anaerobic culture are tissue samples, biopsy specimens, and samples aspirated by needle and syringe. Samples collected by swabs usually are unacceptable because swabs easily capture contaminating flora, they absorb only a small amount of sample, and bacteria tend to adhere to the swab’s fibers. Swabs should be used only for a sample that can be obtained in no other way, such as material from a brain abscess. Sputum, bronchial washings not collected by protected brush, endotracheal aspirates, voided or catheterized urine, stool, ileostomy or colostomy drainage, and gastric or small bowel contents (except in blind loop syndrome) are unsuitable because these specimens may contain normal anaerobic flora.

Many anaerobic bacteria cannot survive exposure to room air or extreme temperatures. For this reason, samples must be collected anaerobically and transported in a special anaerobic transport device, protected from hot or cold temperatures, and processed quickly when they arrive in the laboratory. To avoid needle-stick injuries and leakage, samples collected by needle and syringe should be transferred to an anaerobic transport vial. Ideally, specimens should be processed within 2 to 3 hours of collection. If processing will be delayed, samples should be held at room temperature. However, anaerobes in large volumes of purulent material or tissue can remain viable for longer periods than anaerobes in small volumes or swabs.

Identification Schemes

A direct Gram stain of the specimen before it is cultured is essential to help detect and identify anaerobes and judge their clinical importance. A properly performed Gram stain aids in assessing the quality of the specimen, provides information about the potential for a mixed anaerobic and aerobic infection, and directs the culture workup. Bacterial vaginosis, a condition characterized by the absence of *Lactobacillus* and the presence of Gram-variable coccobacilli, is best diagnosed by Gram stain alone rather than culture. In other infections, the Gram stain may expedite treatment by providing the clinician with important information about the presence and probable identity of anaerobes. Finally, the Gram stain results should be correlated with the culture results and specimen source to help assess the clinical relevance of the organisms isolated.

The extent to which an anaerobic isolate should be identified depends on the site from which the sample was collected and the clinical circumstances; isolates from sterile sites are usually studied more completely than isolates from nonsterile sites. In an effort to simplify procedures and economize resources, experts have developed flowcharts that laboratories may use. These recommendations use a combination of 4 levels of identification: reporting the presence of anaerobes without further identification, ruling out certain pathogens, identifying isolates to the genus level, and identifying isolates to the species level.
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One scheme\(^1\) for identification of anaerobic cocci isolates from nonsterile sites recommends that the laboratory report simply note the presence of gram-negative or gram-positive anaerobic cocci. If the isolate is a gram-negative bacillus, *Bacteroides fragilis* should be ruled out by lack of growth on a *Bacteroides* bile esculin plate. Likewise, if the isolate is an anaerobic spore-forming gram-positive bacillus with double zone hemolysis on blood agar, it should be tested to determine if it is *Clostridium perfringens*, and the result should be reported as either “*C. perfringens*” or “*Clostridium* species, not *C. perfringens*.” Anaerobic, non–spore-forming, gram-positive bacilli can be reported as such without further identification.

A flowchart has also been developed for anaerobic isolates from sterile sites.\(^1\) In this scheme, anaerobic gram-negative and gram-positive cocci can be either reported as present or fully identified. Gram-positive bacilli and gram-negative non–*Fusobacterium*-like bacilli are identified to the species level, but gram-negative, indole-negative fusiform bacilli can be either fully identified or reported as “*Fusobacterium*-like.”

Baron and Citron\(^3\) developed a low-cost identification scheme that identifies most anaerobic isolates to the genus level. In this scheme, gram-negative anaerobic cocci are all reported as *Veillonella* species and gram-positive anaerobic cocci are all reported as *Peptostreptococcus* species. However, gram-positive anaerobic cocci from deep soft tissue infections or those in pure culture should be identified to the species level. This is because *Finegoldia magna* (formerly *Peptostreptococcus magnus*) is often found in these sites and is often resistant to clindamycin, an antibiotic that is frequently used to treat anaerobic infections. For anaerobic gram-negative and gram-positive bacilli, Baron and Citron\(^3\) developed flowcharts that identify the organism to the genus level using spot and rapid tests along with colony appearance.

Methods to identify anaerobes include a combination of rapid disk, spot, and biochemical tests; microbiochemical systems; and rapid enzymatic systems. In many cases, rapid disk, spot, and biochemical tests enable quick and economical identification of anaerobic isolates to the genus level. For isolates that require identification to the species level, microbiochemical systems such as API 20A (bioMérieux, Inc) and Minitek (BD Biosciences) are available. Rapid enzymatic systems such as the Anaerobe ANI card and Rapid ID 32A (bioMérieux, Inc), Rapid Anaerobe ID (Dade Microscan, Inc), Crystal Anaerobe ID Kit (BD Biosciences), and Rapid ID-ANA (Remel, Inc) eliminate the need for growth of isolates and differentiate many species that cannot be identified by microbiochemical systems.

Other methods used to identify anaerobes include conventional gas liquid chromatography and 2 newer technologies. Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry is now used in some laboratories, and sequencing of genetic markers is often used in laboratories that have molecular assessment technologies.
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Special considerations

The following specimens and situations may warrant special consideration:1,2

- *C. difficile* infection is best detected by toxigenic culture or molecular detection of the toxin B gene. Enzyme immunoassays that detect toxins A and B have been shown to lack sensitivity.
- If only 1 of several blood draws for blood culture contains an anaerobe, the organism may be a contaminant or the result of transient bacteremia, and a complete identification may not be necessary. This is more likely if the organism is part of normal skin flora such as a gram-positive coccus or *Propionibacterium* species. However, if the organism is present in multiple samples, it should be completely identified.
- The practice of inoculating and processing broths with routine nonshunt cerebrospinal fluid specimens is controversial, because this often results in growth of contaminants and adds little to the recovery of pathogens. However, if the specimen is from a patient with a ventricular shunt, a thioglycolate broth should be inoculated and incubated for up to 10 days. In this situation, *P. acnes* may be a pathogen.
- Cultures of specimens that could harbor *Actinomyces* species should be held at least 10 to 14 days. This includes samples from jaw and neck abscesses, the sinus tract, lacrimal glands, the tonsillar area, and intrauterine devices. *Actinomyces* should also be suspected if the Gram stain shows sulfur granules and gram-positive branching bacilli.

Conclusion

Experts recommend that, in addition to a Gram stain, all requests for anaerobic culture include a simultaneous aerobic culture of the specimen. This is because specimens for anaerobic culture often contain anaerobic and aerobic bacteria that contribute to the infection. Experts also agree that anaerobic specimens from sterile sites should be identified to the genus and species level as much as possible. However, protocols vary for identification of anaerobes from nonsterile sites or from sterile sites that grow mixed flora. The most effective protocols are customized for each laboratory and consider the technical expertise of personnel, available resources, budget constraints, and the needs of the laboratory’s clients.

References


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