EDUCATIONAL COMMENTARY – ANAEROBIC BACTERIA

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Learning Outcomes

Upon completion of this exercise, the participant will be able to:

- Discuss clues that indicate an anaerobic infection.
- Discuss the advantages and disadvantages of presumptive versus definitive identification of anaerobic bacteria.
- Apply general guidelines for determining which anaerobic isolates require susceptibility testing.

Anaerobic bacteria are found both in the body as normal microbial flora (i.e., endogenous anaerobes) and in the environment (i.e., exogenous anaerobes). Members of the genus *Clostridium* cause most exogenous anaerobic infections; less frequently, *Sarcina ventriculi*, *Fusobacterium ulcerans*, *Desulfovibrio desulfuricans*, and *Desulfomonas* spp. are implicated.

However, it is the endogenous anaerobes that cause most human infections. This, and the fact that most anaerobic infections are polymicrobial, can make it hard to evaluate the clinical significance of a particular anaerobic isolate. It may be merely a contaminant, it may be the sole cause of the infection, or it may contribute to the infection along with other bacteria.

Indications of Infection With Anaerobic Bacteria

A key indication that an anaerobic isolate is the cause of infection is its growth from a suitable specimen that has been properly collected. In general, specimens suitable for anaerobic culture are those that are collected by tissue biopsy or aspirated by needle and syringe, because these are unlikely to be contaminated by endogenous anaerobic bacteria. By contrast, most specimens collected by swab are unsuitable for anaerobic culture because they are likely to be contaminated by endogenous anaerobes. The **Table** lists specimens that are suitable and unsuitable for anaerobic culture by anatomic site.

Table. Specimens Suitable and Unsuitable for Anaerobic Culture.

Anatomic Site	Specimens Suitable for Anaerobic Culture	Specimens Unsuitable for Anaerobic Culture
Respiratory	Bronchial washings (only if obtained with double-lumen plugged catheter) Percutaneous lung aspirate or biopsy Transtracheal aspirate	Bronchial washings or brushings Expectorated sputum Nasopharyngeal swab Secretions obtained by nasotracheal or orotracheal suction Throat swabs
Genitourinary	Endometrial biopsy tissue obtained with endometrial suction curette Suprapubic bladder aspirate Uterine contents obtained by protected swab Culdocentesis aspirate	Urethral swabs Vaginal or cervical swabs Voided or catheterized urine
Gastrointestinal	Tissue obtained by biopsy Gastric or small bowel contents (only in blind loop syndrome)	Stool specimens Rectal swabs Gastric or small bowel contents (except in blind loop syndrome) Ileostomy or colostomy drainage
Normally sterile fluids	Bile Blood Cerebrospinal fluid Aspirated synovial fluid Peritoneal (ascitic) fluid Thoracentesis (pleural) fluid	N/A
Wounds, abscesses, tissues	Bone marrow Decubitus ulcer (from base of lesion after debridement) Material aspirated from abscesses Sulfur granules from draining fistulas Tissues obtained by biopsy or autopsy	Swabs from superficial skin lesions (wounds, abscesses, burns, cysts, ulcers)

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Besides culturing only suitable specimens, the microbiologist should look for the following specific clues that indicate an anaerobic infection:

- Infection near a mucosal surface
- Infection resistant to aminoglycoside therapy
- Foul odor (*Porphyromonas* and *Fusobacterium* spp.)
- Large quantity of gas (*Clostridium* spp.)
- Sulfur granules (*Actinomyces* spp., *Propionibacterium* spp., and *Eubacterium nodatum*)
- Black color or brick-red fluorescence under long-wave ultraviolet light (pigmented *Prevotella* and *Porphyromonas*)
- Distinctive morphology on Gram stain (*Bacteroides*, *Fusobacterium*, *Clostridium*)

Identification of Anaerobic Bacteria

Identification of anaerobic isolates, which can be done presumptively or definitively, is important for three reasons. First, knowing which bacterium is causing the infection can help the physician choose an empiric therapy that is most likely to be effective. Second, the identity of an anaerobic isolate often indicates the likely source of the infective process. For example, *Clostridium septicum* isolated from blood cultures may indicate cancer or other diseases of the colon. Last, identification of anaerobic isolates helps build a database of information about their role in infections.

Presumptive identification of anaerobic isolates has become increasingly popular because definitive identification is both costly and time-consuming, and in most cases presumptive identification suffices to help physicians choose appropriate therapy. Presumptive identification is based on colony morphology, Gram stain appearance, and a variety of rapid, inexpensive tests. These include:

- **Aerotolerance** test to determine whether the organism is a true anaerobe
- **Fluorescence** under long-wave (366 nm) ultraviolet light. Many pigmented *Porphyromonas* and *Prevotella* spp. fluoresce brick-red; *Fusobacterium nucleatum* and *Clostridium difficile* fluoresce chartreuse, and *Veillonella* spp. fluoresce red.
- **Special potency antimicrobial disks** (5 µg vancomycin, 1 mg kanamycin, 10 µg colistin) to verify that the isolate is a true gram-negative bacillus
- **Sodium polyanethol sulfonate (SPS) disk** to presumptively identify a gram-positive coccus as *Peptostreptococcus anaerobius*
- **Nitrate disk** to determine the bacterium's ability to reduce nitrate
- **Bile disk** to presumptively identify an anaerobic, gram-negative bacillus as *Bacteroides fragilis*
- **Catalase** test to differentiate aerotolerant *Clostridium* strains from *Bacillus* spp.
- **Spot indole** test to determine an isolate's ability to produce indole from tryptophan.

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- **Motility test** to aid in identifying motile, gram-negative anaerobes such as *Campylobacter* and *Mobiluncus*
- **Lecithinase and lipase reactions** to help identify species of *Clostridium*

Not all anaerobic isolates can be identified with presumptive tests, and the decision whether to definitively identify these is based on the specific circumstances. In mixed infections definitive identification usually does not affect treatment. Other situations, however, do require definitive identification. Anaerobic bacteria should be definitively identified if they grow in a pure culture from a normally sterile site, if there is solid evidence for clinical relevance, or if susceptibility testing will be performed. Techniques used for definitive identification include:

- Pre-reduced anaerobically sterilized (PRAS) and nonPRAS tubed biochemical test media
- Miniaturized biochemical-based and preexisting enzyme-based systems
- Gas-liquid chromatography (GLC) analysis for end products of glucose fermentation
- High-resolution GLC analysis of whole-cell long chain fatty acid methyl esters (FAME)

Because no commercial identification system reliably identifies all anaerobic bacteria, reference laboratories typically use a combination of PRAS biochemicals and GLC analysis.

Susceptibility Testing of Anaerobic Bacteria

Susceptibility testing of anaerobic bacteria is controversial. Issues include which isolates to test, which antimicrobial agents to use, and which method to use. Because many anaerobic bacteria are fastidious, susceptibility testing is more costly and time-consuming compared with testing other bacteria. Also, because most commonly encountered anaerobic bacteria respond well to first-line antibiotics, empiric therapy based on presumptive identification usually cures the infection.

Nevertheless, some situations do warrant susceptibility testing of anaerobic isolates. These include:

- Bacteria known to be virulent or resistant to antibacterial agents (*Bacteroides* spp., *Porphyromonas* and *Prevotella* spp., *Clostridium perfringens*, *C. ramosum*, *C. septicum*, certain *Fusobacterium* spp., and *Bilophila wadsworthia*)
- Empiric therapy that fails to resolve the infection
- Lack of precedent for empiric therapy
- Severe infection
- Requirement for long-term therapy
- Certain specific infections (brain abscess, endocarditis, infection of a prosthetic device or vascular graft, joint infection, osteomyelitis, and refractory or recurrent bacteremia)

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When designing susceptibility testing batteries, laboratories should incorporate the most current recommendations of the National Committee for Clinical Laboratory Standards (NCCLS document *M11-A6: Methods for Antimicrobial Susceptibility Testing for Anaerobic Bacteria*, 6th ed.). Other factors to consider are the availability of the drug in the hospital formulary, the type of infection, whether the infection is hospital- or community-acquired, existing complications, and known resistance patterns of the bacterium. Drugs to test include penicillin, clindamycin, metronidazole, imipenem, and ampicillin/sulbactam. In addition, ß-lactamase testing may be useful as an adjunct test with *Bacteroides*, *Prevotella*, *Fusobacterium*, and *Clostridium*.

Agar dilution and broth dilution (macro or micro) have been validated for testing anaerobic bacteria. Two other methods—spiral gradient endpoint (SGE) and PDM Epsilometer (E test [AB Biodisk, Solna, Sweden])—may also be used. However, disk diffusion and broth disk elution are not acceptable methods for testing anaerobic bacteria.

Suggested Reading

Engelkirk PG, Duben-Engelkirk J. Anaerobes of clinical importance. In: Mahon CR, Manuselis G. *Textbook of Diagnostic Microbiology*. 2nd ed. Philadelphia, PA: WB Saunders Co; 2000:565-622.

Forbes BA, Sahm DF, Weissfeld AS. Overview and general considerations. In: *Bailey & Scott's Diagnostic Microbiology*. 11th ed. St Louis, MO: Mosby; 2002:511-519.

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