Procedures in Family Practice

Collection and Transportation of Specimens in Anaerobic Infections

Itzhak Brook, MD
Bethesda, Maryland

Management of anaerobic infection depends on appropriate documentation of the bacteria causing the infection. Proper collection of specimens in a manner that avoids contamination by normal flora and prompt delivery to the microbiology laboratory are of utmost importance. Materials appropriate for anaerobic culture include blood specimens, aspirates of body fluids (pleural, pericardial, cerebrospinal, peritoneal, and joint fluids), urine collected by suprapubic aspiration, abscess contents, deep wound aspirates, and specimens obtained by special procedures such as transtracheal aspiration or lung puncture. Unacceptable or inappropriate specimens can be expected to yield normal flora also and therefore have no diagnostic value. These include coughed sputum, throat swabs, feces, gastric aspirates, voided urine, and vaginal swabs. Aspirates of liquid specimen or tissue are always preferred to swabs, although systems for the collection of all culture forms are commercially available.

The proper management of anaerobic infection depends on appropriate documentation of the bacteria causing the infection. Without such an approach the patient may be exposed to inappropriate, costly, and undesired antimicrobial agents with adverse side effects.

Anaerobic infections present special bacteriological problems not encountered in other types of infections and such problems may make the therapeutic approach even more difficult. Generally, bacteriological results will not be available so quickly as in aerobic infections, particularly if the infection is mixed (as are more than one half of the cases). Some laboratories may fail to recover certain or all of the anaerobes present in a specimen. This situation can occur particularly when the specimen is not promptly put under anaerobic conditions for transport to the laboratory. If care is not taken to avoid contamination of the specimen with normal flora, anaerobes may be recovered that have little to do with the patient's illness. As all laboratories are not equipped to identify anaerobes accurately, presumptive results may be very misleading.

Surgical therapy is of the greatest importance in

From the Department of Pediatrics, Uniformed Services University of the Health Sciences, and the Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland. Requests for reprints should be addressed to Dr. Itzhak Brook, Naval Medical Research Institute, National Naval Medical Center, Bethesda, MD 20014.

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anaerobic infections and, in lesser infections, may be all that is required. The types of surgical therapy may vary from drainage of an abscess to excision of necrotic tissue or relief of an obstruction.

The antimicrobial therapy of anaerobic infection, sometimes used as an adjuvant to surgical therapy and sometimes as the sole therapy given, relies to a great extent on proper isolation and identification of the offending bacteria. Penicillin is no longer effective against anaerobic bacteria, except Bacteroides fragilis, and more bacteroides strains, such as B melaninogenicus, are becoming resistant to this drug. Such drugs as clindamycin, chloramphenicol, and metronidazole are active against most anaerobic bacteria and can be used before the exact nature of the bacteria and susceptibilities are known. However, each of these drugs has some untoward effects or toxicity, and less toxic or more appropriate antimicrobial agents can be used when the exact identity and susceptibility of the organisms are known. Using the most specific drug is of particular importance, since many of the anaerobes are changing their "classical" susceptibility, and an isolate of a certain anaerobe that is usually susceptible to an antimicrobial agent may become resistant in a certain patient.

The importance of appropriate cultures for anaerobic bacteria is especially important in mixed aerobic and anaerobic infections. Techniques or media that are inadequate for isolation of anaerobic bacteria, either because of a lack of an anaerobic environment or because of an overgrowth of aerobic organisms, can mislead the clinician to assume that the aerobic organisms recovered are the only pathogens present in an infected site, therefore causing the clinician to direct therapy toward only those aerobic organisms.

The nature of the various organisms in a mixed infection will also influence the choice of drugs. Drugs active against anaerobic bacteria may be quite inactive against the accompanying aerobic or facultative organisms. When mixed infections involve several organisms, two, three, or more drugs may be required to provide effective coverage for each of the organisms in the mixture.

Since anaerobic bacteria frequently can be involved in various infections, ideally, all properly collected specimens should be cultured for these organisms. Special efforts should be made by the physician to isolate anaerobic organisms in infections in which these organisms are frequently recovered, such as abscesses, wounds in and around the oral and anal cavities, chronic otitis media and sinusitis, and aspiration pneumonia, among others.

The most acceptable documentation of an anaerobic infection is through culture of anaerobic microorganisms from the infected site. Three elements requiring the cooperation of the physician and the microbiology laboratory are essential for appropriate documentation of anaerobic infection: collection of appropriate specimens, expeditious transportation of the specimen, and careful laboratory processing.

Collection of Specimens

Specimens must be obtained free of contamination so that saprophytic organisms or normal flora are excluded and culture results can be interpreted correctly. Because indigenous anaerobes are often present on the surfaces of skin and mucus membranes in large numbers, even minimal contamination of a specimen with the normal flora can give misleading results. On this basis specimens can be designated according to their acceptability for anaerobic culture. Materials appropriate for anaerobic cultures should be obtained using a technique that bypasses the normal flora. Unacceptable or inappropriate specimens can be expected to yield normal flora also and therefore have no diagnostic value. Examples of these specimens include coughed sputum, bronchoscopy aspirates, gingival and throat swabs, feces, gastric aspirates, voided urine, and vaginal swabs. Exceptions to these guidelines can be made if in certain instances the clinical condition warrants such a culture. An example is the use of selective media to detect a possible pathogen only, such as Clostridium difficile in stool obtained from a patient with colitis.

Acceptable specimens include blood specimens, aspirates of body fluids (pleural, pericardial, cerebrospinal, peritoneal, and joint fluids), urine collected by percutaneous suprapubic bladder aspiration, abscess contents, deep aspirates of wounds, and specimens collected by special techniques such as transtracheal aspirates or direct lung puncture. Direct needle aspiration is probably the best method of obtaining a culture, whereas use of swabs is much less desirable. Specimens obtained from normally sterile sites may be collected

after thorough skin decontamination (eg, collection of blood, spinal joint, or peritoneal fluids).

Cultures of coughed sputum and specimens obtained from bronchial brushing or bronchoscopy are generally contaminated with normal oral and nasal aerobic and anaerobic flora and are therefore unsuitable for culture. Because the trachea below the thyroglossal membrane is sterile in the absence of pulmonary infection, transtracheal aspiration (TTA), which is done below this site, is a reliable procedure in obtaining suitable culture material for the diagnosis of pulmonary infection.[1,2] An alternative procedure is direct lung puncture. These procedures, when performed by experienced operators, yield important data, and the complication rates are very low. TTA is usually not recommended in patients with severe hypoxia, hemorrhagic diathesis, or severe cough.[3] Rare complications, such as hypoxia, bleeding, subsequent emphysema, or arrhythmia, have rarely been reported in adult patients.[4] TTA has also been successfully utilized in the diagnosis of aspiration pneumonia and lung abscess in children.[2] Cultures obtained through TTA contain fewer pathogens than cultures of expectorated sputum. Side effects of this procedure in children include mild hemoptysis and in rare instances subcutaneous emphysema.

Transportation of Specimens

The ability to recover anaerobes is influenced by the care applied to transportation and laboratory processing of specimens. Unless proper precautionary measures are taken during collection, transport, and laboratory processing, pronounced changes can occur in the aerobic and anaerobic microbial population of a clinical specimen.[5] Sensitivity to oxygen causes some obligate anaerobes to die rapidly upon exposure to air. In clinical samples obligate anaerobes can also be overgrown by facultative anaerobes unless processed rapidly after collection. The organisms, therefore, have to be protected from the deleterious effects of oxygen during the time between the collection of the specimen and their inoculation into the proper anaerobic medium in the microbiology laboratory. Failure to take proper precautions can result in misleading data, which indirectly may be detrimental to the patient.[5-9]

Anaerobes vary in the conditions they require for survival. Some organisms are classified as "moderate" and some as "fastidious" in accordance with their oxygen sensitivity.[10] Among the moderate group (those capable of growing in an oxygen concentration of 2 percent to 8 percent) are Bacteroides fragilis, B oralis, B melaninogenicus, Fusobacterium nucleatum, and Clostridium perfringens. Some fastidious anaerobes will grow in 0.5 percent oxygen, and some are "extremely oxygen-sensitive," such as some strains of B fragilis and peptococci.[11] Low oxidation-reduction potential is another basic requirement for growth of certain anaerobic bacteria such as B vulgatus and C sporogenes.[12] Such conditions usually exist in areas where anaerobes are present as part of the normal flora and at infected sites. The implication of these observations is that specimens must be carefully and rapidly handled in both transport and processing to ensure good recovery of anaerobes.

The specimens should be placed as soon as possible after their collection into an anaerobic transporter. Aspirates of liquid specimen or tissue are always preferred to swabs, although systems for the collection of all three culture forms are commercially available (Figure 1). Several versions of anaerobic transporter are commercially available.

These transport media are very helpful in preserving the anaerobes until the time of inoculation. Liquid specimens may be inoculated into a commercially available anaerobic transport vial, which is devoid of oxygen and sometimes contains an indicator. A plastic or glass syringe and needle may also be used for transport. After the specimen is collected and all air bubbles are expelled from the syringe and needle, the needle tip should be inserted into a sterile rubber stopper (Figure 2). Because air gradually diffuses through the wall of a plastic syringe, no more than 30 minutes should elapse before the specimen is processed. This inexpensive transport device for liquid specimens is especially useful in the hospital, for instance, where the specimen can be rapidly transported to the microbiology laboratory.[9]

Swabs may be placed into sterilized tubes containing carbon dioxide or anaerobically prereduced, sterile Cary-Blair semisolid media. A preferred method utilizes a swab that has been prepared in a prereduced anaerobic tube.

Tissue specimens or swabs can be transported anaerobically in an anaerobic jar or in a petri dish

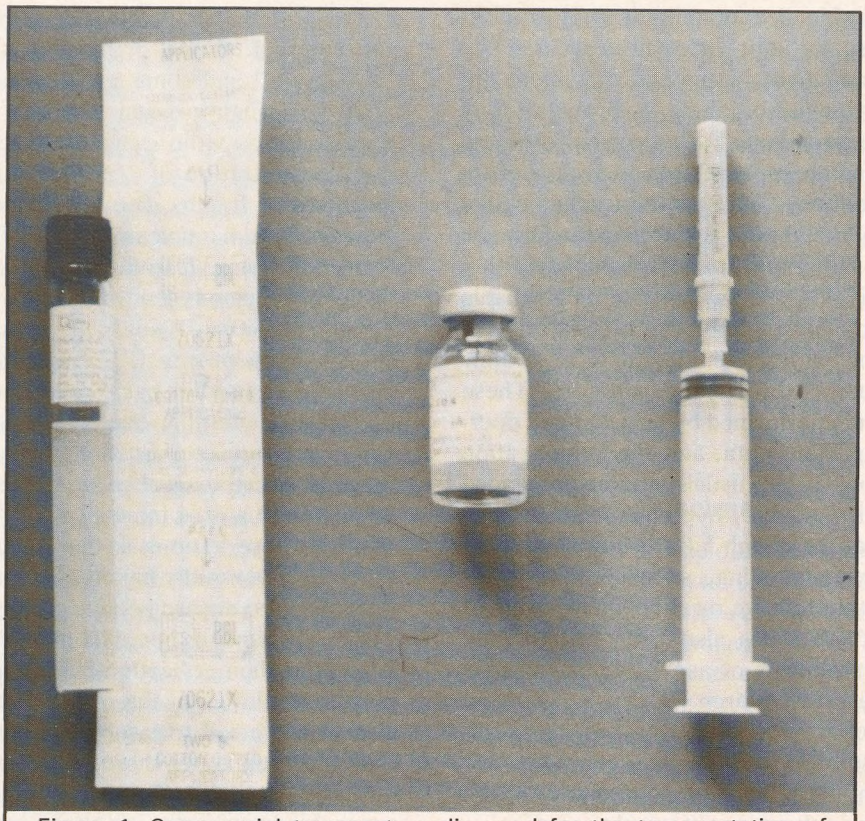

Figure 1. Commercial transport media used for the transportation of anaerobic specimens

placed in a sealed plastic bag made anaerobic by using a catalyzer (Marion Laboratories, Inc, Kansas City, Mo) (Figure 3). Most of the common and clinically important anaerobic bacteria are moderate anaerobes, as shown by the examination of various types of clinical specimens for anaerobes.[11,13]

Because numbers and kinds of microorganisms in clinical materials vary widely, no transport device should be expected to give optimal protection for all anaerobes that may be encountered in specimens. Syed and Loesche[14] reached this conclusion after studying the survival of human dental plaque flora in various transport media. Although some of the transport systems can support the viability of anaerobic organism up to 24 hours,[15,16] all specimens should be transported and processed as rapidly as possible after collection to avoid loss of fastidious oxygen-sensitive anaerobes and the overgrowth of facultative bacteria.

When delay in transportation is expected, refrigeration of the sample may prevent overgrowth of some organisms and preserve their distribution. Several investigators,[15] however, have found refrigeration to be of little benefit.[15]

Conclusions

It is imperative that a physician treating a patient with a suspected anaerobic infection utilize appropriate methods of obtaining cultures of the infected site. This requires that the normal flora are bypassed in obtaining the culture and that an appropriate and rapid transport system is used thereafter. Reliable microbiological data can be obtained only when these proper procedures are performed.

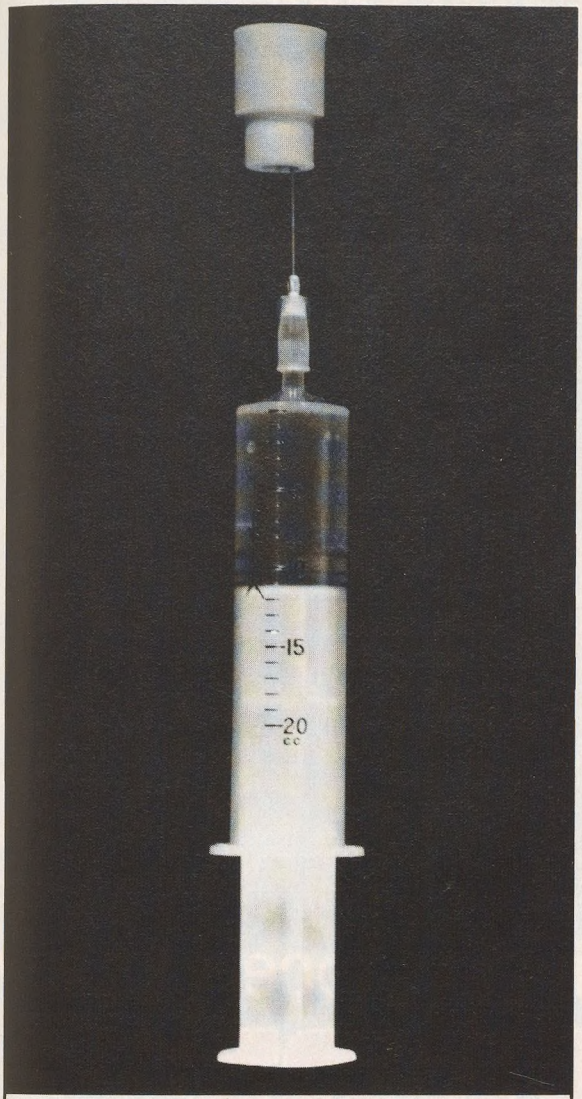

Figure 2. Utilization of a corked syringe and needle for the transportation of an anaerobic specimen

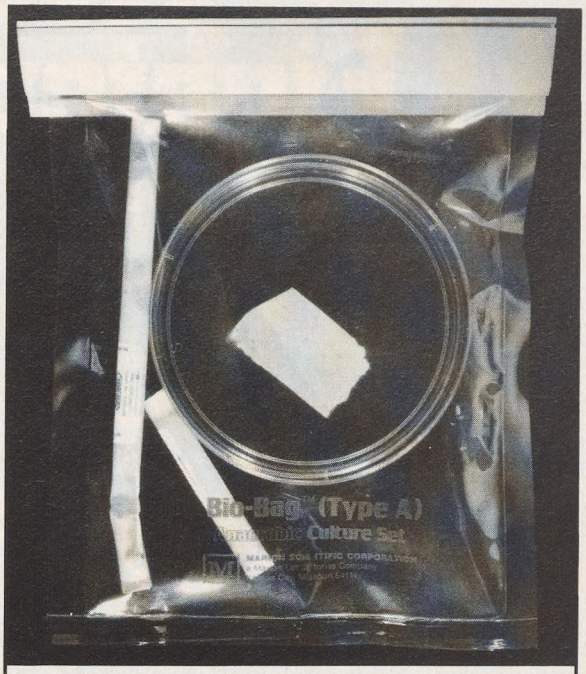

Figure 3. Commercial anaerobic bag system for transportation of tissue or other specimens

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