



Clostridium

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BACKGROUND:

It is not uncommon to receive disease reports involving organisms from the genus *Clostridium*. Often the names of these bacteria are abbreviated. For example *Clostridium difficile* is written as *C. diff*. In fact Clostridia are perhaps the most widely studied anaerobes that cause diseases in humans. In all, there are more than 100 distinct species of bacteria that belong to the genus *Clostridium*. These bacteria can cause an array of human diseases, the most important of which are food poisoning, botulism, tetanus, gas gangrene and pseudomembranous colitis. Typically clostridia are opportunistic pathogens meaning that the organism takes advantage of a breach in a host's natural defenses in order to infect the host.

The common pathogens of this genus are Gram-positive anaerobes. However, due to the biodiversity of this genus it is difficult to make broad generalizations about clostridia. These bacteria are usually anaerobic although some species can become aerotolerant on subculture and a few species (*C. carnis*, *C. histolyticum*, and *C. tertium*) can grow under aerobic conditions. Furthermore, most species are Gram-positive, but a few are Gram-negative.

Aside from a hand full of species clostridia form characteristic spores. The sporulation makes these organisms extremely resistant to disinfectants and drying. In fact, these spores may allow the organisms to persist in the environment for years. Many clostridia are transient or permanent members of the normal flora of the human skin and the gastrointestinal tracts of humans and animals. In addition to being normal flora most clostridia can also be found in the soil of many parts of the world.

Due to the ubiquitous nature of clostridia, many organisms isolated from clinical specimens are merely contaminants and not associated with disease. When determining the importance of a clinical isolate of clostridia, it is important to consider the frequency of isolation of the species, the presence of other microbes of pathogenic potential and the clinical symptoms of the patient.

Characteristics:

Since clostridia are typically anaerobes, they do not create energy by oxidizing sugars to water and carbon dioxide. In the case of anaerobic metabolism, clostridia produce fatty acids and other organic compounds. This metabolic process frequently results in a foul odor. It is interesting to note that some of the odor associated with mud and the decay of plant materials is due to metabolites produced by certain clostridia.

The Gram-stain is useful for identifying clostridia because the cell incorporates the stain while the spore remains unstained. In vitro, clostridia demonstrate optimum growth when plated on blood agar at body temperatures however when the environment becomes hostile, the bacteria produce spores that protect the bacteria from extreme conditions.

All pathogenic clostridia species produce exotoxins which play a central role in the organism's pathogenesis. When the bacteria are in their active form they secrete powerful exotoxins that are responsible for such diseases as tetanus, botulism and gas gangrene.

Epidemiology:

The epidemiology of clostridium organisms is difficult to generalize however the Infectious Disease Epidemiology manual contains disease specific information and should be consulted regarding those diseases.

Specific organisms:

Clostridium tetani is an anaerobic bacterium shaped like a tennis racquet that causes tetanus (lockjaw) in humans. *C. tetani* spores can be acquired from any type of skin trauma involving an infected device leading to tissue contamination. If not treated early, the mortality rates of this disease are high. Immunization is the best way to prevent *C. tetani* infections in children and adults. The process is started early with the first four shots being administered within two years of birth. The initial shots are then followed up with periodic booster shots given every ten years.

C. botulinum produces oval subterminal spores and is motile. Different strains within this species produce one of 8 exotoxin types (A,B,C1,C2,D,E,F,G). Types C and D are encoded by a bacteriophage implanted in the bacteria. Type A is the most potent exotoxin known (10 ng can kill a normal adult). *Clostridium botulinum* produces one of the most potent toxins in existence and causes the deadly botulism food poisoning. Immediate treatment with an anti-toxin must take place for the patient to have a chance at survival. Infantile botulism is acquired in a similar manner but is much milder than the adult version. Honey, is the most common source of the spores which germinate in the child's intestinal tract. Bacterial proliferation and subsequent toxin production cause symptoms which last a few days and then subside without the use of an antitoxin. Infant botulism may occur via germination of spores in the intestinal tract with subsequent toxin production, possibly accounting for some cases of Sudden Infant Death Syndrome (SIDS).

C. perfringens produces large rectangular spores and is non-motile. This species is most often associated with wound infections such as gas gangrene when an anaerobic environment is created by poor blood flow to the wound. This non-motile bacterium is an invasive pathogen that can be contracted from dirt through punctures and lacerations. *C. perfringens* cells proliferate after spore germination occurs and they release their exotoxin. The toxin causes necrosis of the surrounding tissue (Clostridial myonecrosis destroys muscular tissues). Additionally, *C. perfringens* is a major cause of food poisoning in the United States. The disease results from

ingestion of a large number of organisms in contaminated food, usually meat or meat products. *C. perfringens* type A is the usual causative agent. The mortality rate is essentially zero, but elderly and immunologically compromised patients should be closely supervised.

C. difficile produces large oval subterminal spores and two different toxins; toxin A (an enterotoxin causing fluid accumulation in the intestine), and toxin B (a cytopathic agent). Ordinarily, this species can't compete with normal intestinal flora but, when antibiotics eliminate the normal flora, *C. difficile* can produce disease. Pseudomembranous colitis (PC) results predominantly as a consequence of the elimination of normal intestinal flora through antibiotic therapy. Symptoms include abdominal pain with watery diarrhea and leukocytosis. "Pseudomembranes" consisting of fibrin, mucus and leukocytes can be observed by colonoscopy. Untreated pseudomembranous colitis can be fatal in about 27%-44%.

C. sordellii is part of the normal intestinal flora of humans. The organism produces several exotoxins including toxins serologically related to the toxins of *C. difficile*. There are scattered reports in the literature of *C. sordellii* wound infections, most of which involve significant trauma. *C. sordellii* has been occasionally implicated in bone and joint infections, in pulmonary infections, in bacteremia and in fulminate endometritis. Because many clinical laboratories fail to speciate clostridial pathogens, the pathogenic potential of *C. sordellii* is likely underestimated.

C. septicum is a spindle-shaped rod that is motile in young cultures. The organism produces toxins designated alpha, beta, gamma and delta; the alpha toxin is necrotizing and lethal for mice. Whether *C. septicum* is a member of the host's normal flora or whether it takes advantage of a compromised host is uncertain. The organism is not strongly invasive, but has been associated with gas gangrene. Fewer than 200 cases of invasive disease have been reported, but the majority have a malignancy somewhere in the body. The most frequent association is with colorectal cancer, but other types of malignancies have been noted, including leukemia, lymphoma and sarcoma. In one survey of *C. septicum* bacteremia, 49 of 59 (83%) cases had an underlying malignancy and, in 28 of these cases, the portal of entry appeared to be the distal ileum or the colon. Diabetes mellitus is seen in about 20% of cases. In collective review of 162 cases of nontraumatic *C. septicum* infection, 81% of the patients had malignant disease; in contrast, other clostridial species are associated with malignancy in approximately 10% of cases. Thus, in the absence of an overt infection, isolation of *C. septicum* should alert the physician to the possible presence of a malignancy, most likely in the ileum or the colon. Immediate antibiotic therapy is indicated because most patients die quickly of the infection if not treated. Penicillin is the antibiotic of choice, but chloramphenicol, carbenicillin and cephalothin also have been used successfully.

C. tertium is an aerotolerant clostridium that is usually considered nonpathogenic. However, there are scattered reports of this organism causing bacteremia. Most cases have involved neutropenic patients and the gastrointestinal tract appears to be the source of the infection. It is possible that this organism causes many more cases of bacteremia than is currently appreciated. The aerotolerant nature of *C. tertium* may result in its misidentification as a *Bacillus* species.

Other Clostridium: *Clostridium butyricum*, *C. clostridioforme*, *C. innocuum* and *C. ramosum* are isolated with some frequency from clinical specimens and may have an unrecognized clinical significance. These species are often resistant to clindamycin and cephalosporins. *C. ramosum* is usually listed with the ten anaerobic species most frequently isolated from clinical specimens. *C. ramosum* frequently is misidentified, as the Gram reaction is lost easily and spores are difficult to detect. *C. clostridioforme* stains Gram-negative and forms characteristic football-shaped cells, rarely sporulates and may be easily misidentified as *Bacteroides* sp or *Fusobacterium* sp.

Diagnosis:

Clinical:

Clinically, Clostridia infections can present in a number of ways depending on the species and the site of infection. However, since most of these infections are toxin mediated, it may be difficult to isolate the organism. Therefore, the clinical presentation may be the sole basis for the diagnosis.

Gas gangrene: Symptomology and the presence of bacilli in the wound.

Tetanus: Cramping and twitching around a wound, auditory hyperacuity and pain in neck and jaw. Tetanus is similar to strychnine ingestion so must exclude the latter.

Botulism: Difficult to diagnose. Must demonstrate a normal cerebrospinal fluid (CSF) to exclude other possibilities. The toxin is rarely found.

Pseudomembranous colitis: Demonstration of pseudomembranes by colonoscopy is diagnostic.

Laboratory Diagnosis:

C. difficile

Stool should be tested for the presence of *C. difficile* toxins. Testing for toxin is performed by enzyme immunoassay (EIA) or cell cytotoxin assay, which has been the “gold standard” for toxin B. The EIAs are sensitive and easy to perform. Commercially available EIAs that detect both toxins A and B may be used, or an EIA for toxin A may be used in conjunction with cell culture cytotoxicity assay for toxin B. Latex agglutination tests should not be used.

Although *C. difficile* toxin rarely is recovered from stool specimens from asymptomatic adults, it may be recovered from stool specimens from neonates and infants who have no gastrointestinal tract illness. This finding confounds the interpretation of positive toxin assays in patients younger than 12 to 24 months.

C. perfringens

Because the fecal flora of healthy persons frequently includes *C. perfringens*, counts of at least 1 million *C. perfringens* spores per gram of feces obtained within 48 hours of onset of illness are required to support the diagnosis in ill persons. The diagnosis also can be established by detection of *C. perfringens* enterotoxin in stool by commercially available kits. To confirm *C. perfringens* as the cause, the concentration of organisms should be at least 100,000 per gram in the epidemiologically implicated food. Although *C. perfringens* is an anaerobic bacteria, special transport conditions are unnecessary because the spores are durable. Stool, rather than rectal swabs should be collected.

Botulism

A toxin neutralization bioassay in mice is used to identify botulinum toxin in serum, stool, or suspect foods. To increase the likelihood of diagnosis, both serum and stool should be obtained from all persons with suspected botulism. In infant and wound botulism, the diagnosis is made by demonstrating *C. botulinum* organisms or toxin in feces or wound exudate or tissue samples. Toxin has been demonstrated in serum in approximately 1% of infants with botulism. In foodborne cases, serum specimens collected more than 3 days after ingestion of toxin usually are negative, at which time stool and gastric aspirates are the best diagnostic specimens for culture. Since obtaining a stool specimen may be difficult because of constipation, an enema using sterile nonbacteriostatic water can be given.

Enriched and selective media are used to culture *C. botulinum* from stool and foods. *C. botulinum* is a large, usually gram-positive, strictly anaerobic bacillus that forms a subterminal spore.

The reporting source may request the assistance of the health department in sending specimens (stool and blood) to CDC for testing. Consult the Infectious Disease Epidemiology Section on guidelines/ requirements for accepting specimens and the appropriate handling of them.

Stool and blood specimens must be sent to the Central Laboratory in New Orleans to be forwarded to the Centers for Disease Control and Prevention (CDC). Stool specimens (1-2 gms) are to be collected in a clean container (no preservatives) and kept refrigerated. Serum specimens (at least 1 cc) are to be collected in a red-topped tube and either spun down and sera sent or the whole blood sent refrigerated.

Tetanus

There are no laboratory findings characteristic of tetanus. The diagnosis is entirely clinical and does not depend upon bacteriologic confirmation. *C. tetani* is recovered from the wound in only 30% of cases, and can be isolated from patients who do not have tetanus. Laboratory identification of the organism depends most importantly on the demonstration of toxin production in mice.