Genus Clostridia

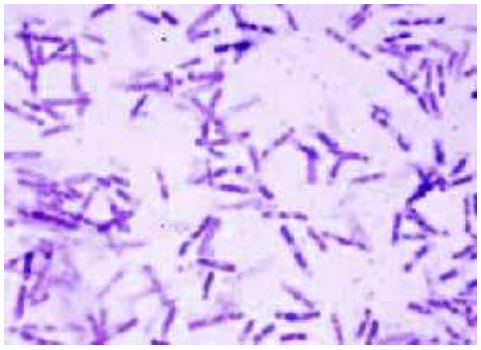
Clostridia are the gram-positive sporefoming anaerobic bacilli that belong to the genus Clostridia family Clostridiaceae. Most of the clostridia are saprophytes, but a few are pathogenic for humans, primarily *C. tetani, C. botulinum, Clostridium perfringens* and *C.difficile*. Nature habitat for Clostridia is the soil, human and animal intestines. Vegetative cells of clostridia are high sensitive to heating, oxygen, disinfectants. Under the unfavorable conditions Clostridia produce spores which are characterized with high resistance to heating. Spores survive about 1-4 hours at 100°C. But all of them are destroyed by autoclaving at 121°C within 20 minutes.

Clostridium of gas gangrene

Clostridium of gas gangrene are a group of morphologically and biologically similar microorganisms that can cause in association with each other as well as with representatives of aerobic and anaerobic opportunists microorganisms severe wound infection - gas gangrene. Themain causative agents of gas gangrene are *C.perfringens, C.novyi, C.septicum, C.hystoliticum.* The dominant agent of gas gangrene is *C.perfringens.*

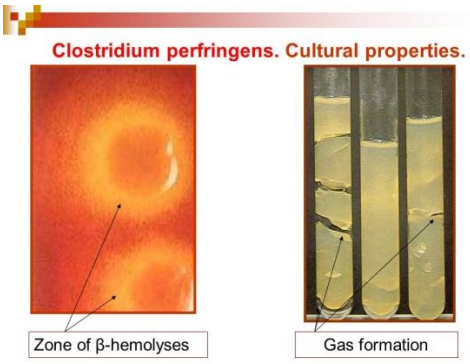
Clostridium perfringens

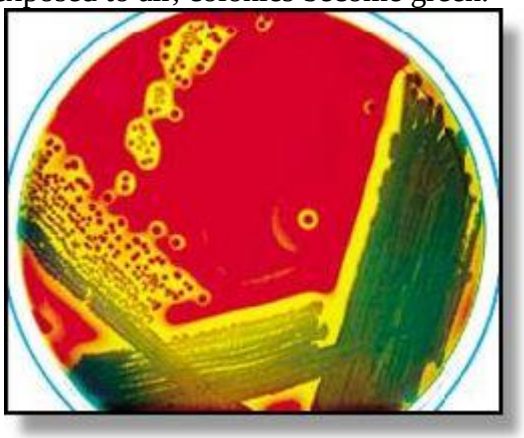
Morphology. *C.perfringens* are large, plump, gram-positive bacillus with straight, parallel sides, rounded or truncated ends. They may occur singly or in chains. *C.perfringens* are capsulated and nonmotile. Spores are central or subterminal.



***C.perfringens* Gram staining**

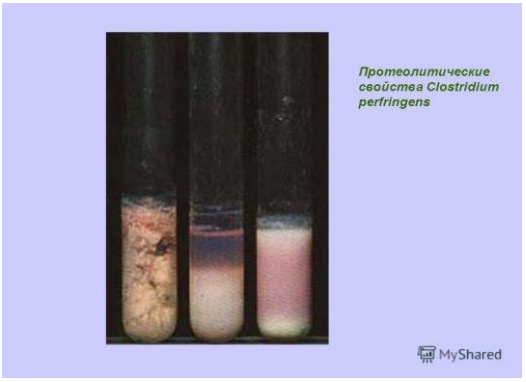
Culture properties. On solid nutrient media (blood agar) *C.perfringens* form S- and R-colonies. S-colonies are smooth, round, with regular edges, gray-white. R-colonies are flat, dry, with uneven edges. Colonies are hemolysed. Under exposed to air, colonies become green.





***C.perfringens* on blood agar**

On Kitt-Tarozzi medium uniform turbidity and gas production after 2-3 hours of inoculating are forming. In Wilson and Blair medium through 1 hour after the inoculation black colonies (due to the reduction of iron) appear.



Proteolitic properties

In the milk via 6-8 hours sponge-like clot (stormy fermentation) is produced.Robertson's cooked meat broth is useful medium. Meat is turned pink but not digested with sour odor.

Fermentative properties. *C.perfringens* have expressed biochemical properties. They ferment glucose, sucrose, lactose, maltose, galactose, glycogen with acid and gas. Indol and VP are negative, MR is positive. Proteolitice activity is weakened. In meat medium they form butyric and acetic acids, and a large amount of gases (H_2S, NH_3, CO_2). They reduce nitrate in nitrite and liquefy gelatin.

Antigenic structure. *C.perfringens* are differentiated into 6 types (A,B,C,D,E,F) on the basis of toxin produced by the strains. Toxins are antigenic and antitoxic sera are used in routine typing of strain. In human pathology a leading role belongs to serovars A, C, D. Serovars A and C, except gas gangrene, cause food poisoning.

Virulent factors. *C.perfringens* produce 12 distinct toxins, besides many other enzymes and biological active soluble substances. The 4 major toxins (α, β, ε, ι) are responsible for pathogenecity. Alpha toxins, having lecithinase activity, cause hemolytic, dermatonecrotizing and lethal effects. Toxins γ,δ, η, κ, λ have lethal and necrotizing properties.Virulent enzymes are represented by hyaluronidase, fibrinolysins, collagenase, proteinase, gelatinase, deoxyribonuclease, etc. They cause profound damage of the cell membranes, which leads to the formation of edema, violation of the redox potential of cells and eventually to cell death. Capsule has antiphagocytic properties.

Epidemiology. Clostridium from human and animals intestines get into the environment (soil, water), where they form spores that are stored for years. The source of infection is the soil. The way of transmission is through the wound.

Organisms enter the wound usually along with foreign particles, e.g. soil, dust, etc. Soil contamination of wounds, massive tissue damage and necrosis create favorable conditions for the development of gas gangrene pathogens. Concomitant aerobic and opportunistic anaerobic microflora help Clostridium vegetation in wound that is, reducing the oxygen, promotes anaerobiosis. Clostridium reproduction is accompanied by production of exotoxins and virulent enzymes that broaden the zone of necrosis. The process spreads particularly intense in the muscle. In the affected area lactic acid and gases (CO_2 and H2) are accumulated, that determine the characteristic symptoms of the disease: edema and crepitation. Soaked in the blood, bacterial toxins and cell's decomposition products cause intoxication, degenerative changes of internal organs are developed.

Apart from this *C.perfringens* may cause gangrenous appendicitis, biliary tract infection, brain abscess, meningitis, panophthalmitis, urogenital infection, etc. Rarely septicemia and endocarditis may occur.

Immunity. Immunity after the disease is not developed.

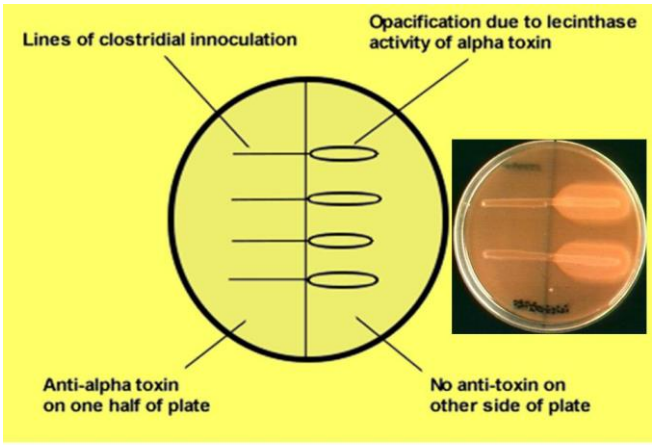
Microbiological diagnosis. Bacteriological method allows to identify causative agents of gas gangrene, detect their serotypes, that are important for specific therapy. Specimens are collected from muscles at the edge of affected area, exudate from area where infection appears more active, necrotic tissue and muscle fragment.At microscopic examination there are a large number of

Gram-positive bacilli, but no leucocytes in the smear. Material is inoculated on Kitt-Tarozzi medium. If the material is grossly contaminated with other organisms, heating at 80°C for 20 minutes may be useful for destroying non-sporing organisms.Anaerobic culture is studied after 48 to 72 hours of incubation. Simultaneously material is inoculated on the Wilson and Blair and milk media. On the second stage detection of turbidity in Kitt-Tarozzi media is detected, Gram stainingof the smear to study morphological properties of bacteriais made, and material on the blood agar, sugar agaris inoculated.

Table Differential signs of the main causative agents of gas gangrene

	Kitt-Tarozzi	**Blood agar**	**Wilson and Blair**	**Milk**
C.perfringens	uniform turbidity, vigorous gas formation within 3-6 hours	round, grey colonies, hemolysis	blackening and column breaks after 1-3 hours	stormy fermentation and gas production after 3-6 hours
C. novyi	a slight turbidity, moderate gas formation	grey sponge-like colonies, hemolysis	blackening without breaks after 24 hours	slow and not typical coagulation
C. septicum	uniform turbidity, vigorous gas formation within 6-12 hours	delicate lace convoluted thread, hemolysis	blackening and column breaks after 6-8 hours	slow and not typical coagulation
C. histolyticum	uniform turbidity without gas formation, rapid dissolution of liver pieces	small transparent “droplets of dew”	unchanged	fast peptonization without clots

Isolated pure culture is further identifed by Nagler's reaction (detection of the presence of alpha-toxin (lecithinase)), biochemical properties, biological method (animal pathogenicity).



Nagler's reaction

Neutralization test is used to detect type of exotoxin.

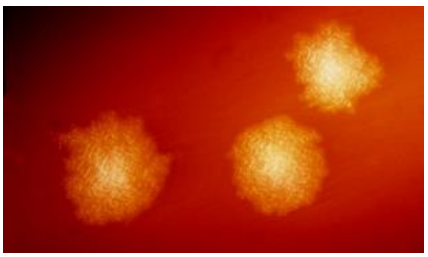
Blood, collected during bacteremia, is cultured in cooked meat medium and glucose broth. It is identified in usual way. In recent years PCR is used to identify causative agents.

Treatment and prophylaxis. Surgical treatment of wounds is the first step in gas gangrene treatment. All damaged, necrotic tissues, foreign bodies and blood clots must be removed from the wound. The major point of thre therapy is adequate local treatment of the wound (antiseptics, antibiotics (penicillin, cephalosporins), hyperbaric oxygenation). Therapy with corresponding antitoxin is necessary to prevent spreading of gas gangrene. Passive immunization with anti-gas gangrene serum is rare in use because it is little effective.

Traumatic wounds should be thoroughly cleansed and debrided. Traditionally, the antibiotic of choice for severe clostridial infection has been penicillin G (20 million units per day in adults). Penicillin G treatment of gas gangrene has become more controversial because of increasing resistance to this drug and data obtained from animal models of infection. In a mouse model of

gas gangrene, antibiotics inhibiting toxin synthesis appeared to be preferable to cell wall–active drugs; clindamycin treatment enhanced survival more than therapy with penicillin; and the combination of clindamycin and penicillin was superior to penicillin alone. For severe clostridial sepsis, clindamycin may be used at a dose of 600 mg every 6 h in combination with high-dose penicillin (3–4 million units every 4 h). Although no clinical trials validate this choice, it is gaining acceptance in the infectious disease community. In cases of penicillin sensitivity or allergy, other antibiotics should be considered, but all should be tested for in vitro activity because of the occasional isolation of resistant strains. Clostridia are frequently, but not universally, susceptible in vitro to cefoxitin, carbenicillin, chloramphenicol, clindamycin, metronidazole, doxycycline, imipenem, minocycline, tetracycline, third generation cephalosporins, and vancomycin. For severe clostridial infections, sensitivity testing should be done before an antimicrobial agent with unpredictable activity is used. Simple contamination of a wound with clostridia should not be treated with antibiotics. Localized skin and soft tissue infection can be managed by debridement rather than with systemic antibiotics. Drugs are required when the process extends into adjacent tissue or when fever and systemic signs of sepsis are present. Surgery is a mainstay of therapy for gas gangrene. Amputation is often required for rapidly spreading infection involving a limb, as the process frequently fails to respond to antibiotics. Hysterectomy is required for uterine myonecrosis. Abdominal wall myonecrosis usually continues despite initial aggressive surgery and antibiotic therapy and requires repeated surgical debridement of all involved muscle.

C.difficile are causative agents of pseudomembranous enterocolitis. This disease is developed after the irrational using of antibiotics.
C.difficile are motile gram-positive bacillus, sporeforming. *C.difficile g*row on the media for anaerobes. Colonies have characteristic morphology -white, dense with rough edges (resembling spots) and have an odor of cresol.



C.difficile colonies

C.difficile produce two exotoxins (A and B). Toxin A is enterotoxin, stimulates guanylate cyclase, has diarrheagenic and lethal action. Toxin B is cytotoxin, damages cells of intestinal epithelium, inhibits protein synthesis, disturbs membrane function.
C.difficile are part of the intestine normal microflora of 3% of healthy adults and in 30-50% of infants. Intestinal lesions are often observed after the treatment with clindamycin, aminopenicillin, cephalosporins. Antibiotics suppress normal microflora (first of all bacteroides, bifidobacteria), which is the foundation of colonization resistance. *C.difficile* survive due to spores formation. Abolition of antibiotics promotes the germination of spores to vegetative cells that produce exotoxins. The disease can also be spread in the hospital. Symptoms include watery diarrhea, fever, nausea, and abdominal pain. Pseudomembranous colitis, toxic megacolon, perforation of the colon, and sepsis are the main complications.
The main method of diagnosis is bacteriological investigation. Material for investigation is feces. Presence of toxins is detected on the cell culture (CPE) or using ELISA. PCR has important value. Vancomycin, metronidazole are used for treatment. Eubiotics are used for recovery of normal microflora.

BOTULISM
C.botulinum

Morphology. *C.botulinum* are gram-positive, non-capsulated and motile rods, peritrichous. They form oval and bulging spores, resemble tennis racket.

C.botulinum stain with gentian violet

Culture properties. *C.botulinum are* strick anaerobe that grow only in the absence of oxygen. The optimum temperature is 20-35°C in neutral or slightly alkaline medium.On the blood agar they form large, irregular, hemolysed colonies. On Kitt-Tarozzi medium they cause turbidity and gas production. In the column of agar colonies resemble tuft of cotton wool.

C.botulinum on blood agar after 48hs of incubation

Fermentative properties. *C.botulinum* ferment glucose and maltose with acid and gas, produce H_2S and ammonia, volatile amines, ketones, acetic, citric and lactic acid, peptonize milk, liquefy gelatin.

Antigenic structure. According to the botulotoxin antigen structure C.botulinum may be divided into 8 serogroups (A, B, C1, C2, D, E, F, G). Serovars A, B and E the most often cause disease in human.

Virulen factors. Botulinum toxin is the most powerful biological poison(lethal dose for human is about 1-2 μg).It is released from bacteria as inactive protein that must be cleaved by either microbial or human protease to expose the active site. It is neurotoxin, which acts slowly by inhibiting release of acetylcholine at neuromuscular junctions, that slows transferring of impulses from motoneurons to muscles, leading to flaccid paralysis.

Epidemiology. *C.botulinum* from animals intestines get into the soil, where they form spores. The way of transmission is alimentary. The spores, got into the food (often meat, fish, vegetables preservations), germinate, forming exotoxin that accumulates in products.

Pathogenesis. *C.botulinum* cause botulism - the food-poisoning infection, resulting from ingestion of food that contain exotoxin. Incubation period is 12 to 36 hours. Vomiting, diplopia, constipation, difficulty in swallowing, speaking and breathing (disphagya, disphonia) are the manifestations, which may be followed by coma, delerium and death in 1 to 7 days. Wound botulism is separated clinical form, it occurs as the result of wound contamination with soil. Toxin is absorbed at the site of infected wound. The symptoms are the same with food-borne botulism except for gastrointestinal symptoms. Infant botulism is resulting of germination of spores in the gut. Clinical symptoms are constipation, inability to suck.

Microbiological diagnosis. Specimens for diagnosis are food, feces, vomit, may be patient's blood. Methods of investigaion are bacteriological for causative agent isolation, isolation of toxin

and it's typing. Detection of toxin is made by biological method (neutralization test) and IHT. For neutralization test, specimens (three portions) are mixed with definite type of antitoxin (A, B, E) and are inoculated intraperitoneally into separate mice. One portion without antitoxin is control. If antitoxin is specific with the type of toxin, antitoxin neutralized toxin and the mouse survives. The rest mice die.

Treatment. Administration of the polyvalent antitoxic serum before the detection of toxinserotype, and monovalent serum after detection of toxinserotype, is used for specific treatment.

Patients should be hospitalized and monitored closely, both clinically and by spirometry, pulse oximetry, and measurement of arterial blood gases for incipient respiratory failure. Intubation and mechanical ventilation should be strongly considered when the vital capacity is <30% of predicted, especially when paralysis is progressing rapidly and hypoxemia with absolute or relative hypercarbia is documented. Serial measurements of the maximal static inspiratory pressure may be useful in predicting respiratory failure. In food-borne illness, equine antitoxin should be administered as soon as possible after specimens are obtained for laboratory analysis. Treatment should not await laboratory analyses, which may take days. The previous trivalent antitoxin preparation (types A, B, and E) is no longer available. Instead, a bivalent preparation containing toxin types A and B and an investigational monovalent type E preparation can be obtained. The bivalent preparation is administered routinely; monovalent type E antitoxin is given in addition when exposure to type E toxin is suspected (after seafood ingestion, for example). In the United States, antitoxin as well as help with clinical management and laboratory confirmation are available at any time from state health departments or from the Centers for Disease Control and Prevention (CDC; emergency number, 770-488-7100). A limited supply of an investigational heptavalent antitoxin (types A through G) is maintained by the U.S. military for emergency use.

After testing for hypersensitivity to horse serum, antitoxin is given as recommended by the CDC; repeated doses are not considered necessary. Anaphylaxis and serum sickness are risks inherent in use of the equine product, and desensitization of allergic patients may be required. If there is no ileus, cathartics and enemas may be used to purge the gut of toxin; emetics or gastric lavage can also be used if the time since ingestion is brief (only a few hours). Neither the use of antibiotics to eliminate an intestinal source of possible continued toxin production nor the administration of guanidine hydrochloride and other drugs to reverse paralysis is of proven value.

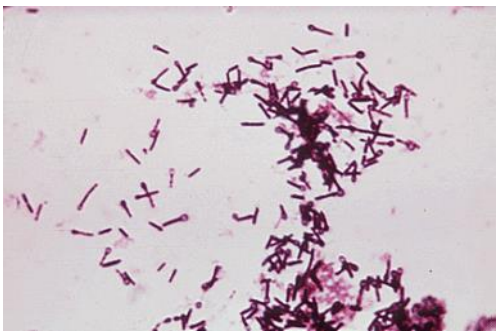
Treatment of infant botulism requires supportive care and administration of human botulism immune globulin, which can be obtained by calling the California Department of Health Services at 510-231-7600 or by following the instructions at www.infantbotulism.org. Neither equine antitoxin nor antibiotics have been shown to be beneficial. In wound botulism, equine antitoxin is administered. The wound should be thoroughly explored and debrided, and an antibiotic such as penicillin should be given to eradicate C. botulinum from the site, even though the benefit of this therapy is unproven. Results of wound cultures should guide the use of other antibiotics.

Botulinum toxins are being employed for a variety of cosmetic and therapeutic purposes, and new uses are being evaluated. Generalized botulism-like weakness complicating therapy (iatrogenic botulism) has been reported but is rare.

Prophylaxis. Non specific prophylaxis includes proper food canning and preservation.Active immunization with toxoid is effective.

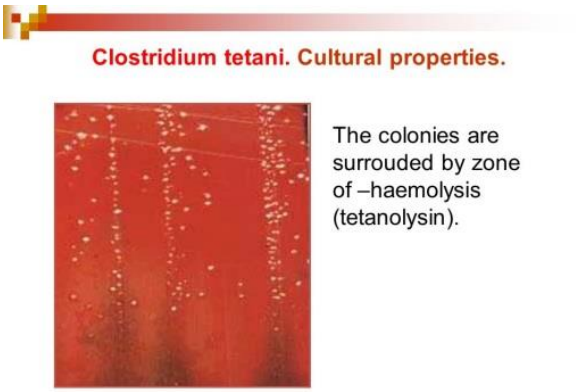
TETANUS
C.tetani

Morphology. *C.tetani are* slender, long slightly curved, gram-positive bacilli, occurring singly or in chain. Spores are spherical, terminal and bulging, giving the bacilli drumstick appearance. They are non-capsulated and motile.



C.tetani

Culture properties. *C.tetani are* an obligatory anaerobes that grow only in absence of oxygen. The optimum temperature is 37°C and pH 7.4. They grow fairly well in Kitt-Tarozzi medium, blood agar, sugar agar, Wilson and Blair medium. On the blood agar colonies have tendency to swarm over the surface; they are surrounded with α-hemolytic zone. In deep agar culture colonies are like spherical fluffy balls, about 1-3mm, with radial filamentous edges. On solid media they form flat as a thin film with uneven edges colonies.



C. tetani colonies

Fermentative properties. The most strains don't ferment carbohydrates, don't produce lipase, urease, don't reduced nitrates, decompose gelatin.

Antigenic strucuture. *C.tetani* possess O- and H-antigens. O-antigen is common for all representatives of the genus. The flagellar antigen differentiates *C.tetani* into 10 types. Tetani toxin (neurotoxin) is pharmacologically and antigenically identical.

Virulent factors. Clostridium tetani produces two types of toxin: hemolysin (tetanolysin) and neurotoxin (tetanospasmin). Tetanolysin causes lysis of erythrocytes. Tetanospasmin is a powerful neurotoxin, responsible for all clinical signs of tetanus. It acts on inhibitory neurons of central nervous system and blocks the release of the glycine and γ-butric acid (neurotransmitters).

Epidemiology. The source of the infection is human and animal (*C.tetani* are saprophytes of intestine). Standing out from the faeces, pathogen is widely distributed in the environment, especially in the soil, forming the spores. Disease follows the injury such as puncture or battle wounds, septical abortion, surgery operation carried out with non-sterile instruments, etc.

Pathogenesis. Tetanus results from contamination of wound by *Clostridium tetani*. Germination and multiplication occur if certain factors like necrotic tissue, ionisable calcium salts and lactic acid are present. Toxin is absorbed from the area of infection and through the motor nerve endings reaches anterior horn cell. In inhibitory neurons, tetanospasmin blocks transmission of a nervous impulse. The absence of inhibitory influence permits to simultaneous spasms of muscles, producing muscle rigidity and clenching of the *jaw (trismus*) and *arching of the back (opistotonus).*

Opistotonus

The incubation period is 2 days to several weeks and it depends upon the site, nature of wound, doses, toxigenecity of organism and immune status of patient.

There are many clinical types of tetanus. Tetanus neonatorum occurs from contamination of cut surface of umbilical cord in infants. It has high rate of fatality. Post abortal and puerperal tetanus results from the infection of genital tract with unsterile instrument and dressing. Puerperal tetanus is rare but most dangerous. Cephalic tetanus occurs from the wounds of head. Splanchnic tetanus affects mainly muscles of deglutition and respiration with dysphagia.

Immunity. Post infectious immunity is not developed.

Microbiological diagnosis. Microbiological diagnosis is used only for confirmation of clinical diagnose. Microscopy of wound exudates and *C.tetani* demonstration give presumptive diagnose. Bacteriological method is used for confirmation and identification of isolated from wound clostridia. Material (necrotic tissues, blood, exudate from wound) is inoculated on the special media. Isolated microorganisms are identified by biochemical properties. Tetanospasmin can be detected in the wound discharge with neutralization test in animals.

Treatment. Penicillin, tetracycline, cephalosporines can be used for etiotropic treatment. Antitoxic serum and human anti-tetanus immunoglobulin are used for specific treatment.

ANTIBIOTIC THERAPY Although of unproven value, antibiotic therapy is administered to eradicate vegetative cells—the source of toxin. The use of penicillin (10–12 million units IV, given daily for 10 days) has been recommended, but metronidazole (500 mg every 6 h or 1 g every 12 h) is preferred by some experts on the basis of this drug's excellent antimicrobial activity and the absence of the GABA-antagonistic activity seen with penicillin. The drug of choice remains unclear: one nonrandomized clinical trial found a survival benefit with metronidazole, but another study failed to find a difference among benzathine penicillin, benzyl penicillin, and metronidazole. Clindamycin and erythromycin are alternatives for the treatment of penicillin allergic patients. Additional specific antimicrobial therapy should be given for active infection with other organisms.

ANTITOXIN Given to neutralize circulating toxin and unbound toxin in the wound, antitoxin effectively lowers mortality; toxin already bound to neural tissue is unaffected. Human tetanus immune globulin (TIG) is the preparation of choice and should be given promptly. The dose is 3000–6000 units IM, usually in divided doses because the volume is large. The optimal dose is not known, however, and results from one study indicated that a 500-unit dose was as effective as higher doses. Pooled IVIg may be an alternative to TIG, but the specific antitoxin concentration in this formulation is not standardized. The value of administering antitoxin before wound manipulation or of injecting a dose proximal to the wound or infiltrating the wound is unclear. Additional doses are unnecessary because the half-life of antitoxin is long. Antibody does not penetrate the blood-brain barrier. Intrathecal administration should be considered experimental. Equine tetanus antitoxin (TAT) is not available in the United States but is used elsewhere. It is cheaper than human antitoxin, but the half-life is shorter, and its administration commonly elicits a hypersensitivity reaction and serum sickness.

Prophylaxis. Tetanus toxoid, which is the component of different vaccines (DPT, DT-anatoxin, TABte), is used for routine immunization. Antitoxic serum and human anti-tetanus immunoglobulin are used for passive prophylaxis.