The Neisseriae

The family Neisseriaceae includes the genera Neisseria, Kingella, Eikenella, Simonsiella, Alysiella, and several unnamed species **The neisseriae are gram-negative cocci that usually occur in pairs.** ***Neisseria gonorrhoeae (gonococci) and Neisseria meningitidis (meningococci*****) are pathogenic for humans and typically are found associated with or inside polymorphonuclear cells.** Some neisseriae are normal inhabitants

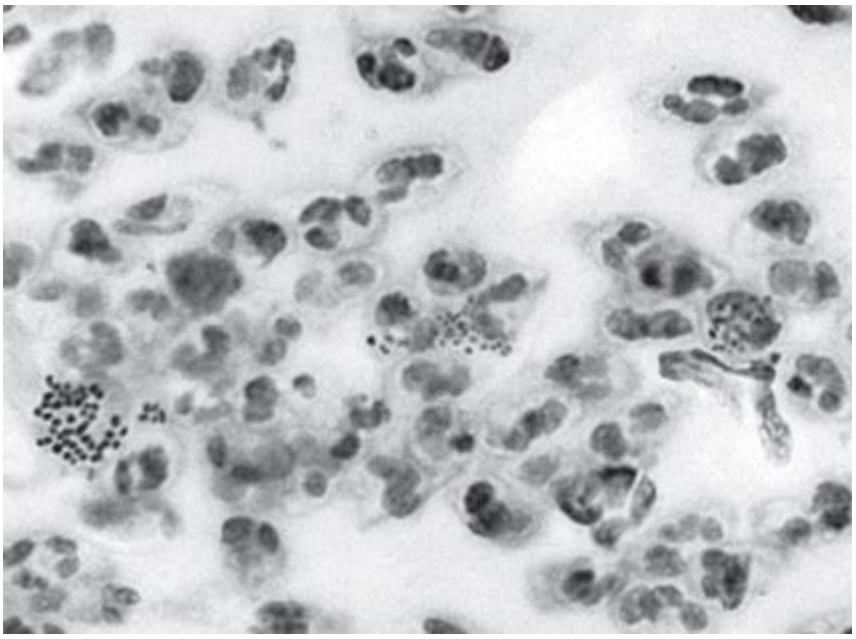
of the human respiratory tract, rarely if ever cause disease, and occur extracellularly .

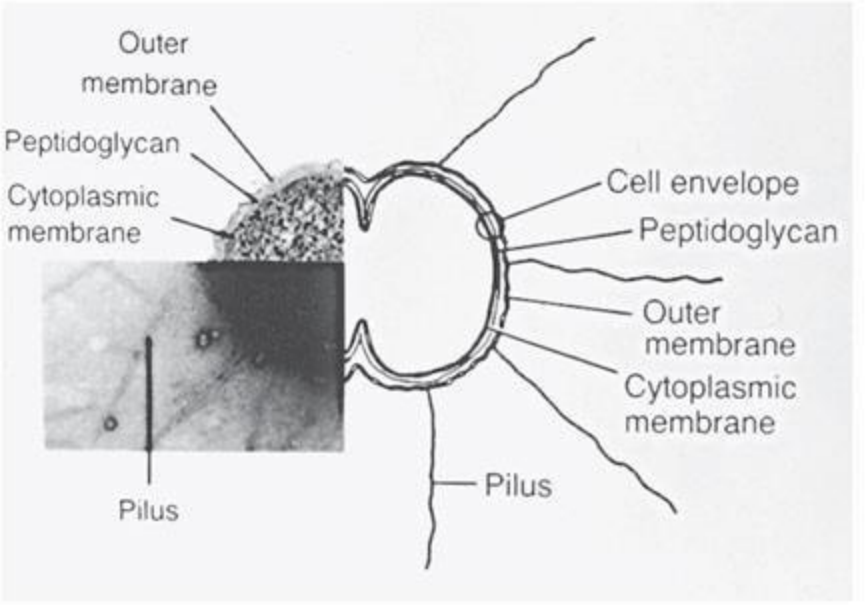
(comparison) : Gonococci and meningococci are closely related, with 70% DNA homology, and are differentiated by a few laboratory tests and specific characteristics:

1 - Meningococci have polysaccharide capsules, whereas gonococci do not.

2 - and meningococci rarely have plasmids whereas most gonococci do.

3 - Most importantly, the two species are differentiated by the usual clinical presentations of the diseases they cause: Meningococci typically are found in the upper respiratory tract and cause meningitis, while gonococci cause genital infections.





Morphology & Identification

A. TYPICAL ORGANISMS

The typical neisseria is a gram-negative, nonmotile diplococcus, approximately 0.8 µm in diameter . Individual cocci are kidney-shaped; when the organisms occur in pairs, the flat or concave sides are adjacent.

B. CULTURE

In 48 hours on enriched media (eg, Mueller-Hinton, modified Thayer-Martin), gonococci and meningococci form convex, glistening, elevated, mucoid colonies 1–5 mm in diameter. Colonies are transparent or opaque, non pigmented, and non hemolytic. *Neisseria flavescens, Neisseria subflava, and Neisseria lactamica* have yellow pigmentation. *Neisseria sicca* produces opaque, brittle, wrinkled colonies. *M catarrhalis* produces non pigmented or pinkish-gray opaque colonies.

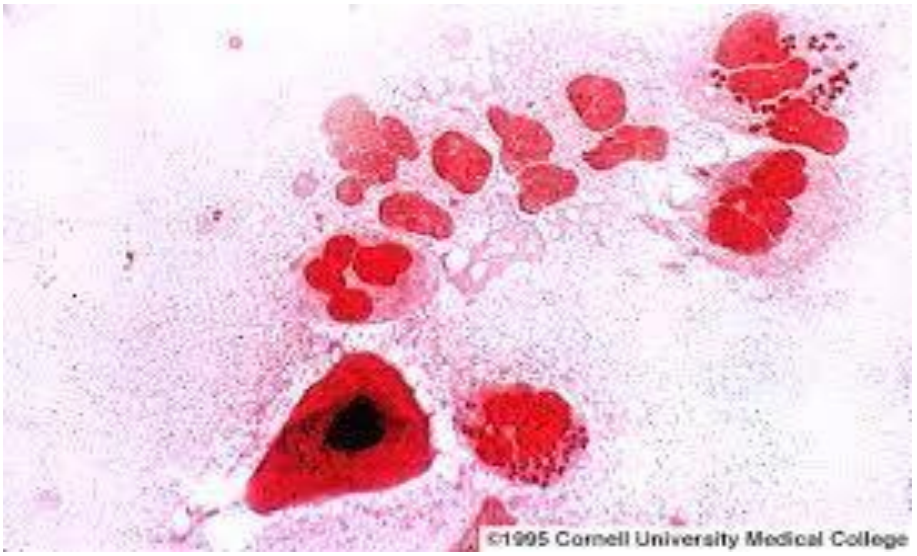
C. GROWTH CHARACTERISTICS

The neisseriae grow best under aerobic conditions, but some will grow in an anaerobic environment. They have complex growth requirements. Most neisseriae ferment carbohydrates, producing acid but not gas, and their carbohydrate fermentation patterns are a means

of distinguishing them . **The neisseriae produce oxidase and give positive oxidase reactions; the oxidase test is a key test for identifying them**. When bacteria are spotted on a filter paper soaked with tetramethyl paraphenylenediamine hydrochloride (oxidase), the neisseriae rapidly turn dark purple. Meningococci and gonococci grow best on media containing complex organic substances such as heated blood, hemin, and animal proteins and in an atmosphere containing 5% CO2 (eg, candle jar). Growth is inhibited by some toxic constituents of the medium, eg, fatty acids or salts. The organisms are rapidly killed by drying, sunlight, moist heat, and many disinfectants. They produce autolytic enzymes that result in rapid swelling and lysis in vitro at 25 °C and at an alkaline pH .

NEISSERIA GONORRHOEAE

Gonococci ferment only glucose and differ antigenically from the other neisseriae. Gonococci usually produce smaller colonies than those of the other neisseriae. Gonococci that require arginine, hypoxanthine, and uracil (Arg– , Hyx– , Ura– auxotype) tend to grow most slowly on primary culture. Gonococci isolated from clinical specimens or maintained by selective subculture have typical small colonies containing piliated bacteria. On nonselective subculture, larger colonies containing non piliated gonococci are also formed. Opaque and transparent variants of both the small and large colony types also occur; the opaque colonies are associated with the presence of a surface-exposed protein, Opa.



Antigenic Structure

N gonorrhoeae is antigenically heterogeneous and capable of changing its surface structures in vitro—and presumably in vivo—to avoid host defenses. Surface structures include the following:

A - (FIBRIAE) Pili are the hair-like appendages that extend up to several micrometers from the gonococcal surface. They enhance attachment to host cells and resistance to phagocytosis. They are made up of stacked pilin proteins (MW 17,000–21,000).

The pilins of almost all strains *of N gonorrhoeae* are antigenically different, and a single strain can make many antigenically distinct forms of pilin

B. POR

Por protein extends through the gonococcal cell membrane. It occurs in **trimers to form pores in the surface through which some nutrients enter the cell. Por proteins may impact intracellular killing of gonococci within neutrophils by preventing phagosome-lysosome fusion**. The molecular weight of Por varies from 34,000 to 37,000. Each strain of gonococcus expresses only one of two types of Por, but the Por of different strains is antigenically different .

C. OPA PROTEINS

These proteins function in adhesion of gonococci within colonies and in attachment of gonococci to host cells .

D. RMP (PROTEIN III)

This protein (MW about 33,000) is antigenically conserved in all gonococci. It is a reduction-modifiable protein (Rmp) and changes its apparent molecular weight when in a reduced state**. It associates with Por in the formation of pores in the cell surface.**

E. LIPOOLIGOSACCHARIDE (LOS) In contrast to the enteric gram- negative rods , gonococcal LPS does not have long Oantigen side chains and is called a lipooligosaccharide. Its molecular weight is 3000–7000. Gonococci can express more than one antigenically different LOS chain simultaneously.

Toxicity in gonococcal infections is largely due to the endotoxic effects of LOS. In a form of molecular mimicry, gonococci make LOS molecules that structurally resemble human cell membrane glycosphingolipids. A structure The gonococcal LOS and the human glycosphingolipid of the same structural class react with the same monoclonal antibody, indicating the molecular mimicry. The presence on the gonococcal surface of the same surface structures as human cells helps gonococci evade immune recognition.

F. OTHER PROTEINS Several antigenically constant proteins of gonococci have poorly defined roles in pathogenesis.

Pathogenesis

Gonococci exhibit several morphologic types of colonies , but only piliated bacteria appear to be virulent. Opa protein expression varies depending on the type of infection**. Gonococci that form opaque** colonies are isolated from men with symptomatic urethritis and from uterine cervical cultures at mid cycle**. Gonococci that form transparent** colonies are frequently isolated from men with asymptomatic urethral infection, from menstruating women, and from invasive forms of

gonorrhea, including salpingitis and disseminated infection. Antigenic variation of surface proteins during infection allows the organism to circumvent host immune response.

Gonococci attack mucous membranes of the genitourinary tract, eye, rectum, and throat, producing acute suppuration that may lead to tissue invasion; this is followed by chronic inflammation and fibrosis.

In males, there is usually urethritis, with yellow, creamy pus and painful urination. The process may extend to the epididymis. As suppuration subsides in untreated infection, fibrosis occurs, sometimes leading to urethral strictures. Urethral infection in men can be asymptomatic.

In females, the primary infection is in the endocervix and extends to the urethra and vagina, giving rise to mucopurulent discharge. It may then progress to the uterine tubes, causing salpingitis, fibrosis, and obliteration of the tubes. Infertility occurs in 20% of women with gonococcal salpingitis. Chronic gonococcal cervicitis or proctitis is often asymptomatic

Gonococcal **bacteremia leads to skin lesion**s (especially hemorrhagic papules and pustules) on the hands, forearms, feet, and legs and to tenosynovitis and suppurative arthritis, usually of the knees, ankles, and wrists. Gonococci can be cultured from blood or joint fluid of only 30% of patients with gonococcal arthritis**. Gonococcal endocarditis** is an uncommon but severe infection. Gonococci sometimes cause meningitis and eye infections in adults. Complement deficiency is frequently found in patients with **gonoccal bacteremia. Gonococcal ophthalmi**a **neonatorum**, an infection of the eye of the newborn, is acquired during passage through an infected birth canal.

Diagnostic Laboratory Tests

A. SPECIMENS

Pus and secretions are taken from the urethra, cervix, rectum, conjunctiva, throat, or synovial fluid for culture and smear. Blood culture is necessary in systemic illness

B. SMEARS

Gram-stained smears of urethral or endocervical exudate reveal many diplococci within pus cells. Stained smears of endocervical exudates have a sensitivity of about 50% and a specificity of about 95% when examined by an experienced microscopist. Cultures of urethral exudate from men are not necessary when the stain is positive, but cultures should be done for women. Stained smears of conjunctival exudates can also be diagnostic, but those of specimens from the throat or rectum are generally not helpful.

C. CULTURE

Immediately after collection, pus or mucus is streaked on enriched selective medium (eg, modified Thayer-Martin medium) and incubated in an atmosphere containing 5% CO2 (candle extinction jar) at 37 °C. To avoid overgrowth by contaminants, the selective medium contains antimicrobial drugs (eg, vancomycin, 3 µg/mL; colistin, 7.5 µg/mL; amphotericin B, 1 µg/mL; and trimethoprim, 3 µg/mL). If immediate incubation is not possible, the specimen should be placed in a CO2-containing transport-culture system. Forty-eight hours after culture, the organisms can be quickly identified by their appearance on a Gram-stained smear, by oxidase positivity, and by co agglutination, immuno fluorescence staining, or other laboratory tests. The species of sub cultured bacteria may be determined by fermentation reactions .

D. NUCLEIC ACID AMPLIFICATION TESTS

Several Food and Drug Administration-cleared nucleic acid amplification assays are available for direct detection *of N gonorrhoeae* in genitourinary specimens.

E. SEROLOGY

Serum and genital fluid contain IgG and IgA antibodies against gonococcal pili, outer membrane proteins, and LPS. Some IgM of human sera is bactericidal for gonococci in vitro. In infected individuals, antibodies to gonococcal pili and outer membrane proteins can be detected by immunoblotting, radioimmunoassay, and ELISA (enzyme-

linked immunosorbent assay) tests. However, these tests are not useful as diagnostic aids for several reasons: gonococcal antigenic heterogeneity; the delay in development of antibodies in acute infection; and a high background level of antibodies in the sexually active population.

NEISSERIA MENINGITIDIS

Antigenic Structure At least 13 serogroups of meningococci have been **identified by immunologic specificity of capsular polysaccharides**. The most important sero groups associated with disease in humans are A, B, C, Y, and W-135. Meningococcal antigens are found in blood and cerebrospinal fluid of patients with active disease.

The outer membrane proteins of meningococci have been divided into classes on the basis of molecular weight. All strains have either class 1, class 2, or class 3 proteins; these are analogous to the Por proteins of gonococci and are responsible for the serotype specificity of meningococci.

Meningococcal LPS is responsible for many of the toxic effects found in meningococcal disease.

Pathogenesis

Humans are the only natural hosts for whom meningococci are pathogenic**. The nasopharynx is the portal of entr**y. There, the organisms attach to epithelial cells with the aid of pili; they may form part of the transient flora without producing symptoms. From the nasopharynx, organisms may reach the bloodstream, producing bacteremia**; Meningitis** is the most common complication of meningococcemia. It usually begins suddenly, with intense headache, vomiting, and stiff neck, and progresses to coma within a few hours. During meningococcemia, there **is thrombosis of many small blood vessels in many organs, with perivascular infiltration and petechia**l hemorrhages. There may be **interstitial myocarditis, arthritis, and skin lesions.**

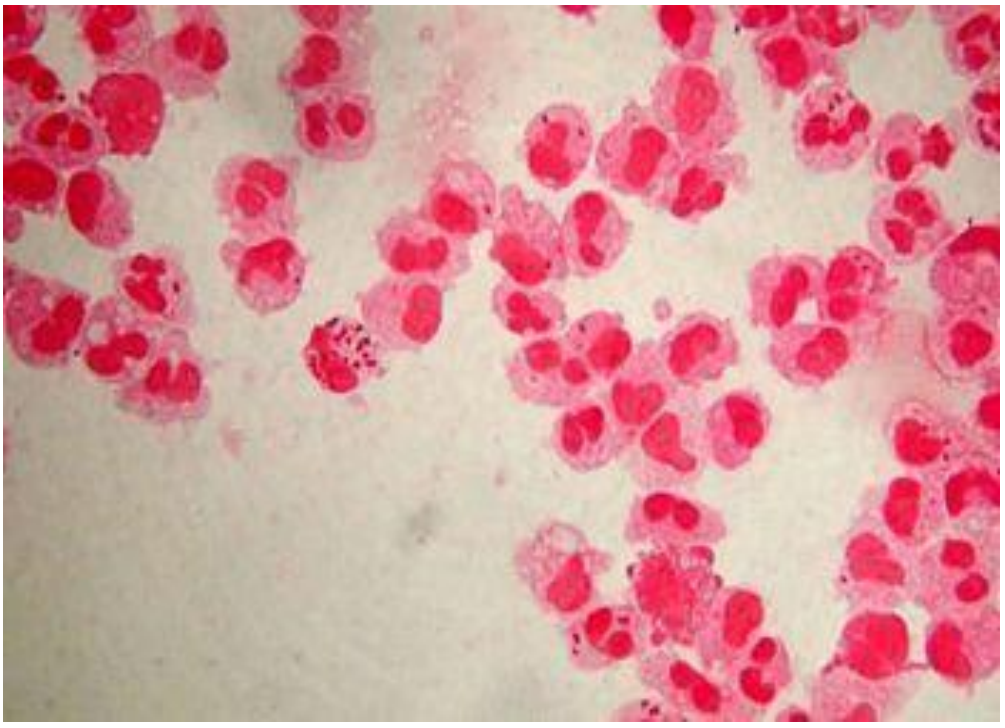
Neisseria bacteremia is favored by the absence of bactericidal antibody (IgM and IgG), inhibition of serum bactericidal action by a blocking IgA antibody, or a complement component deficiency (C5, C6, C7, or C8). Meningococci are readily phagocytosed in the presence of a specific opsonin.

Diag**nostic Laboratory Tests**

A. SPECIMENS

Specimens of blood are taken for culture, and specimens **of spinal fluid** are taken for smear, culture, and chemical determinations. **Nasopharyngeal swab** cultures are suitable for carrier surveys. Puncture material from petechiae may be taken for smear and culture.

B. SMEARS Gram-stained smears of the sediment of centrifuged spinal fluidpetechial aspirate often show typical neisseriae wpolymorphonucleukocytes or extracellularly.polymorpor extracellularl



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C. CULTURE

Culture media without sodium polyanethol sulfonate are helpful in culturing blood specimens**. Cerebrospinal fluid specimens are plated on "chocolate" agar and incubated at 37 °C in an atmosphere of 5% CO2**

(candle jar). Freshly drawn spinal fluid can be directly incubated at 37 °C if agar culture media are not immediately available**. A modified Thayer-Martin medium with antibiotics (vancomycin, colistin, amphotericin) favors the growth of neisseriae, inhibits many other bacteria,** and is used for nasopharyngeal cultures. Presumptive colonies of neisseriae on solid media, particularly in mixed culture, can be **identified by Gram stain and the oxidase test**. Spinal fluid and blood generally yield pure cultures that can be further identified by carbohydrate fermentation reactions and agglutination with type-specific or polyvalent serum.

D. SEROLOGY

Antibodies to meningococcal polysaccharides can be measured by latex agglutination or hemagglutination tests or by their bactericidal activity. These tests are done only in reference laboratories.

Epidemiology, Prevention, & Control

Meningococcal meningitis occurs in epidemic waves , Five to 30% of the normal population may harbor meningococci (often nontypeable isolates) in the nasopharynx during interepidemic periods. During epidemics, the carrier rate goes up to 70–80%. A rise in the number of cases is preceded by an increased number of respiratory carriers.

More important is the reduction of personal contacts in a population with a high carrier rate.

Spore-Forming Gram-Positive Bacilli: Bacillus & Clostridium Species

The gram-positive spore-forming bacilli are the Bacillus and Clostridium species. These bacilli are ubiquitous, and because they form spores they can survive in the environment for many years**. Bacillus species are aerobes, whereas clostridia are anaerobes. is caused by *Bacillus anthracis.*** Anthrax remains an important disease of animals and occasionally of humans, *and B anthracis* is a major agent of bioterrorism and biologic warfare. **Bacillus cereus causes food poisoning** and occasionally eye or other localized infections.

Clostridia cause several important toxin-mediated diseases: *Clostridium tetani, te**tanus**; Clostridium botulinum, **botulism;** Clostridium perfringens, **gas gangrene**; and Clostridium difficile, pseudomembranous **colitis***

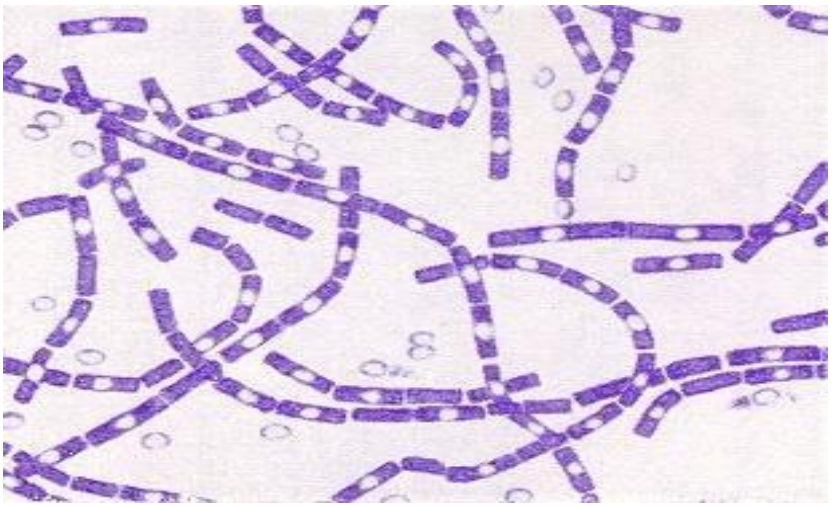
■ *BACILLUS SPECIES*

The genus bacillus **includes large aerobic, gram-positive rod**s occurring in chains.

Morphology & Identification

A. TYPICAL ORGANISMS

The typical cells, measuring 1 × 3–4 µm, have square ends and are arranged in long chains; **spores are located in the center** of **the non motile bacilli.**



B. CULTURE

Colonies *of B anthracis* are round and have a "cut glass" appearance in transmitted light. Hemolysis is uncommon with B anthracis but common with the saprophytic bacilli. Gelatin is liquefied, and growth in gelatin stabs resembles an inverted fir tree.

C. GROWTH CHARACTERISTICS

The spores are resistant to environmental changes, withstand dry heat and certain chemical disinfectants for moderate periods, and persist for years in dry earth. Animal products contaminated with anthrax spores (eg, hides, bristles, hair, wool, bone) can be sterilized by autoclaving

BACILLUS ANTHRACIS

Pathogenesis Anthrax is primarily a disease of herbivores—goats, sheep, cattle, horses, etc; other animals (eg, rats) are relatively resistant to the infection. **Humans become infected incidentally by contact with infected animals or their products. In animals, the portal of entry is the mouth and the gastrointestinal tract. Spores from contaminated soil** find easy access when ingested with spiny or irritating vegetation.

In humans, the infection is usually acquired by the entry of spores through injured skin (cutaneous anthrax) or rarely the mucous membranes (gastrointestinal anthrax), or by inhalation of spores into the lung (inhalation anthrax). The spores germinate in the tissue at the site of entry, and growth of the vegetative organisms results in formation of a gelatinous edema and congestion. Bacilli spread via lymphatics to the bloodstream, and they multiply freely in the blood and tissues shortly before and after the animal's death.

B anthracis that **does not produce a capsule is not virulent and does not induce anthrax in test animals.** The poly-D-glutamic acid capsule is antiphagocytic. The capsule gene is on a plasmid.

Anthrax toxin is made up of three proteins: protective antigen (PA), edema factor (EF), and lethal factor (LF). PA binds to specific cell receptors, and following proteolytic activation it forms a membrane channel that mediates entry of EF and LF into the cell. EF is an adenylyl cyclase; with PA it forms a toxin known as edema toxin. **LF plus PA form lethal toxin, which is a major virulence factor and cause of death in infected animals .**

In inhalation anthrax ("woolsorter's disease"), the spores from the dust of wool, hair, or hides are inhaled, phagocytosed in the lungs, and

transported by the lymphatic drainage to the mediastinal lymph nodes, where germination occurs. This is followed by toxin production and the development of hemorrhagic mediastinitis and sepsis, which are usually rapidly fatal.

Clinical Findings In humans, approximately 95% of cases are cutaneous anthrax and 5% are inhalation. Gastrointestinal anthrax is very rare .Cutaneous anthrax generally occurs on exposed surfaces of the arms or hands, followed in frequency by the face and neck.

Diagnostic Laboratory Tests

Specimens to be examined are fluid or pus from a local lesion, blood, and sputum. Stained smears from the local lesion or of blood from dead animals often show chains of large gram-positive rods. Anthrax can be identified in dried smears by immunofluorescence staining techniques

When grown on blood agar plates, the organisms produce non hemolytic gray to white colonies with a rough texture and a ground-glass appearance. Comma-shaped outgrowths (Medusa head) may project from the colony. Gram stain shows large gram-positive rods. Carbohydrate fermentation is not useful. In semisolid medium,

anthrax bacilli are always non motile, whereas related nonpathogenic organisms (eg, B cereus) exhibit motility by "swarming.

. Contact with infected animals or with their hides, hair, and bristles is the source of infection in human

Control measures include (1) disposal of animal carcasses by burning or by deep burial in lime pits, (2) decontamination (usually by autoclaving) of animal products, (3) protective clothing and gloves for handling potentially infected materials, and (4) active immunization of domestic animals with live attenuated vaccines. Persons with high occupational risk should be immunized.

BACILLUS CEREUS

Food poisoning caused by Bacillus cereus has two distinct forms: the emetic type, associated with fried rice, and the diarrheal type, associated with meat dishes and sauces.

. ■ CLOSTRIDIUM SPECIES

The clostridia are large anaerobic, gram-positive, motile rods. Many decompose proteins or form toxins, and some do both. Their natural habitat is the soil or the intestinal tract of animals and humans, where they live as saprophytes.

Morphology & Identification

A. TYPICAL ORGANISMS

Spores of clostridia are usually wider than the diameter of the rods in which they are formed. In the various species, **the spore is placed centrally, sub terminally, or terminally. Most species of clostridia are** motile and possess peritrichous flagella. A gram stain of a Clostridium species with terminal spor.

B. CULTURE

Clostridia are **anaerobes and grow under anaerobic conditions**; a few species are aero tolerant and will also grow in ambient air. . In general, the clostridia grow well on the blood-enriched media used to grow anaerobes and on other media used to culture anaerobes as well.

C. COLONY FORMS

Some clostridia produce large raised *colonies (eg, C perfringens);* others produce smaller colonies (*eg, C tetani). Some* clostridia form colonies that spread on the agar , Clostridium Gram stain. Individual gram positive bacilli are present (short arrow). Some bacilli have terminal spores (long arrow**). Many clostridia produce a zone of hemolysis on blood agar,*C perfringens* typically produces multiple zones of hemolysis around colonies.**

D. GROWTH CHARACTERISTICS

Clostridia can ferment a variety of sugars; many can digest proteins. Milk is turned acid by some and digested by others and undergoes "stormy fermentation" (ie, clot torn by gas) with a third group (eg, *C perfringens).* Various enzymes are produced by different species .

E. ANTIGENIC CHARACTERISTICS

Clostridia share some antigens but also possess specific soluble antigens that permit grouping by precipitin tests.

CLOSTRIDIUM BOTULINUM

***Clostridium botulinum*, which causes botulism,** is worldwide in distribution; **it is found in soil and occasionally in animal feces.** Spores of the organism are highly resistant to heat, withstanding 100 °C for several hours. Heat resistance is diminished at acid pH or high salt concentration. Toxin During the growth *of C botulinum* and during autolysis of the bacteria, toxin is liberated into the environment**. Seven antigenic varieties of toxin (A–G) are known. Types A, B, and E (and occasionally F) are the principal causes of human illness**. The toxin is a
150,000-MW protein that is cleaved into 100,000-MW and 50,000-MW proteins linked by a disulfide bond.

Botulinum toxin action is absorbed from the gut and binds to receptors of presynaptic membranes of motor neurons of the peripheral nervous system and cranial nerves. Proteolysis—by the light chain of botulinum toxin—of the target **SNARE proteins in the neurons inhibits the release of acetylcholine at the synapse, resulting in lack of muscle contraction and paralysis.** *C botulinum t*oxins are among the most toxic substances known: The lethal dose for a human is probably about 1–2 µg. The toxins are destroyed by heating for 20 minutes at 100 °C.

Pathogenesis

The most common offenders are spiced, smoked, vacuum-packed, or canned alkaline foods that are eaten without cooking. **In such foods, spores** of C botulinum **germinate; under anaerobic conditions, vegetative forms grow and produce toxin. The toxin acts by blocking release of acetylcholine at synapses and neuromuscular junctions .**

Epidemiology, Prevention, & Control

Since spores *of C botulinum* are widely distributed in soil, they often contaminate vegetables, fruits, and other materials. . When such foods are canned or otherwise preserved, they either must be sufficiently heated to ensure destruction of spores or must be boiled for 20 minutes before consumption. The risk from home-canned foods can be reduced if the food is boiled for more than 20 minutes before consumption. **Toxoids are used for active immunization of cattle .**

CLOSTRIDIUM TETANI

Clostridium tetani, **which causes tetanus**, is worldwide in distribution in the soil and in the feces of horses and other animals. **Several types *of C tetani c*an be distinguished by specific flagellar antigens. All share a common O (somatic) antigen**, which may be masked, and all produce the same antigenic type of neurotoxin, tetanospasmin. Toxin The vegetative cells *of C tetani* produce the toxin tetanospasmin (MW 150,000) that is cleaved by a bacterial protease into two peptides (MW 50,000 and 100,000) linked by a disulfide bond.

Pathogenesis

C tetani is not an **invasive organism**. The infection remains strictly localized in the area of devitalized tissue (wound, burn, injury, umbilical stump, surgical suture) into which the spores have been introduced. The volume of infected tissue is small, and the disease is almost entirely a toxemia.

Germination of the spore and development of vegetative organisms that produce toxin are aided by (1) necrotic tissue, (2) calcium salts, and (3) associated pyogenic infections, all of which aid establishment of low oxidation reduction potential. The toxin released from vegetative cells reaches the **central nervous system** and rapidly becomes fixed to receptors in the spinal cord and brain stem and and

cause Hyperreflexia, muscle spasms, and spastic paralysis result. **Extremely small amounts of toxin can be lethal for humans.**

Clinical Findings

The incubation period may range from 4–5 days to as many weeks. The disease is characterized by tonic contraction of voluntary muscles. Muscular spasms often involve first the area of injury and infection and then the muscles of the jaw (trismus, lockjaw), which contract so that the mouth cannot be opened. Gradually, other voluntary muscles become involved, resulting in tonic spasms**. Death usually results from interference with the mechanics of respiration. The mortality rate in generalized tetanus is very high.**

Diagnosis

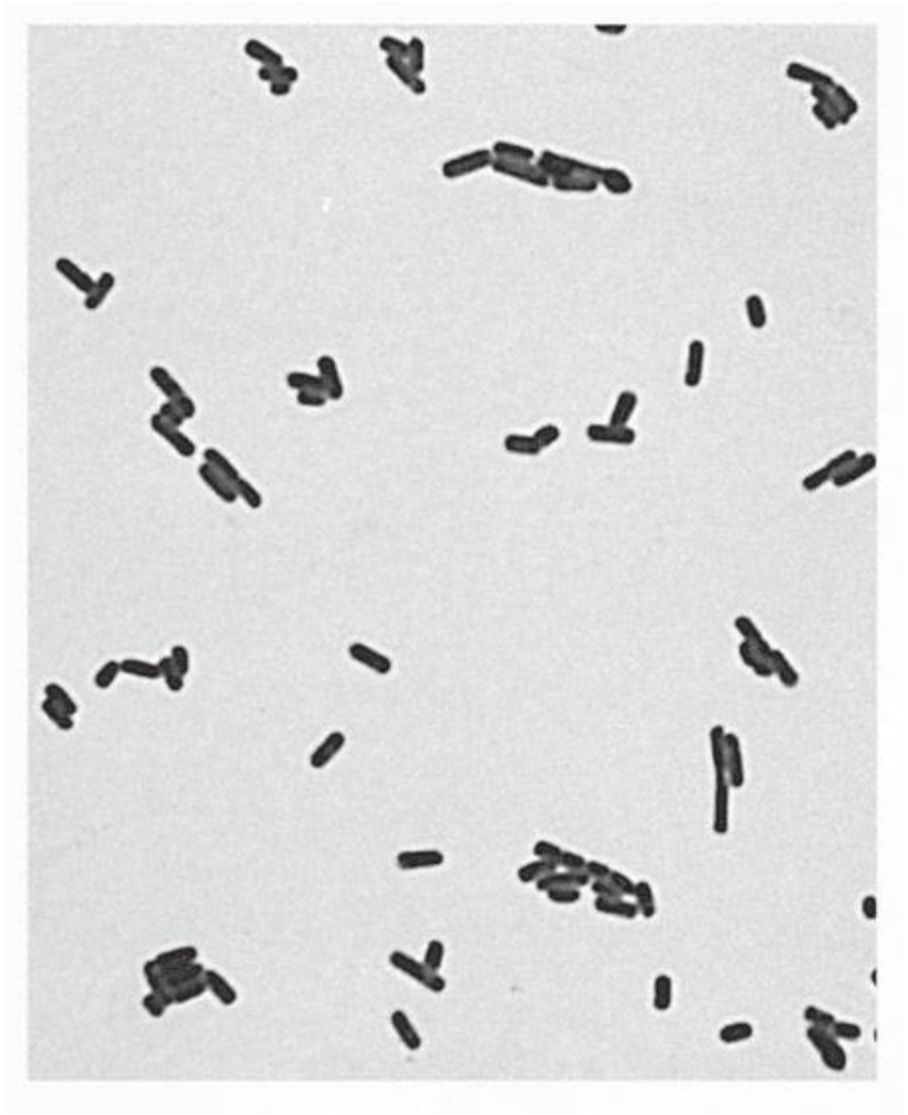
The diagnosis rests on the clinical picture and a history of injury, although only 50% of patients with tetanus have an injury for which they seek medical attention. The primary differential diagnosis of tetanus is strychnine poisoning. Anaerobic culture of tissues from contaminated wounds may yield *C tetani,* Proof of isolation *of C tetani* must rest on production of toxin and its neutralization by specific antitoxin.

Prevention & Treatment

The results of treatment of tetanus are not satisfactory. Therefore, prevention is all-important**. Prevention of tetanus depends upon** (1) active immunization with toxoids; (2) proper care of wounds contaminated with soil, etc; (3) prophylactic use of antitoxin; and (4) administration of penicillin.

CLOSTRIDIA THAT PRODUCE INVASIVE INFECTIONS

Clostridium perfringens



Clostridium perfringens can produce invasive infection (**including myonecrosis and gas gangrene) if introduced into damaged tissue**. The invasive clostridia produce a large variety of toxins and enzymes that result in a spreading infection. Many of these toxins have lethal, necrotizing, and hemolytic properties.

The alpha toxin *of C perfringens* type A **is a lecithinase**, and its lethal action is proportionate to the rate at which it splits lecithin (an important constituent of cell membranes) to phosphorylcholine and diglyceride. **The theta toxin** has similar hemolytic and necrotizing effects but is not a lecithinase**. DNase and hyaluronidase, a collagenas**e that **digests collagen of subcutaneous tissue and muscle**, are also produced.

Some strains of C perfringens produce a **powerful enterotoxin, especially when grown in meat dishes**.

The action *o*f *C perfringens enterotoxin* involves marked hypersecretion in the jejunum and ileum, with loss of fluids and electrolytes in diarrhea. Much less frequent symptoms include nausea, vomiting, and fever.

Pathogenesis &Clinical Findings

From a contaminated wound (eg, a compound fracture, postpartum uterus), the infection spreads in 1–3 days to produce crepitation in the subcutaneous tissue and muscle, foul-smelling discharge, rapidly progressing necrosis, fever, hemolysis, toxemia, shock, and death.

C perfringens food poisoning usually follows the ingestion of large numbers of clostridia that have grown in warmed meat dishes. The toxin forms when the organisms sporulate in the gut

Diagnostic Laboratory Tests

Specimens consist of material from wounds, pus, and tissue. The presence of large gram-positive rods in Gram-stained smears suggests gas gangrene clostridia; spores are not regularly present

CULTURE

Material is inoculated into chopped meat-glucose medium and thioglycolate medium and onto blood agar plates incubated anaerobically. The growth from one of the media is transferred into milk. A clot torn by gas in 24 hours is suggestive *of C perfringens*. Once pure cultures have been obtained by selecting colonies from anaerobically incubated blood plates, they are identified by biochemical reactions (various sugars in thioglycolate, action on milk), hemolysis, and colony form.

Lecithinase activity is evaluated by the precipitate formed around colonies on egg yolk media.

Final identification rests on toxin production and neutralization by specific antitoxin. *C perfringens* rarely produces spores when cultured on agar in the laboratory.

Prevention & Control

Early and adequate cleansing of contaminated wounds and surgical debridement, together with the administration of antimicrobial drugs directed against clostridia (eg, penicillin), are the best available preventive measures.