In: Advances in Medicine and Biology. Volume 16 ISBN 978-1-61122-731-4
Editor: Leon V. Berhardt, pp.

Chapter IX

Identification, and Clinical Relevance of Gram–Positive Spore Formers

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Abstract

The gram positive spore former constitutes a group of widely distributed infectious microorganism which has posed serious threat to public health. This group comprises the *Bacillus*, *Clostridium*, *Staphylococcus* and *Streptococcus species*. The authors reviewed the current trends involved in the mode of action in causing disease of man and animals. In this study, key gaps knowledge in identification, diagnosis, pathology, epidemiology, treatment, prevention and control were brought to the fore, with aim of providing knowledge in dealing with quick diagnosis, cure/ prevention of diseases caused by these groups of microorganisms. Some of the medically important diseases discussed include: anthrax, botulism and tetanus. These diseases have caused serious strain on the budgets of most nations in providing prompt diagnosis, treatment and control of such diseases for their citizenry.

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Introduction

Gram positive bacteria have two major groups when phylogenetically classified. They are the low G+C and high G+C gram positive bacteria. The low G+C includes the clostridia, the bacilli and lactobacilli, mycoplasmas. The clostridia and lactobacilli are endospore formers, occurring as cocci and rods. However, this possession of endospores consequently does not mean close relatedness. Among the gram positive group, the uniqueness of their different peptidoglycan is frequently used for easy identification of the different groups. Aside from the peptidoglycan layer, the gram-positive bacteria have polysaccharides and teichoic acids present in their structures. The peptidoglycans which are sometimes also called murein are heteropolymers of glycan strands, which are cross-linked through short peptides. The murein consists of chains of alternating residues of *N*-acetylglucosamine and *N*-acetyl muramic acid. The muramic acid is a unique substance associated with bacterial cell walls. These chains are cross-linked by short polypeptide chains consisting of both L- and D-aminoacids. However, among the gram-negative bacteria, the peptidoglycan is simple in structure and comparatively uniform throughout most genera.

Most gram positive bacteria are harmless free living saprophytes, whereas in some species are major human pathogens. For the basis of this contribution, we would like to discuss some of the groups with high level of medical importance, where we would be highlighting on their morphology, pathology, diagnosis and treatments. These groups include the *Bacilli* and *Clostridium* species.

Bacilli: Among the gram positive spore forming bacilli are the *Bacillus* and *Clostridium* species. This gram positive spore formers could almost be found in every environment, due to the ability to form spores that can thrive in the environment for many years. *Bacillus* species are aerobes while clostridia are anaerobes. Several species of this group cause medically important disease in humans. Notable is anthrax disease caused by the *Bacillus anthracis*. Anthrax affects predominantly animals and occasionally humans. The bacterium is a major agent of bioterrorism and biological warfare. *Bacillus cereus* causes food poisoning and occasionally eye or other localized infections. *Clostridium* species cause several important toxin –mediated diseases e.g. *Clostridium tetani* causes tetanus, *Clostridium botulinum* causes botulism, *Clostridium perfringes* causes gas gangrene and *Clostridium difficle* causes pseudo membranous'' colitis.

The Bacillus Species

This genus is aerobic, occurring as rods in chains. Most of the members of this genus are saprophytic organisms and they are prevalent in soil, water and air. Examples include *Bacillus cereus* and *Bacillus subtilis*. Some are insect pathogens and can grow in foods, producing enterotoxin or emetic toxin that causes food poisoning. It may occasionally produce disease in immunocompromised humans (e.g. meningitis, endocarditis, conjunctivitis or acute gastroenteritis). *B. anthracis*, which causes anthrax, is the principal pathogen of the genus and a zoonotic disease.

Bacillus Anthracis

Morphology and Identification

The typical cells of *Bacillus anthracis*, measures between 1 x 3-4 µm, with rectangular ends arranged in long chains. The spores are located in the center, oval in shape and refractile. Colonies of *B. anthracis* are round and have a cut glass appearance in transmitted light. Typical colonies have a wavy margin and small projections. The organism is a gram positive and survives between 12-45^{o}C. Hemolysis is uncommon with *B. anthracis* but common with saprophytic bacilli. The saprophytic bacilli utilize simple sources of nitrogen and carbon for energy and growth. The spores are resistant to environmental changes and can withstand dry heat at 140^{o}C for 1-3hrs but can be destroyed when autoclaved at right temperature and pressure. To certain chemical disinfectants, they can be resistant to it for moderate periods and persist for years when found in dry earth. Animal products contaminated with anthrax spores (e.g. hides, bristles, hair, wool, bone) can be sterilized only by autoclaving.

Pathogenesis

Anthrax is primarily a disease of herbivores –goats, sheep, cattle and horses. Other animals (e.g. rats) are relatively resistant to the infection. Human becomes infected incidentally by contact with infected animals and their products. In animals, the portal of entry is the mouth and the gastrointestinal tract. Spores from contaminated soil may find easy access when ingested with spiny or irritating vegetation. In humans, the infection is by the entry/ inoculation of spores through injured skin (cutaneous anthrax forming lesions known as malignant pustule) or rarely the mucous membranes (gastrointestinal anthrax) or by inhalation of spores into the lung (inhalation anthrax- called the wool-sorters disease). This condition could be very fatal due to consequent inflammation, septicemia and hemorrhage. At the site of entry, the spores germinate in the tissue into vegetative organisms resulting in formation of a gelatinous edema and congestion. *Bacillus anthracis* spreads via lymphatics to the blood stream, and they multiply freely in the blood and tissues /organs such as lung shortly before and after the animal's death. Anthrax toxin is made up of three proteins Lethal Factor (LF), Edema Factor (EF), Protective Antigen (PA).

Mode of Action of the Toxin in Cells

The Protective Antigen (PA) binds to specific cells receptors and following proteolytic activation, a membrane channel is formed that mediates entry of Edema Factor (EF) and Lethal Factor (LF) into the cell. The binding between the lethal factor (LF) and the protective antigen (PA) form a lethal toxin. This toxin is the major virulence factor which causes death in infected animals. When injected into laboratory animals, the lethal toxin can quickly kill the animals. In inhalation anthrax, when the spores from the dust on wool, hair or hides are inhaled; it is phagocytosed in the lungs and transported by the lymphatic drainage to the mediastinal lymph nodes, where germination occurs followed by toxin production and the development of hemorrhagic mediastinitis and sepsis, which are usually rapidly fatal.

Pathology

In susceptible animals such as mammals, *Bacillus anthracis* proliferate at the site of entry. The capsules remain intact, and the organisms are surrounded by a large amount of proteinaceous fluid containing few leukocytes from which they rapidly disseminate and reach

blood stream. In the resistant animals, the *Bacillus anthracis* proliferate for few hours by which time a massive accumulation of leukocytes is formed and the capsule gradually disintegrates and disappears. In humans, approximately 95% of cases are cutaneous anthrax and 5% are inhalation .Gastrointestinal anthrax is very rare. It has been reported from Africa, Asia and USA following occasions where people have eaten meat from infected animals. Cutaneous anthrax generally occurs on exposed surfaces of the arms or hands, face and neck. A puritic papule develops between 1-7 days after entry of the organisms or spores through a scratch. Initially it resembles an insect bite, which progresses to papule rapidly changing into a vesicle or small ring of vesicles that coalesce and necrotic ulcer develops. The lesions typically are 1-3 cm in diameter and have a characteristic central black eschar. Systemic signs and symptoms of fever, malaise, and headache may occur. After 7-10 days the eschar is fully developed. Eventually it dries, loosens and separates. Healing is by granulation and leaves a scar. It may take many weeks for the lesion to heal and the edema to subside. Antibiotic therapy does not appear to change the natural progression of the disease. In patients, cutaneous anthrax can lead to sepsis, which could lead to systemic infection –including meningitis and death. The incubation period of inhalation anthrax may be as long as six weeks. The fatality rate is high in settings of known exposure and may be higher when the diagnosis may not be initially suspected. Abdominal pain, vomiting and bloody diarrhea are exceptional clinical signs.

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. Virulent anthrax cultures kill mice or guinea pigs upon intraperitoneal infection.

Resistance / Immunity

Active immunity to anthrax can be induced in susceptible animals by vaccination with live attenuated bacilli, with spore suspensions or with protective antigens from culture filtrates. It is advisable that animals that graze in suspected areas should be immunized for anthrax annually.

In United States, the anthrax vaccine available is an aluminium hydroxide –precipitated preparation of protective antigen from sterile filtrate of cultures of a virulent non-encapsulated strain. Two inoculations of this vaccine yield significant protection from inhalation anthrax in rhesus monkeys. In Russia, a live attenuated spore based vaccine has been widely used in large field trials.

Treatment

Many antibiotics are effective against anthrax in humans, but treatment must be started early. Ciprofloxacin is recommended for treatment of anthrax. Many antibiotics are active against *B. anthracis*, penicillin G, along with gentamycin or streptomycin has been used previously to treat anthrax. In cases of potential exposure to *B. anthracis* as an agent of biological warfare, prophylaxis with ciprofloxacin or doxcycline should be continued for

4weeks while three doses of the vaccine are given; or for 8weeks if no vaccine is administered.

Prevention and Control

Soil contaminated with an anthrax spores from the carcasses of dead animals may remain viable for decades. Spores can germinate in soil at pH of 6.5 at proper temperature. Grazing animals infected through injured mucous membranes serve to perpetuate the chain of infection. Contact with infected animals or with their hides, hair and bristles is the source of infection in humans.

Control measures include:

- Disposal of animal carcasses by burning or deep burial in lime pits.
- Decontamination (usually by autoclaving) of animals' products.
- Protective clothing and gloves for handling potentially infected materials.
- Active immunization of domestic animals with live attenuated vaccines.

Bacillus Cereus

Bacillus cereus is a large gram positive bacillus resembling *B. anthracis,* except that it is motile and lacks the glutamic capsule. Food poisoning caused *Bacillus cereus* has two distinct forms

- The emetic type associated with fried rice.
- Diarrheal type associated with meat dishes and sauces.

B. cereus is a soil organism that commonly contaminates rice. When large amounts of rice are cooked and allowed to cool slowly, the *B. cereus* spores germinate and the vegetative cells produce the toxin during log phase growth or during sporulation. The diarrheal form has an incubation period of 1-24hrs and is manifested by profuse diarrhea with abdominal pain and cramps; fever and vomiting are uncommon. The enterotoxin may be preformed in the food or produced in the intestine. The presence of *B. cereus* in a patients stool is not sufficient to make a diagnosis of *B. cereus* disease since the bacteria may be present in normal stool specimens at concentration of 10^5 bacteria or more per gram of food. *B cereus* causes eye infections when the organisms are introduced into the eye by foreign bodies associated with trauma. *B. cereus* has also been associated with localized infections and with systemic infections, including endocarditis, meningitis, pneumonia and osteomyletis.

Other *Bacillus* species rarely associated with human disease include:

- *B. thuringiensis*
- *B. popillae*
- *B. sphaericus*
- *B. larvae*
- *B. lentimorbus*

These are mainly pathogens for insects and some have been used as commercial insecticides. Genes for *B. thuringiensis* coding for insecticidal compounds have been inserted into the genetic material of some commercial plants.

Clostridium Species

The *Clostridia* are large anaerobic, gram positive motile rods. Pleomorphism is common and various forms may be seen in stained smears from cultures or wounds. 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. Among the pathogens are the organisms causing botulism, tetanus, gas gangrene and pseudo membranous colitis.

Morphology and Identification

Spores of *Clostridia* occur wider in diameter than most rods shaped bacteria.The spores occur centrally, subterminally or terminally with most motile species possessing peritrichous flagella.

Clostridia grow well on the blood enriched media used to grow anaerobes and on other media used to culture anaerobes as well. Many *Clostridia* produce zone of hemolysis on blood agar. Their colonies could be raised or small. The *Clostridia* can ferment a variety of sugars, and many can digest proteins. They cause stormy fermentation (clot torn by gas).

Clostridium Botulinum

Clostridium botulinum causes botulism and it's widespread. It can be found in soil, vegetables, fruits, leaves, silage, manure, mud from lakes and sea and occasionally in animal feces. The widespread occurrence of *C. botulinum* in nature is as a result of its ability to produce a potent neurotoxin in food and resistance of the spores to inactivation agents.These attributes combine to make it a formidable pathogen of humans and a host of animals and birds. Types of *C. botulinum* are distinguished by the antigenic type of toxin they produce. Spores of the organism are highly resistant to heat, withstanding 100ºC for several hours. Heat resistance is diminished at acidic (low) pH or high salt concentration. *C. botulinum* is motile with peritrichous flagellation and spores are oval or subterminal. As *Clostridium botulinum* grows, autolysis of the bacteria can occur, causing toxin to be 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. Type A and B have been associated with a variety of foods and type E predominantly with fish products. Type C produces a disease in birds called limberneck, while types D cause botulism in mammals. The molecular weight of this toxin is about 150000MW. Botulinum toxin is absorbed from the gut and binds to receptors of presynaptic membrane of motor neurons of the peripheral nervous system and cranial nerves. The toxins inhibit release of acetylcholine at the synapse, resulting in lack of muscle contraction and paralysis. The lethal doses for a human is probably about 1- 2 µg. The toxins are destroyed by heating for 20 minutes at 100ºC.

Pathogenesis

Pathogenesis occurs as a result of intoxication from ingestion of food in which *C. botulinum* has grown and produced toxin. The most susceptible are people that feed on spiced, smoked, vacuum-packed or canned alkaline foods (preserved hams, large sausages, home preserved meats and vegetables) that are eaten without cooking. In such foods, it may not exhibit signs of spoilage. Spores of *C. botulinum* germinate; under anaerobic conditions, forming vegetative forms that grow and produce toxin. Symptoms begin 18-24hrs after ingestion of the toxic food with visual disturbances (in coordination of eye muscles, double vision) inability to swallow and speech difficulty. Death occurs from respiratory paralysis or cardiac arrest. Gastrointestinal symptoms are not regularly prominent. There is no fever and patients remain fully conscious until shortly before death. The mortality rate is high. Patients who recover do not develop antitoxin in the blood.

In United States, infant botulism is more common than the classic form of paralytic botulism associated with the ingestion of toxin – contaminated food. The infants in the first months of life develop poor feeding, weakness and sighs of paralysis ("floppy baby"). Infant, botulism may be one of the causes of sudden infant death syndrome. *C. botulinum* and botulinum toxin can occur in the feces. It is assumed that *C. botulinum* spores could be in the babies' food producing toxin in the babies' gut.

Honey has been implicated as a possible vehicle for the spores. Most of these infants recover with supportive therapy alone.

Diagnostic Laboratory Tests

Toxin can often be demonstrated in serum from the patient and may be found in leftover foods. Mice injected intraperitoneal die rapidly. Toxin may be demonstrated by passive hemogglutination or radioimmunoassay.

Treatment

Potent antitoxins to three types of botulinum toxins have been prepared in horses. Since the type responsible for an individual case is usually not known, trivalent (A, B, E) antitoxin must be promptly administered intravenously with customary precaution. Adequate ventilation must be maintained by mechanical aspirator; if necessary. The priorities involved in treatment are to remove unabsorbed toxin from the stomach and intestinal tract, to neutralize unfixed toxin by giving polyvalent antitoxin and to give relevant intensive care and support.

Epidemiology, Prevention and Control

Spores of *C. botulinum* are widely distributed in soil; they often contaminate vegetables, fruits and other materials. Canned foods or otherwise preserved, must be boiled for 20minutes before consumption. Strict regulation of commercial canning has largely overcome the danger of widespread outbreaks, but commercially prepared foods have caused deaths. A chief risk factor for botulism lies in home canned foods, particularly string beans, corn, peppers, olives, peas and smoked fish or vacuum packed fresh fish in plastic bags. Toxic food may be spoiled or rancid, and cans may swell or the appearance may be innocuous. 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 in South Africa. Botulism toxin is considered to be a major agent for bioterrorism and biological warfare.

Clostridium Tetani

Clostridium tetani causes "tetanus" and is widely distributed in soil, in feces of horses and other animals. The organism under microscope appears as straight, slender rod with rounded ends. The fully developed terminal spore gives the organism the appearance of a drum stick with a large round ends. The tetanus bacillus is an obligate anaerobe, which is motile via numerous peritrichous flagella. It grows well in cooked meat broth and produces a thin spreading film when grown on enriched blood agar. Spores of some strains cannot be destroyed by boiling in water for up to 3hrs, or dry heat at 160^{o}C for 1hr, or 5% phenol for 2weeks. However, the spores could be destroyed using glutaraldehyde. Several types of *C. tetani* can 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.

The vegetative cells of *C. tetani* produce the toxin tetanospasmin (MW 150000) that is cleared by a bacterial protease into two peptides (MW 50000 and 100000) linked by a disulfide bond. The toxin initially binds to receptors on the presynaptic membranes of motor neurons. It then migrates through a transport system to the cell bodies of these neurons to the spinal cord and brain stem. The toxin diffuses to terminals of inhibitory cells, (including both glycinergic inter neurons and aminobutyric- acid secreting neurons from the brain stem. The toxin degrades synaptobrevin; a protein required for docking of neurotransmitter that resides on the presynaptic membrane. Release of the inhibitory glycine and γ-aminobutyric acid is blocked, and the motor neurons are not inhibited. Hyperreflexia, muscles pain and spastic paralysis then results. Extremely small amounts of toxin can be lethal for humans.

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) from which the spores have been introduced. Germination of the spore and development of vegetative organism that produce toxin are aided by Necrotic tissue, presence of calcium salts and associated pyogenic infections, all of which aid establishment of low oxidation-reduction potential. The toxins released from vegetative cells reach the central nervous system and rapidly becomes fixed to receptors in the spinal cord and brain stem. 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 (lockjaw) which contract so that the mouth cannot be opened. Gradually other voluntary muscles become involved, resulting in tonic spasms. Any external stimulus may precipitate a titanic generalized muscle spasm. The patient is fully conscious; and pain may be intense. Death usually results from interference with mechanics of respiration.

Diagnosis

The primary differential diagnosis of tetanus is strychnine poisoning. Anaerobic culture of tissues from contaminated wounds may yield *C. tetani* but neither preventive nor therapeutic use of antitoxin should ever be withheld pending such demonstration. Proof of isolation of *C. tetani* must rest on production of toxin and its neutralization by specific antitoxin.

Control

Tetanus is a totally preventable disease. Tetanus toxoid immunization should be mandatory. Tetanus toxoid is produced by detoxifying the toxin with formalin and then concentrating it. Aluminum-salt-adsorbed toxoids are employed. Three injections of the immunization should be given. First is the initial course immunization then another dose about 1year later. This initial immunization should be carried out in all children during the early year of life. Thereafter, booster injections may be given to maintain serum level of more than 0.01 unit antitoxin per millimeter. Normally during vaccination, tetanus toxoid is combined with diphtheria toxoid and pertussis vaccine.

Prevention and Treatment

The results of treatment of tetanus are not satisfactory, thus prevention is all important. Prevention of tetanus depends upon: Active immunization with toxoids, Proper care of wounds contaminated with soil, Prophylactic use of antitoxin and administration of penicillin. Intramuscular administration of human antitoxin gives adequate systemic protection for 2-4 weeks. It neutralizes the toxin that has not been fixed to nervous tissue. Patients who develop symptoms of tetanus should receive muscles relaxants, sedation and assisted ventilation. Sometimes they are given very large doses of antitoxin (3000 – 10000 units of tetanus immune globulin) intravenously in an effort to neutralize toxin that has not yet been bound to nervous tissue. Surgical debridement is vitally important because it removes the necrotic tissues that are essential for proliferation of the organisms. Penicillin strongly inhibits the growth of *C. tetani* and stops further toxin production. Antibiotics may also control associated pyogenic infection.

When an individual previously immunized sustains a potentially dangerous wound, an additional dose of toxoid should be injected to restimulate antitoxin production.

Other Groups of Medically Important Gram Positive Non Spore Formers Include: *The Staphylococci*

The *Staphylococci* are Gram-positive spherical cells, usually arranged in grape-like irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal flora of the skin and mucous membranes of the humans; others cause suppuration, abscess formation; a variety of pyogenic infections and even fatal septicemia. The pathogenic *Staphylococci* often hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins.

The most common type of food poisoning is caused by a heat-stable staphylococcal enterotoxin. The genus staphylococcus has at least 30 species. The three main species of medical importance are *Staphylococcus aureus, Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*.

Staphylococcus aureus is coagulase – positive, which differentiates it from other species. *S. aureus* is a major pathogen for humans. Almost every person will have some type of *S. aureus* infection during his/her lifetime, ranging in severity from food poisoning or minor skin infections to severe life- threatening infections. The coagulase negative staphylococci are

normal human flora and sometimes cause infection, often associated with implanted appliances and devices, especially in very young, old and immunocompromised patients. *S. saprophyticus* is a relatively common cause of urinary tract infections in young women.

Morphology and identification

Typical Organisms

Staphylococci are spherical cells about 1µm in diameter arranged in irregular clusters. Young cocci strains strongly stain gram- positive, on aging, many cells become gram – negative. Staphylococci are non- motile and do not form spores. They are found in cocci pairs, clusters, tetrads and chains.

Culture and Growth Characteristics

Staphylococci grow on most bacteriological media under aerobic or microaerophilic conditions. They grow rapidly at 37°C, but form pigments best at room temperature 20-25°C. Colonies on solid media are round, smooth, raised and glistening. Staphylococci produce catalase, which differentiates them from *Streptococci*. *Staphylococci* slowly ferment many carbohydrates, producing lactic acid but not gas. *Staphylococci* are relatively resistant to drying, heat 50°C for 30 minutes. They are resistant to many antimicrobial drugs e.g. Penicillin G, Ampicillin, Ticarcillin, Piperacillin, and Naficillin (Methicillin and Oxacillin). Several properties resident on the cell wall of *Staphylococcus* causes it to be antigenic; this ranges from the presence of peptidoglycan, teichoic acid to protein A.

Enzymes and Toxins

Staphylococci can cause diseases by their ability to multiply and spread widely in tissues and through their production of many extracellular substances. Some of these substances are enzymes, others are considered to be toxins though they may function as enzymes. Some of these include:

Catalase

Staphylococci produce catalase, which converts hydrogen peroxide (H_2O_2) into water and oxygen. The catalase test differentiates the *Staphylococci* which are positive, from the *Streptococci*, which are negative.

Coagulase and Clumping Factor

S. aureus produces coagulase, an enzyme – like protein that clots oxalated or citrated plasma. Coagulase binds to prothrombin; together they become enzymatically active and initiate fibrin polymerization. Coagulase may deposit fibrin on the surface of *Staphylococci*, perhaps altering their ingestion by phagocytic cells or their destruction within such cells. Clumping factor is a surface adhering compound that is responsible for adherence of the organisms to fibrinogen and fibrin. When mixed with plasma, *S. aureus* forms clumps. Clumping factor is distinct from coagulase. Other enzymes produced by *S. aureus* include hyaluronidase or spreading factor, a staphylokinase used for (fibrinolysis); proteinases, lipases, and β – lactamases,

Exotoxins

Leukocidin

The toxin of *S. aureus* has two components. It can destroy white blood cells of humans and rabbits. The two components act synergistically on the white blood cell membrane to form pores and increase cation permeability. Other toxins include the exfoliate toxins which yield the generalized desquamination of the staphylococcal scalded skin syndrome.

Toxic shock syndrome toxin: Toxic shock syndrome is manifested by an abrupt onset of high fever, vomiting, diarrhea, rash, cardiac and renal failure in the most severe cases. Toxic shock syndrome associated *S. aureus* can be found in the vagina, in wounds or other localized infections, or in the throat but virtually never in the bloodstream.

Enterotoxins: These are multiple (A-E, G-I, K-M) enterotoxins. They are heat stable and resistant to action of gut enzymes. As an important cause of food poisoning, enterotoxins are produced when *S. aureus* grows in carbohydrate and protein foods. Ingestion of 25µg of enterotoxin B results in vomiting and diarrhea. The emetic effect is probably the result of central nervous system stimulation (vomiting center) after the toxin acts on neural receptors in the gut.

Pathogenesis

Staphylococci, particularly *S. epidermidis* are members of the normal flora of the human skin, respiratory and intestinal tracts. Nasal carriage of *S. aureus* occur in 20- 25% of humans. *Staphylococci* are also found regularly on clothing, bed linen and other fomites in human environment. The pathogenic capacity of a given strain of *S. aureus* is the combined effect of extracellular factors and toxins together with the invasive properties of the strain. Pathogenic, invasive *S. aureus* produces coagulase, tend to produce yellow pigment and to be hemolytic. Non-pathogenic and non-invasive such as *S. epidermidis* are coagulase-negative and tend to be non-hemolytic. Such organisms rarely produce suppuration but may cause diseases in immunosuppressed persons. *S. saprophyticus* causes urinary tract infections in young women. A staphylococcal infection appears as a “pimple” hair follicle infection or abscess. There is usually an intense, localized, painful inflammatory infection that undergoes central suppuration and heals when the pus is drained. The wall of fibrin and the cells around the core of the abscess tends to prevent the spread of the organisms. *S. aureus* infections can also result from direct contamination of a wound. When *S. aureus* disseminates and bacteremia ensues, endocarditis, meningitis or pulmonary infection can result. Secondary localization within an organ or system is accompanied by the symptoms and signs of organ dysfunction and intense focal suppuration.

Diagnostic Laboratory Test

Specimens used for the diagnosis include: Surface swab, pus, blood, tracheal aspirate, or spinal fluid for culture, depending upon the localization of the process.

Smears: Typical staphylococci are seen in stained smears of pus or sputum.

Culture: Specimens inoculated on blood agar plates give rise to typical colonies in 18hours at 37°C but hemolysis and pigment production may occur not until several days later. *S. aureus* ferment mannitol. Specimens contaminated with mixed flora can be cultured on

media containing 7.5% NaCl; the salt inhibits other flora but not *S. aureus*. Other tests include biochemical tests coagulase, susceptibility, serologic and typing tests.

Epidemiology and Control

Staphylococci are ubiquitous human parasites. The chief sources of infection are from shredded human lesions, fomites contaminated from such lesions, and the human respiratory tract and skin. Contact spread of infection has assumed added importance in hospitals, where large proportion of the staff and patient carry antibiotic–resistant staphylococci in the nose or on the skin. Cleanliness, hygiene and aseptic management of lesions can control the spread of staphylococci from lesions. Aerosols (glycols) and ultraviolet irradiation of air have little effect. In hospitals, the areas at highest risk of severe staphylococcal infections are the newborn nursery, intensive care units, operating rooms, and cancer chemotherapy wards.

Treatments

Most persons harbor *Staphylococci* on the skin and in the nose or throat. Even if the skin can be cleared of *Staphylococci*, re-infection by droplets will occur almost immediately. Because pathogenic microbes are commonly spread from one lesion to other areas of the body by fingers, clothing, antisepsis is important to control recurrence.

Serious multiple skin infections (acne, furunculosis) occur most often in adolescents. Similar skin infections occur in patients receiving prolonged courses of corticosteroids.

In acne, lipases of *Staphylococci* and *Corynebacteria* liberate fatty acids from lipids and thus cause tissue irritation. Tetracyclines are used for long term treatments. It is difficult to eradicate pathogenic *Staphylococci* from infected persons, because the organisms rapidly develop resistance to many antimicrobial drugs and the drugs cannot act in suppurative lesions.

Bacteremia, endocarditis, pneumonia and other severe infections due to *S. aureus* require prolonged therapy with β-lactamase resistant penicillin. Vancomycin is often reserved for use with nafcillin –resistant *Staphylococci* if the infection is found to be due to non-β-lactamase producing *S. aureus.* Penicillin G is the drug of choice, but only a small percentage of *S. aureus* strains are susceptible to it.

S. epidermidis infections are difficult to cure because they occur in prosthetic devices where the bacteria can sequester themselves in a biofilm. *S. epidermidis* is more often resistant to antimicrobial drugs than *S. aureus*; approximately 75% of *S. epidermidis* strains are nafcillin –resistant.

Penicillin G –resistant *S. aureus* strains from clinical infections often produce penicillinase.

The Streptococci

The *Streptococci* are Gram – positive spherical bacteria that characteristically form pairs or chains during growth. They are widely distributed in nature. Some are members of the normal human flora; *Streptococci* elaborate a variety of extracellular substances and enzymes.

Streptococci are a heterogeneous group of bacteria and no one system suffices to classify them. Twenty species including *Streptococcus pyogenes* (group A), *Streptococcus agalactiae* (group B) and the *Enterococci* (group D) are characterized by combinations of features;

colony growth characteristics; hemolysis patterns on blood agar (α-hemolysis, β-hemolysis or non-hemolysis).

Classification of Streptococci:

The classification of *Streptococci* into major categories has been based on a series of observations:

Colony morphology and hemolytic reactions on blood agar, Serologic specificity of the cell wall group- specific substance (Lancefield classification), Biochemical reactions and resistance to chemical and physical factors. Ecologic features: *Streptococci* of particular medical interest include: *Streptococcus pyogenes, Streptococcus agalactiae*, *Streptococcus faecalis* and *Streptococcus bovis*. *S. pyogenes* is the main human pathogen associated with local and systemic invasion and post streptococcal immunologic disorders. They belong to the group A antigen containing *Streptococci.*

Streptococcus agalactiae: These are the group B *Streptococci*. They are members of the normal flora of the female genital tract and an important cause of neonatal sepsis and meningitis. They are β- hemolytic and produce zones of hemolysis that are only slightly larger than the colonies.

Streptococcus pneumoniae: They are pneumococci and are α-hemolytic. Their growth is inhibited by optochin and colonies are bile soluble. Other species include: Viridians *Streptococci*, which are the most prevalent members of the normal flora of the upper respiratory tract and are important for the healthy state of the mucous membranes there.

Morphology and Identification

Individual cocci are spherical or ovoid and are arranged in chains. *Streptococci* are Gram positive. However, as culture ages and the bacteria die, they loose their Gram-positivity and appear to be Gram- negative, and this can occur after overnight incubation.

Most group A, B and C strains produce capsules composed of hyaluronic acid. Most streptococci grow in solid media as discoid colonies, usually 1-2mm in diameter. Growths of streptococci tend to be poor on solid media or in broth unless enriched with blood or tissue fluids.

Toxins and Enzymes

More than 20 extracellular products that are antigenic are elaborated by group A streptococci, including streptokinase. (fibrinolysin). Streptokinase is produced by many strains of group A β-hemolytic *Streptococci.* It transforms the plasminogen of human plasma into plasmin, an active proteolytic enzyme that digests fibrin and other proteins.

Hyaluronidase splits hyaluronic acid, an important component of the ground substance of connective tissue. This hyaluronidase aids in spreading infecting microorganisms (spreading factor).

Hemolysins: Many streptococci are able to hemolyse red blood cells into varying degrees. Complete disruption of erythrocytes with release of hemoglobin is called β-hemolysis. Incomplete lysis of erythrocytes with the formation of green pigment is called α-hemolysis. β-hemolytic group A,

S. pyogenes when cultured in Blood agar elaborates to be β- hemolysis (streptolysins- with complete disruption of erythrocytes and release of hemoglobin).

Streptolysin Q and S: Streptolysin Q is responsible for some of the hemolysis seen when growth cuts deep into the medium in blood agar plates. Streptolysin S is the agent responsible

for hemolytic zones around streptococcal colonies growing on the surface of the blood agar plates. It is elaborated in the presence of serum.

Pathogenesis

Infections can be divided into several categories: Diseases attributable to invasion by β-hemolytic group A streptococcus, Diseases attributable to local infection with β- hemolytic group A *S. pyogenes* and their by products, Infective endocarditis, Invasive group A streptococcal infections, streptococcal toxic shock syndrome and scarlet fever and diseases attributable to invasion by β- hemolytic group A *Streptococci* (*S. pyogenes*). Here, the portal of entry determines the principal clinical picture. In each case, there is a diffuse and rapidly spreading infection that involves the tissues and extends along the lymphatic pathways only with minimal local suppuration.

Erysipelas: if the portal of entry is the skin, erysipelas results with massive brawny edema and a rapidly advancing margin of infection.

Cellulitis: Streptococcal cellulitis is an acute, rapidly spreading infection of the skin and subcutaneous tissues. It follows infection associated with mild trauma, burns, wounds, or surgical incisions, pain, tenderness, swelling and erythema occur. Cellulitis is differentiated from erysipelas by two clinical findings; in cellulitis, the lesion is not raised and the line between the involved and uninvolved tissue is indistinct.

Sepsis: Infection of traumatic or surgical wounds with *Streptococci* results in sepsis or surgical scarlet fever. The most common infection due to β-hemolytic *Streptococci* is streptococcal sore throat. Virulent group A *Streptococci* adhere to the pharyngeal epithelium by means of lipoteichoic acid-covered surface pili. In neonates and small children, the sore throat occurs as sub acute nasopharyngitis with a thin serious discharge, and little fever but with a tendency of the infection to extend to the middle ear, the mastoid, and the meninges. The cervical lymph nodes are usually enlarged. The illness may persist for weeks. In older children and adults, the disease is more acute and is characterized by intense nasopharyngitis, tonsillitis, and intense redness, and edema of the mucous membranes with purulent exudates, enlarged tender, cervical lymph nodes; and usually a high fever.

Infective Endocarditis

Acute endocarditis occurs when in the course of bacteremia; hemolytic streptococci, pneumococci, and other bacteria may settle on normal or previously deformed heart values, producing acute endocarditis. Rapid destruction of the valves frequently leads to fatal cardiac failure in days or weeks, unless a prosthetic device can be inserted during antimicrobial therapy.

Various *Streptococci*, particularly *enterococci,* cause urinary tract infections. Anaerobic *Streptococci* occur in the normal female genital tract, the mouth, and the intestine. They may give rise to suppurative lesions, sometimes alone, but most often in association with other anaerobes, particularly bacteroides. The pus usually has a foul odor.

Diagnostic Laboratory Tests

Specimens to be obtained depend on the nature of the streptococcal infection. A throat swab, pus or blood is obtained for culture. Serum is obtained for antibody determinations. Smears from pus often show single cocci or in pairs, rather than definite chains. Cocci are sometimes gram-negative because the organisms are no longer viable and have lost their

ability to retain blue dye (crystal violet) and be gram-positive. When specimens suspected of containing *streptococci* are cultured on blood agar plates, cultures will grow hemolytic group A *streptococci* (e.g. in sepsis), within hours or a few days. Certain α-hemolytic streptococci and enterococci may grow slowly on blood cultures. However, in cases of suspected endocarditis cultures occasionally do not turn positive within few days.

Epidemiology, Preventions and Control

Many *Streptococci* are members of the normal flora of the human body. They cause disease only when established in parts of the body where they do not normally occur. To prevent such accidents, particularly in the course of surgical procedures on the respiratory, gastrointestinal and urinary tracts that results in temporary bacteremia, antimicrobial agents are often administered prophylactically to persons with known heart valve deformity. The ultimate source of group A *Streptococci* is a person harboring the organisms. The individual may have a clinical or sub clinical infection or may be a carrier, distributing *Streptococci* directly to other persons via droplets from the respiratory tract or skin. The nasal discharges of a person harboring β- hemolytic *Streptococci* are the most dangerous sources for spread of these microbes. The infected udder of a cow yields milk that may cause epidemic spread of β-hemolytic *Streptococci*. Immunologic grouping and typing of *Streptococci* are valuable tools for epidemiologic tracing of the transmission chain.

Control procedures are directed mainly at the human source:

- Detection and early antimicrobial therapy administration is crucial. Prompt eradication of *Streptococci* from early infections can effectively prevent the development of post streptococcal disease. This requires maintenance of adequate penicillin levels in tissues for 10days; Erythromycin is an alternative drug of choice.
- Eradication of group A *Streptococci* from carriers. This is especially important when carriers are in areas such as obstetric delivery rooms, operating rooms, classrooms or nurseries. Unfortunately, it is often difficult to eradicate β-hemolytic *Streptococci* from permanent carriers and individuals may occasionally have to be shifted away from sensitive areas for some time.
- Dust control, ventilation, air filtration, ultraviolet light and aerosol mists are all of doubtful efficacy in the control of streptococcal transmission. Milk should always be pasteurized.
- Group B *Streptococci* account for most cases of neonatal sepsis at present. They are derived from the mother's genital tract where carriage is asymptomatic. Neonatal illness maybe favored by deficiency of maternal antibody. Group B streptococcal disease in the newborn can be prevented by drug prophylaxis in a mother with positive cultures and in the setting of premature labor or prolonged rupture of the membranes.

Treatments

All β-hemolytic group *Streptococc*i are sensitive to penicillin G and most are sensitive to erythromycin. Some are resistant to tetracycline. A-hemolytic *Streptococci* and *Enterococci* vary in their susceptibility to antimicrobial agents. Aminoglycosides often enhance the rate of bactericidal action of penicillin on *Streptococci*, particularly *Enterococci*. In acute

streptococcal infections, every effort must be made to rapidly eradicate *Streptococci* from the patients, eliminate the antigenic stimulus (before day 8), and thus prevent post streptococcal disease.

Antimicrobial drugs are also very useful in preventing reinfection with β-hemolytic group A *Streptococci* in rheumatic fever patients.

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