# Lecture

# Staphylococci

## Gram positive cocci Genus:

## Staphylococcus

- General characteristics

Staphylococci are non sporulating, non-motile gram-positive cocci. Microscopically, these organisms are typically found in grape-like clusters and tetrads, as well as in pairs and sometimes in short chains. Usually unencapsulated or have limited capsule formation. When grown on blood agar, staphylococci form small (1 to 2 mm), smooth, round colonies that are often pigmented and may be surrounded by a zone of B-hemolysis.

Staphylococci are very hardy organisms and can resist drying, withstand 10% NaCl broth, and will survive at temperatures between 10° and 45o C. Because staphylococci are facultative anaerobes, they will grow in the presence or absence of oxygen. Staphylococci are catalase positive. They reside on mucous membranes, skin and anterior naris.

Classification of species depends mainly on:

1. Aerobic acid production from different carbohydrates.
2. Coagulase activity.
3. Haemolysis.
4. Nitrate reduction.
5. Genetic methods: ribotyping, DNA - DNA hybridization.

Coagulase production separates the staphylococci into two major groups:

**A- Coagulase positive:** *The coagulase-positive species S. aureus, S. intermedius, S. delphini, S. schleiferi subsp. coagulans and the coagulasevariable species S. hyicus are regarded as potentially serious pathogens.*

**B-** ***Coagulase negative****: S.epidermidis, S.saprophyticus and many other species.*

In general, the staphylococci are variably sensitive to antimicrobial drugs. Resistance could be produced by: -

1. **Production of B- lactamase (penicillinase):** It is controlled by a plasmid, thus they are resistant to penicillins and cephalosporins (more than 95% of staphylococci isolates are resistant to those antibiotics).

2. **Methicillin Resistance Staphylococcus aureus (MRSA):** It is independent of B- lactamase production, the responsible genes may be resides in the plasmid or mostly in the chromosome. This character is important especially in nosocomial infection. Bacteria that are resistant to methicillin are also resistant to all other antibiotics in the beta-lactam class, which includes penicillin derivatives and cephalosporins. The only antibiotics available to kill MRSA are powerful and potentially toxic options such as vancomycin
3. **Plasmids - mediated resistance** to tetracycline, erythromycin, and aminoglycosides.

**I-*Staphylococcus aureus***

❖ ***Antigenic structure***

i. **Tcichoic acids** (PG linked); lipoteichoic acids (membrane associated).Regulates cationic environment.

2. **Protein A**: It is responsible for agglutination test known as COAGGLUTINATION it combined with Fc portion of IgG molecule. The Fab portion of IgG bound to protein A is free to combine with a specific antigen. Thus, it reduces the false positive reactions.
3. **Capsular polysaccharides**. Eleven serotypes have been reported. Types 1 and 2 are highly encapsulated, mucoid strains that are virulent for experimental animals, but rarely encountered among clinical isolates of S. aureus. These strains produce a "microcapsule" which may be antiphagocytic.
4. **Peptidoglycan:** can lead to fever and alterative complement activation that leads to inflammation followed by leukopenia, thrombocytopenia, and shock.
5. **Adhesins:** Specific cell wall associated surface proteins of S. aureus that bind to matrix proteins such as fibronectin, fibrinogen (clumping factor), collagen, bone sialoprotein, etc. These binding activities are thought to be involved in binding and colonization of various body sites and specific cell types.

❖ **Toxins and enzymes.**

**1- Staphylolysins: exotoxins and they are a, ß, y, & haemolysins:**

- Alpha- haemolysin; It is a dermonecrotic protein, dissolves rabbit erythrocytes, damages platelets and has powerful action on vascular smooth muscles.
- Beta- haemolysin: Dissolves sheep erythrocytes upon incubation for 1 hr at 37°C, and 18 hr at 10°C (this is called hot and cold reaction). Antigenicity is distinct from other haemolysins. Toxic for many kinds of cells including human erythrocytes.
- Gamma- haemolysin: is lytic for erythrocytes from different mammalian species and also cytotoxic for leukocytes. Gamma-toxin has also been proposed to play a role in the pathogenesis of toxic shock syndrome (TSS) together with toxic shock syndrome toxin 1(TSST-1).
- Delta- haemolysin: Dissolves erythrocytes of sheep, mice, guinea pigs, rabbits, horses, rats, as well as human. Leukotoxic as well as dermonecrotic.

**2- Leukocidin (Panton-Valentine Leukocidin):**

Found in 5% of all S. aureus strains and in 50% of ones from abscesses. Lethal to PMNs, disrupts their membranes through pore formation which leads to increased permeability.

**3- Enterotoxins:**

It is a neurotoxin affects the vomiting center in the CNS, which causes vomiting and diarrhea. The infection needs the presence of the bacteria, while the toxication does not.

**4- Toxic shock syndrome toxin-1 (TSST-1):**

Found in almost all S. aureus strains, isolated from individuals: suffering from TSST. About 90% of healthy individual have antibodies. against TSST-1. TSST patients either do not have antibodies or have them at very low levels.

**5- Exfoliatine toxin (ET):**

It is involved in Staphylococcal scalded skin syndrome (SSSS). Mostly phage group. II strains of S. aureus controlled by a plasmid or chromosomal gene or both.

**6- Coagulase:**

Plasma clotting protein deposits fibrin on the surface of staphylococci, perhaps altering their ingestion by phagocytic cells or their destruction within such cells. Coagulase is considered as an invasive factor rather than spreading factor.

**7- Clumping Factor (fibrinogen binding protein):**

Clumping factor allows S. aureus to adhere to fibrinogen. Coagulase and clumping factor are distinct entities.

**8- Other enzymes:**

Such as hyaluronidase, staphylokinase, proteases, lipases, B-lactamase and thermostable nucleases (DNases, RNases).

**e. Toxic shock syndrome:** a multisystem, febrile illness with hypotension, vomiting, diarrhea, rash with subsequent palmer and plantar desquamation. Hyperaemia of mucous membranes can occur regularly.

❖ **Diagnostic lab tests:**

**a) Specimens:** it depends on the localization of infection: surface swab, pus, blood, tracheal aspirate, spinal fluid. Antibody determination in serum is rarely of value in the beginning of infection but when there is chronic infection antibodies are very important to be detected.

**b) Stained smears:** "after taking the specimens" typical staphylococci appear; gram positive bacteria in grape shaped could be seen in stained smear of pus and sputum.

**c) Culture:** on blood agar, typical colonies appear in 18 hrs at 37 °C after several days we could see the haemolysis and pigment production. In case of mixed flora, we could use media containing 7.5% NaCl (Staph 110 and

mannitol salt agar. On the latter they develop yellow color). On Baired Parker agar colonies appear black surrounded by clear halo.

**d) Coagulase test:** it separates staphylococci into two groups; coagulase positive and coagulase negative.

**e) Catalase test:** it differentiates the staphylococci, which are positive, from the streptococci, which are negative.

**f) Serological tests:** Antibodies to teichoic acid specially can be detected in prolonged, deep infections (e.g. endocarditis). These serologic tests have low practical value.

**g) PCR**.

❖ **Pathogenicity**

**a. Infections of the skin and associated structures:**

Pyoderma (purulent skin infections): abscesses, furuncles, carbuncles, and impetigo.

**b. Staphylococcal Scalded Skin Syndrome (SSSS):**

Most often observed in infants and young children. The syndrome begins as erythema around the mouth and nose and spreads rapidly to affect the skin of the neck, the trunk, and sometimes the extremities. The epidermal necrolysis, mediated by the toxin exfoliation results in extensive areas of denuded skin. The intraepithelial split in SSSS is in the granulosa layer.

**c. Gastrointestinal tract Staphylococcal diseases:**

I. Staphylococcal enteritis: patients receiving chemotherapeutic agents especially via the oral route (reduce the normal flora).

II. Staphylococcal food poisoning.

**d. Deeper infections:**

They are either primary infections or may stem from an infection that has metastasized form a cutaneous infection or from carrier site. They are not likely to occur in healthy individuals but rather in those who have a precondition such as extensive surgery, burns, diabetes, cystic fibrosis, lower respiratory tract viral infections, ulcers and immunodefective (or immunosupressed) individuals, S.

aureus can cause infection in many tissues and systems and organs: e.g. endocarditis, cystitis, meningitis, pneumonia, septicaemia, infection of post-operative wounds and others like osteomyelitis.

**e. Toxic shock syndrome:** a multisystem, febrile illness with hypotension, vomiting, diarrhea, rash with subsequent palmer and plantar desquamation. Hyperaemia of mucous membranes can occur regularly.

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g) **PCR.**

❖ **Epidemiological (nosocomial) infections**

In case of hospital acquired infection or an epidemic spread out in the hospital, due to S.aureus it is so important to diagnose the type or strain of S.

aureus that cause the infection. This goal can be achieved by following a. kind of tests known as "EPIDEMIOLOGICAL MARKERS":

1- **Biotyping:** not all isolates that had been isolated from different sites, are 100% identical in all characters. .
2- **Phage typing:** there are about 100 phages (can infect more than 22 specified types of S. aureus) classified into four groups called lytic groups.
3- **Antibiogram:** similar or identical patterns of resistance to antibiotics can also be used as presumptive evidence of the relatedness of strains in epidemiological tracing. Methicillin resistance is very important.
4- **Serotyping:** there are more than 30 specific antigens (agglutinogens)
5- **Geneting methods:** DNA hybridization or plasmid profile. . 6) Polymerase chain reaction (PCR)

❖ **Treatment**

Gram positive bacteria usually treated by penicillin but that were until 1970 then resistant strains appeared and penicillin becomes to cause allergy. So we can use: Cephalosporin, Methicillin, Oxacillin or an aminoglycoside. Refampin (in case of endocarditis, since it can reach inside the valve). In case of methicillin resistance vancomycin is appropriate alternative.

❖ **Sources of infections**

1. Patients with lesions are discharging staphylococci.
2. Healthy carriers: they are healthy, but carrying the microorganisms, hence they considered as source of infection, especially food handler. "
3. Animals: some of them have strains that affect humans and could be transmitted to human by direct contact with animals raised at homes and cause disease.

❖ **Prophylaris**

No vaccine had been developed against S. aureus because antigens. are very wide, toxins have variable mode of action. However, cleaning is very important to prevent infection.

## II- COAGULASE NEGATIVE STAPHYLOCOCCI (CONS)

Interest had increased in CONS when we started using clinical prosthetic devices. They may cause wound infection, UTI, bacteraemia, catheters infections, vascular grafts, and infections of prosthetic devices.

**S. epidermidis:** It a major skin inhabitant it causes endocarditis, colonization of prosthetics, bacteraemia, wound infections, and UTI especially in elderly hospitalized patients, sensitive to novobiocin.

**S. saprophytics:** Causes 10-20% of primary Urinary Tract Infections (UTI) in young women (16-35 yrs). They are resistant to novobiocin.

# Lecture 2:

# Streptococci

## *Gram positive cocci*

## *Genus: Streptococcus*

- **General characteristics**

Gram positive cocci that appear in chains or clusters, most species are facultative anaerobes except one group "strict aerobes" which inhabits the intestinal tract and female genital tract. All streptococci are catalase negative. Some streptococci elaborate capsular polysaccharide. Hair-like pili project through the capsule of group a streptococci. The pili consist partly of M protein and are covered with lipoteichoic acid.

They are fastidious requiring many amino acids, vitamins, purine and pyrimidinc bases. Primary habitat of upper respiratory tract of human.

- **Classification**

1 - Depending.on haemolutic behaviour on blood agar:

a. **Alpha haemolytic /** incomplete haemolysis / viridans group.
b. **Beta haemolytic /** complete haemolysis / major human pathogens.
c. **Gamma haemolytic /** no haemolysis / not primary pathgens.

2- Rebecca Lancefield and co-workers / classification depending on antigenic characteristics of cell wall; carbohydrate C- substance in beta haemolytic streptococci / serological groups A-V (except I and J).

3- Biochemical reactions and resistant to physical and chemical factors.

4- Molecular genetics and ecologic features.

- *Group A streptococci (Streptococcus pyogenes)*

❖ **Antigenic structure**

**1-Capsule:** most group A, B, and C strains produce capsules composed of hyaluronic acid. Those who has capsule can impede the phagocytosis. This capsule is antigenic but not immunogenic.

**2-Cell wall proteins M, R, T.**

- **M protein:** this substance is a major virulence factor of group A S. pyogenes. When M protein is present, the Streptococci are virulent, and they are able to resist phagocytosis. The M protein (found in fimbriae) binds

fibrinogen from serum and blocks the binding of complement to the underlying peptidoglycan. It is immunogenic. There are more than 80 different types of M protein. Strains of S. pyogenes that produce certain M protein types are rheumatogenic, whereas strains of S. pyogenes that produce other M protein types are nephritogenic.

- **T. protein:** this antigen has no relationship with virulence of Streptococci. It permits differentiation of certain types of streptococci, especially those whom isolated from impetigo patients, by agglutination with specific antisera.
- **Cell surface proteins:** R protein is used as an epidemiologic marker and has no known role in virulence. F protein (fibronectin binding), G protein binds the Fc portion of antibodies, and P substance, which probably make up most of the streptococcal cell body.

**3- Cell wall carbohydrates:** according to them, Streptococci are classified into serological groups (Lancefield groups). The serologic specificity is determined by an amino sugar. For group A Streptococci, this is rhamnose-N-acetylglucosamine; for group B, rhamnose-glucosamine polysaccharide; for group C, rhamnose-N-acetylgalactosamine; for group D, glycerol teichoic acid containing D-alanine and glucose; for group F, glucopyranosyl-N-acetylgalactosamine. Group a sugar (also called C-substance) can cross-react with heart valves glycoprotein. C-substance found to cause arthritis to some animals.

**4- Cell wall peptidoglycan:** causes fever, lysis of red blood corpuscles in rabbits and other animals, and dermal necrosis.

**5- Cytoplasmic membrane antigens:** cross- reacts with tissues of heart, kidneys, and connective tissues. Group D specific antigen belongs to the cytoplasmic membrane antigens.

- **Extracellular streptococcal products**

The Streptococci elaborate extacrellular products in vitro. Many of these products are believed to be important in the pathogenesis of streptococcal infections. They are used in diagnosing such infections.

**1-Pyrogenic exotoxin** (formerly kriown as Erythrogenic toxin): Three streptococcal pyrogenic exotoxins (SPE), are recognized; A, B and C. In the non-immune individual, it causes rash that is characteristic of scarlet fever.

**2- Cardiohepatic toxin and nephrotoxin:** a low molecular weight compound excreted by virulent strains of Streptococci is capable of producing lesions in the heart and liver tissue when injected into susceptible animals.

**3- Haemolysins:** the haemolytic activity of many streptococcal strains is due to the production of two distinct extracellular haemolysins called streptolysin O (SLO) and streptolysin S (SLS).

**SLO:** is oxygen labile and immunogenic. Detection of antistreptolysin o antibodies is extensively used in the diagnosis of streptococcal infections. SLO has been implicated as a factor in the pathogenesis of rheumatic fever, a post streptococcal complication.

**SLS:** is oxygen stable, responsible for the zone of beta haemolysis seen, around surface colonies of streptococci on blood agar plates. It is capable of lysing mammalian red blood corpuscles and leukocytes, it is non-immunogenic. Intravenous injections of SLS in rabbits cause intravascular haemolysis and acute liver necrosis.

- **Spreading factors:**

**a. Hyaluronidase:** can digest host connective tissue hyaluronic acid, as well as the organism's own capsule. Depolymerization could therefore enhance the spread of streptococci in the tissue. However, it could be used as treatment in certain cases.

**b. Protease:** a proteolytic activity has been shown in strains causing soft tissue necrosis or toxic shock syndrome.

**c. Streptokinase:** produced by most of group a streptococci. It can interact with the proenzyme plasminogen of human serum, converting it to plasmin. Plasmin can digest fibrin and other serum factors important in the formation of blood clots. This activity isnow used in the treatment of acute myocardial infarction.

**d. Nucleases (streptodornases A-D):** At the site of infection, the host's inflammatory response results in the accumulation of nuclear exudates from dead or injured white blood cell. Virulent streptococci can produce nucleases such as ribonuclease and deoxyribonuclease that will digest DNA and RNA of the exudates thus facilitating the spreading of streptococci.

**e. Other extracellular products:** such as C5a peptidase: is an extracellular enzyme that degrades complement component C5a, the main factor that attracts phagocytes to sites of complement deposition.

This large repertoire of products is important in the pathogenesis of S. pyogenes infections. Even so, antibodies to these products are relatively insignificant in protection of the host.

- **Pathogenesis**

Ninety percent of streptococcal diseases caused by .group a beta haemolytic Streptococci. Streptococcal diseases are divided into two categories:

**I - Suppurative diseases:**

**a. Impetigo:** a highly contagious skin disease found primarily in children, involves the infection of epidermal layers of skin. The ... infection begins as small blisters that can spread to adjacent areas.

**b. Cellulites:** inflammatory condition associated with streptococcal invasion of subcutaneous tissue. This type of disease results in gangrene and invasion of blood stream.

**c. Erysipelas:** involves the infection of dermis characterized by a spreading inflammation with massive brawny oedema and a rapidly advancing margin of infection.

**d. Necrotizing fasciitis (streptococcal gangrene):** this is an infection of the subcutaneous tissues and fascia. There is extensive and very rapidly spreading

necrosis of the skin and subcutaneous tissues. Group A streptococci that cause necrotizing fasciitis have sometimes been termed "flesh-eating bacteria".

**e. Puerperal fever:** uterine infection that frequently accompanies delivery when aseptic techniques are not followed.

**f. Streptococcal sore throat:** in children and adults, the disease is acute and is characterized by intense nasopharyngitis, tonsillitis, and intense redness and oedema of the mucous membranes, with purulent exudates; enlarged, tender cervical lymph nodes; and (usually) high fever.

**g. Sepsis:** infection of traumatic or surgical wounds with streptococci results in sepsis or surgical scarlet fever.

**h- Toxic shock:** is caused by a few strains that produce a toxic shock-like toxin.

**II- Non suppurative diseases**

**a. Scarlet fever:** associated with the formation of erythrogenic toxin by group A streptococci.

**b. Rheumatic fever:** M protein cross reacts with sarcolemma. Antibodies cross-react with heart tissue, fixes complement, and cause damage accompanied by inflammation of the joints. Highest incidence found in age group 5-19 years.

**c. Acute glomerulonepbritis (AGN):** Antigen-antibody complexes may be deposited in kidney, fix complement, and damage glomeruli. Only a few M-types are nephritogenic. d. Erythema nodosum: skin condition, small red nodules appear under the surface of the skin. It could be the result of hypersensitivity to the peptidoglycan of the streptococcal cell wall.

- **Identification**

Preliminary identification:

1- 1 - Haemolytic behaviour. Cultures on sheep blood agar plates are the gold standard,
2- Bacitracin sensitivity: group A streptococci are sensitive to Bacitracin, while group B streptococci are resistant.
3- Fluorescent antibody.

4- Phadebact coagglutination test: Major beta haemolytic groups A, B, C, and G detected by this kit.

5- Streptex: latex particles are conjugated to group specific streptococcal antibodies mixed with unknown growth of streptococci result in agglutination.

6- Directly from throat swab (large numbers of streptococci required) is mixed with nitrous acid (extraction) in combination with coagglutination. The test required 30 minutes.

7- PCR.

- **Diagnostic test for post streptococcal diseases:**

1- Determination of anti streptolysin O titer (ASOT). An elevated ASO is a useful diagnostic marker for rheumatic fever. In contrast to rheumatic fever, antistreptolysin O (ASO) titers are low in acute glomerulonephritis.

2- Streptozyme test (slide agglutination test): Sheep red blood corpuscles are sensitized with extracellular products from group a streptococci mixed with diluted patient serum results in agglutination. The extracellular products are Hyaluronidase (HA), deoxyribonuclease (DNase), nicotinamide adenine dinucleotidase (NADase), and streptokinase (SK).

- **Treatment and prevention**

**Penicillin** is the drug of choice for treatment of group a beta haemolytic streptococci. In case of individuals sensitive to penicillin, erythromycin is a suitable alternative. **Vaccines** are prepared from streptococcal type specific M protein against group a infections.

- **Other groups**

1. Group B Streptococci: they found in oral cavity, intestinal tract and vagina S. agalactiae, which surrounded by a polysaccharide capsule. They cause neonatal meningitis by transporting to the baby during delivery, UTI, and puerperal fever.
2. Group C Streptococci: cause infections in many animals' species but rarely in human. S. dysgalactiae subsp. equisimilis cause human illnesses such as

endocarditis, pneumonia, meningitis, and wound infections. Outbreaks of disease associated with unpasteuresid milk or cheese made from unpasteurized milk. S. equi causes strangles in horses. S. equi subsp. zooepidemicus infections are infrequent in human e.g. meningitis and pneumonia.

3. Group D Streptococci: alpha or gamma haemolytic on sheep blood agar, of intestinal origin (Enterococci): E. faecium, s. bovis, s. equinus, Enterococcus durans, Enterococcus faecalis, the latter is of medically important since is causes UTI and abdominal lesions.

- **Non-Lancefield Group Streptococci**

Includes the viridans Streptococci (S. mutans, S. sanguis; S. salivarius; S. *mitis) and S. pneumonia.*

***Streptococcus pneumonia***

Formerly, Diplococcus. Inhabitants of the upper respiratory tract, gram positive cocci that occur singly, in pairs, or in chains. The clinical specimens are Lancet shaped and surrounded by a capsule, only encapsulated (smooth form) strains are virulent. On repeated subculture in the laboratory, the capsule is lost (rough form). The pneumococci are facultative anaerobes that are very fastidious in their cultural requirements. Some strains need and elevated level of CO2 (5-10%) for initial isolation.

The addition of blood to culture media supplies the enzyme catalase. Under aerobic conditions pneumococi produce hydrogen peroxide, which can: be toxic to the pneumococci. The enzyme catalase acts to remove the accumulated hydrogen peroxide.

On blood agar pneumococcal colonies exhibit alpha haemolysis and closely resemble colonies of alpha haemolytic Streptococci.

**Virulence factors**

1- **Capsular polysaccharide:** the primary virulence factor of the pneumococci. The capsule prevents binding of antibody to the cell wall of the pneumococcus and thus inhibits phagocytosis.

2- **Pneumolysin:** all pneumococci produce pneumolysin. This protein is related to S. pyogenes streptolysin O. Pneumolysin is toxic to pulmonary endothelial cells. Pneumolysin deficient pneumococci are less virulent than the toxin producing isolates. Most patients with pneumococcal disease exhibit an antibody response to pneumolysin and pneumolysin immunization is protective in animals.

3- **Pneumococcal surface protein A (PspA):** found on all pneumococci, and highly variable both immunologically and in molecular mass. Passive immunization with anti PspA is protective.

4- **Neuraminidase:** like pneumolysin, is released upon autolysis. Despite the involvement of this enzyme in the colonization by other bacteria, there is no proven role in pneumococcal virulence.

5- **SigA protease:** all pneumococci produce SIgA protease, which is a property shared with other species causing pneumonia and meninigitis. This enzyme could play a part in establishment of the microorganisms in the nasopharynx.

- **Infections produced by Pneumococci**

1- **Primary infections:** caused by certain serotypes and responsible of approximately 75% of pneumonia cases and responsible of more than half of lethal cases caused by pneumococcal bacteraemia. Lobar pneumonia, Septicaemia, Peritonitis, Purulent meningitis and purulent otitis are examples of primary infections.

2- **Secondary infections:** Produced by any serotype, 40-70% of the population is carriers of pathogenic S. pneumoniae. Many authorities believe that pneumonia and related pneumococcal infections are acquired endogenously through lowered host resistance rather than exogenously by direct contact.

- **Laboratory diagnosis of pneumococci.**

Specimens: sputum, laryngeal swabs, transtracheal aspirates, blood, and CSF. A definitive diagnosis of pneumococcal pneumonia can be made only if the microorganisms are isolated directly from the blood or from other clinical specimens by plating onto blood agar. Alternatively, by identification of specific

pneumococcal antibodies by counter Immunoelectrophoresis of body fluids: pleural fluid, blood; and urine.

The pneumococci can be differentiated from other alpha haemolytic streptococci by the following procedures:

**1-** Quellung reaction: it is the most accurate and specific test of identification of the pneumococci. Sputum or exudative material is spread on. a slide and mixed with antiserum against type specific polysaccharide or the polyvalent antiserum results capsular swelling. A capsular halo can be observed around the diplcocci.

**2-** Optochin susceptibility test: optochin is an antimicrobial drug derived from quinine. When disks containing the drug are placed on blood agar previously seeded with pneumococci, zones of inhibition can be observed around the optochin. Alpha haemolytic Streptococci are resistant to optochin.

**3-** Bile solubility test: the pneumococci pruduce an enzyme (amidase) that cleaves specific covalent bonds in the peptidoglycan layer. This enzyme is activated by ..e or bile salts such as sodium deoxycholate solution are added to a broth culture of pneumococci, the cells are rapidly lysed. While, Alpha haemolytic streptococci are not lysed by bile.

**4-** Mouse virulence test: most pneumococcal strains, when injected intraperitoneally into mice, can induce death of animals within 24-48 hours. Alpha haemolytic streptococci,' used under the same procedures, are: not lethal to mice.

**Treatment of pneumococci**

Penicillin is the drug of choice. In penicillin allergic patients, erythromycin or one of its derivatives azithromycin, can be used. Vancomycin is the drug of choice for the penicillin resistant pneumococci.

**Prevention of pneumococci.**

In 1983, a polyvalent vaccine containing polysaccharide antigen from 23 types of S. pneumoniae was recommended for children over 2 years. In 1998, a vaccine containing pneumococcal polysaccharide coupled to a carrier

protein (diphtheria toxoid) as the immunogen was shown to be effective in young children. The vaccine contained the polysaccharide of the seven most common pneumococcal serotypes. This vaccine was approved by the FDA in 2000.

# LECTURE 3

# GRAM NEGATIVE COCCI

[Document subtitle]

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## THE GRAM NEGATIVE COCCI and related organisms

### I-Neisseria

### General characteristics:

1. All are fastidious Gram negative diplococci with a bean shaped configuration, the flat or concave sides are adjacent.

2. Flagella and swimming motility are absent. The cells are nonmotile in liquid media but surface-bound motility ("twitching motility') is frequently observed.

3. All species are aerobic or facultatively anaerobic. The pathogens. grow better in 5 to 10% CO2.

4. All species produce cytochrome oxidase (oxidase positive).

The medically important members are **N. meningitidis (the meningococcus) and N. gonorrhoeae (the gonococcus),** both are pathogenic for humans (obligate parasite) and typically are found associated **with or inside polymorphonuclear cells.** The organisms": are sensitive to low temperature and drying.

### Neisseria meningitidis (the meningococcus)

Isolated by Weichselbaum in 1887

- **Antigenic composition of meningococci**

Antigens are organized in three ways:

**1. Serogroups:** meningococci are divided into at least 12 serogroups based on chemical and structural characteristics of capsular polysaccharides. Serogroups:

A, B, C, X, Y, Z, 29E, and W-135 are of medical important. A, B, and C produce epidemics. Serogroups X, Y, and Z produce meningitis, single cases; and found in carriers of the disease.

**2. Serotypes:** serotyping based on **outer membrane proteins of the cell wall (e.g. porio proteins).** These serotypes have become important in studies of the epidemiology of infection and in the development of new vaccines.

**3. Immunotypes:** immunotyping based on lipopolysaccharides.

Neisserial LPS is distinguished from enteric LPS by its highly-branched basal oligosaccharide structure and the absence of · repeating O-antigen subunits. For these reasons, neisserial LPS is referred to as **Lipooligosaccharides (LOS).**

❖ **Pathogenicity of meningococci**

The most important virulence factors contributing to disseminated disease are pili, IgA1 protease, LOS, outer membrane proteins, and capsule polysaccharides.

Infection with N. meningitidis has two presentations, **meningococcemia,** characterized by skin lesions, and acute bacterial meningitis**.** The fulminant form of disease (with or without meningitis) is characterized by multisystem involvement and high mortality.

Infection is by aspiration of infective bacteria, which attach to epithelial cells of the nasopharyngeal and oropharyngeal mucosa by the aid of pili, cross the mucosal barrier, and enter the bloodstream. It is not clear whether blood-borne bacteria may enter the central nervous system and cause meningitis.

The mildest form of disease is a transient bacteremic illness characterized by a fever and malaise; symptoms resolve spontaneously in 1 to 2 days. The most serious form is the fulminant form of disease complicated by meningitis.

Chills, fever, malaise, and headache are the usual manifestations of infection. Signs of meningeal inflammation are also present.

The majority of people who contract bacterial, meningitis and meningococcal septicemia survives and make a full recovery; however, some are left with major residual effects such as deafness, 'mental retardation, and behavioral defects.

❖ **Diagnostic laboratory tests of meningococci**

**Specimens:** specimens of blood are taken for culture, and specimens. Of spinal fluid (lumbar puncture) are taken for smear, culture, and. chemical determination.

**Smears:** gram stained smears of the sediment of centrifuged spina! fluid often show typical Neisseriae within polymorphonuclear, leukocytes or extracellularly.

**Culture:** specimens are cultured on media such as chocolate agar, · Mueller-Hinton agar or modified. Thayer-Martin medium with antibiotics (vancomycin, colistin, amphotericin). Yielded cultures can be further identified by carbohydrate fermentation reactions.

Oxidase test.

Sugar fermentation test.

Serology: it usually performed on CSF, such as capsular swelling, fluorescent antibody staining, and counter Immunoelectrophoresis (CIE). Negative capsular swelling tests and negative CIE test results do not necessarily rule out a meningococcal infection because serogroups other than A and C frequently possess insufficient capsular antigens to be detected by these methods.

❖ **Prevention and control of meningococci**

**A) Chemotherapy**

Antimicrobial drugs; Sulpha drugs were drug of choice until bacteria developed resistance against these drugs. Therefore penicillin is the drug of choice. In cases of penicillin allergic patients, chloramphenicol or ampicillin with moxalactam could be used.

Ciprofloxacin, ceftriaxone or rifampicin may be used prophylactically as a means of preventing the disease state in carriers.

**B) Active immunization**

Immunizing materials have been developed against serogroups A, C, Y, and W-135 meningococci. However, Group C vaccine does not induce protective levels of antibody in children younger than 2 years. Group B are poorly immunogenic.

❖ **Epidemiology.**

Meningococcal are endemic worldwide. They inhabit mainly the nasopharynx and transmitted via droplets or close kissing contact. Of. the young adult population, 10-30% carry meningococci. Carriage is highest among enclosed populations. The most vulnerable age groups are children aged < 5 years, particularly those < 2 years, followed by teenagers and young adults. This disease is more common during autumn and winter seasons.

**Neisseria gonorrhoeae (gonococcus GC) ·**

Isolated in 1879 by Neisser. They considered as one of the most common sexually transmitted pathogen.

❖ **Antigenic structure of gonococci**

Neisseria gonorrhoeae is antigenically heterogeneous and capable of changing its surface structures in vitro, and presumably in vivo, to avoid host defenses. Surface structures include: pili, porins proteins, opacity associated proteins (opa), LOS and others. Gonococci do not have capsular polysaccharides.

❖ **Virulence factors of gonococci**

The following factors are currently regarded as possible agents of gonococcal pathogenicity:

**1) Pili:** mediate initial attachment of gonococci to epithelial cells. Gonococci produce four colony forms: T1, T2, T3, and T4. Only the bacteria in T1 and T2

colonies are piliated, and are regarded as virulent because they produce infection in volunteers.

**2) Por proteios (protein I):** when the gonococcal membrane is in intimate contact with the host cell membrane, the Por protein is transferred to the host cell, resulting in alterations in ionic permeability of the host cell plasma membrane. May contribute to the intracellular survival of gonococci inside of neutrophils,

**3) Opa (protein II):** afimbrial adhesins that mediate firm attachment of gonococci to epithelial cells.

**4) IgA protease:** cleaves and inactivates the SIgA subclass Igal. Nonpathogenic Neisseria species do not produce IgA protease.

**5) Epithelial endocytosis:** the gonococci attach to the epithelial cells of the cervix, are subjected to endocytosis, multiply within the cell, and are protected from the phagocytic activities of leukocytes.

**6) Endotoxin (LOS):** mediates' most of the toxic damage in the epithelial cells, and regulates complement activation on the surface of the organism. It also is implicated in the attachment of gonococci to host cells by piliated and nonpiliated organisms. The sialylation of the LPS results in the conversion of a serum-sensitive organism to serum-resistant. Gonococcal endotoxin is probably responsible for some disseminated gonococcal infections (DGI).

❖ **Gonococcal diseases**

**A. Gonorrhea**

Gonorrhea is one of the most common bacterial venereal diseases. Complicating the high incidence of disease is the increasing appearance of multiple antibiotic resistance. The disease is generally spread via sexual activity.

Disease in men occurs after an incubation period of from 2-14 days. Onset of disease is usually marked by mild discomfort in the Acinetobacter calcoaceticus an opportunist causes variety of infections especially hospital

associated infections; Wound infections. Meningitis, Pneumonia, Bacteraemia, and Urethritis.

***III- Moraxella***

The moraxella group includes six species. They are nonmotile, nonfermentative, and oxidase positive. On staining, they appear as small gram negative bacilli, coccobacilli, or cocci. They are members of the normal flora of the upper respiratory tract and occasionally cause bacteraemia, endocarditis, conjunctivitis, meningitis, or other infections. Most of them are susceptible to penicillin,

**A) Moraxella catarrhalis .**

It was previously named Branhamclla catarrhalis and before that Neisseria catarrhalis. It is a member of the normal flora in nasopharynx. It causes bronchitis, pneumonia, sinusitis, otitis media, and conjunctivitis.

B) Moraxella lacunata: Causes conjunctivitis. bactoria in TI and T2 colonios ao piliated, and are regarded as virulent because they produco infection in volunteors.

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Diseaso in men occurs after an incubation period of from 2-14 days. Onset of discase is usually marked by mild discomfort in the urethra, followed a fow hours later by dysuria and a purulent yellowish green urethral discharge. In women, symptoms usually begin within 7-. 21 days after infection. A significant portion of infected. womca is asymptomatic (70 - 80%). However, onset is sometimes severe, with dysuria and vaginal discharga. The cervix and deeper reproductivo organs are the sites most frequently infected.. .

Complications lo mony. postgonococcaf urethritis, a common 'scquela, actually results from infection with othec organisms (c.8. Chlamydia trachomatis). Epididymitis is uncommon and usually unilateral. Prostatitis and urethral. Stricture are less common. Ascending infection may result in infertility. Io women, salpingitig: (pelvio inflammatory diseaso) is the most important clinical problem. This syndroma has two important consequences: 1) sterility and

ectopic pregnancy, and 2), jusceptibility to: chronic infectionsDGI with: bacteromia is more common among women than men.

B. Extragenital infections:

**i. Pharyngitis and conjunctivitis:** Infants born to a mother with cervicovaginal gonorrhoa may develop gonococcal conjunctivitis.

**ii. Gonococcal arthritis** may be preceded by symptomatic bacteremia.

The onset typically is acute, with fever, sovero pain and limitation of movement in one or a few joints.

❖ **Pathogenesis of gonococci**

Gonococci initially attach to host epithelial cells via pilt. Closer attachment is then quickly mediated by the Opa protein. Secretory IgA proteaso protects the organisms from antibodies present at the mucosal surface. Some epithelial cells are damaged by gonococcal LOS, but others are probably invaded by the organism. After attachment and initial colonization, the sequence of events probably includes:

1) Entry of gonococci into the host cell by endocytosis.

2) Intracollular replication inside the endocytic vesicle. (Host cell killing of bacteria within the vesicles is inhibited by the membrano perturbing activities of Por).

3) Transport of the vesiclo to: tho basal of the cell, fusion with tho cell membrane, and release of the gonococci into the subepithelial tissue, tha lamina propria.

4). Multiplication in the lamina propria aided by iron acquisition systems,

5) The gonococci have opportimities to spread because of the proximity of the lamina propria to regional lymphatics and blood vessels, DGI happens in approx. 1% of cases.

❖ **Laboratory diagnosis of gonococc.**

Laboratorý identification procedures include the following:

**1) Specimens:** The specimens selected for diagnosing gonorrhon: depond Op tho gender; ago and sexual preference of the patient: Urethral and cervical specimens routinely are collected from meni. And women, rospectively. Howover, the organist may be isolated from blood, tho nasopharynx, skin lesions, CSP and the anal canal of homosexual.

**2) Direct examination:** Gram stain of purulent materials may reveal Gram-negativo diplococcic in polymorphonuclear leukocytes.

**3) Culturo:** Specimons are cultured on media such as supplemented chocolate agar of Thayer-Martin medium in an elevated CO2 eavironment". Blood cultures and cultures of synovial aspirates or skin lesions should bo attempted for patients with suspected disseminated gonococcal infection (DGI).

**4) Oxidase test.**

**5) Superoxol test:** catalaso test using 30% H2O2 was used to differentiate N. gonorrhoear from other Neisseria species.

**6) Carbohydrate fermentation.**

**7) Serological tests:**

a. Direct fluorescent antibody technique.

b. Staphylococcal coagglutination techniquo.

**8) Molecular methods:** The microbiological diagnosis of gonorrhea based on culture or selective medium produces about 80-95%. Sensitivity, with false-negative results attributed to poor specimen storage; transport problems, and inhibition of growth by the compononts of selective modia. As an alternative diagnostic test: molecular techniques have been developed and offor tho

promiso of: eliminating: transport and specimen collection issues, which are bolicvod to affect test sensitivity in the field setting.

❖ **Treatmort of gonacocci**

The recommended. Treatment for uncomplicated infections is a third-generation cephalosporin or a fluoroquinolono plus an antibiotio: (c.g. doxycyclino or orythromycin) effectivaagainst possible coinfection with Chlamydia trachomatis. Sox partners should be referred. And treated. The current CDC Treatment Guidelines: recommend treatment of all gonococcal infoctions with antibiotic regimons effectivo against resistant strains. The recommended antimicrobial agents are ceftriaxono, cefiximo, ciprofloxacin, or oflaxacin.

Prophylaxis of "ophthalmia neonatorum" can be achieved by local application of 0.5 % erythromycin ophthalmic ointment or 1% tetracycline ointment.

No vaccino was prepared until now. The patients failed to develop a solid immunity.

***II- Acinetobacter***

Includes many species of gram negative diplococci or coccobacilli. Widely distributed in nature and aro part of the normal human flora (skin).

Acinetobaciar calcoaceticus an opportunist causos varioty of infections ospocially hospital associated infections; Wound infections, Meningitis, Proumonia, Bacteraania, and Urethritis.

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**B) Morarella lacunata:** Causes conjunctivitis.

# SPORE FORMER GRAM POSITIVE BACILLI

Lecture four

## *I- Bacillus*

Saprophytes found in soil, water, air and vegetation. The mostly important genera are B. anthracis, B. cereus, B. thuringiensis, B. subtilis and B. megaterium.

### B. anthracis

### General characteristics

Members of this species are non-motile, facultative anaerobes, large bacilli measuring 4-8 um in length and 0.5-1.0 um in width. They have square ends and arranged in long chains, usually encapsulated with centric spore. Colonies on agar plate are large, opaque, white, medusa head, and have "cut glass" appearance in transmitted light.

### Antigenic structure

**1. Capsular polypeptide:**

This capsule composed of Polyglutamic acid and it is antiphagocytic.

**2. Somatic polysaccharides:**

Found in cell wall, it does not considered as a virulence factor and it is not immunogenic.

**3. Protein exotoxin complex:**

The toxin components include the edema factor, lethal factor and protective antigen. The latter protects the former two from body proteases before entering host cells, and it also induces protective antibodies when used as a vaccine.

**Pathogenicity**

B. anthracis is a pathogen of animals (herbivorous). Spores found in soil and on vegetation ingested, inhaled or gain entrance through abraded skin or mucosa.

The spores germinate in the tissue and transformed into vegetative cells, which produce the exotoxin. The capsule inhibits phagocytosis. In human, the disease considered as an occupational hazard.

**Diseases in human**

**1- Skin infection (cutaneous anthrax):**

The organism enters through abraded skin, after 12-36 hrs a papule is formed which changed into a vesicle, then to a pustule, eventually into a dark necrotic area surrounded by a rim of edema.

**2- Wool sorters disease**

Inhaled spores settle in the respiratory tract, Producing local hemorrhage and edema.

**3- Septicemia**

It is rare; however it leads to meningitis.

**4- Gastrointestinal anthrax**

Occurs in countries where contaminated meats are sold. Toxin produced in the intestinal tract forms a necrotic lesion in the iluem or cecum. High fatality rate is occurring.

**Treatment**

Penicillin is the drug of choice. Streptomycin, tetracycline and erythromycin are alternative drugs. Treatment before the appearance of bacteremia is important because the antibiotics are no effective against the toxin.

**Prevention**

Anthrax is an endemic in many areas of the world, and can produce epizootic outbreaks. The control of this disease can be achieved via:

A) Vaccination of cattle.

B) Cremation or burial with quick lime of infected animals.

C) Restricted movement of livestock.

Control in human is provided by immunization with toxoid. Recovery from the disease produces permanent immunity for human and animals.

***II- Clostridium***

Saprophytes found in soil, water, air and vegetation. Gram positive spore forming bacilli. Measuring 3-8 um in length and 0.4-1.2 um in width. Obligate anaerobes. Some of them are non-motile. Most of them are unencapsulated. Few species parasitize intestinal tract of human and animals. Produce highly toxic exotoxins.

***C. botulinum***

Found in soil and occasionally in animal feces. Spore are highly resistant to heat, withstanding 100°C for 3-5 hours. Diminish at acid pH or high salt concentration. During growth and autolysis, the bacterium liberates toxins.

Seven distinct antigenic varieties (A-G). Type A toxin is a complex consisting of neurotoxin and heamagglutinin which protect the neurotoxin from stomach acid and enzymes. Probably 1-2 ug is lethal to human.

**Pathogenicity**

C. botulinum causes botulism "an intoxication resulting from the ingestion of food in which C. botulinum has grown and produced toxin". Spiced, smoked vacuum packed or canned alkaline foods are eaten without cooking. The toxin acts by blocking release of acetylcholine at synapses and neuromuscular joints producing respiratory paralysis. Symptoms begin after 18-36 hrs. after ingestion of toxic food; visual disturbance, inability to swallow, speech difficulty and death occurs from respiratory paralysis or cardiac arrest.

Botulism is a disease of high mortality rate. There is no fever, patient remains fully conscious until short time before death. Those who recover does not develop antitoxin in the blood.

**Laboratory diagnosis**

Toxin can often be demonstrated in serum from the patient, and toxin may be found in leftover food. Mice injected intraperitoneally die rapidly. The antigenic type of toxin is identified by neutralization with specific antitoxin in mice. C botulinum may be grown from food remains and tested for toxin production, but this is rarely done and is of questionable significance. Toxin may be demonstrated by ELISA, passive hemagglutination or radioimmunoassay.

**Treatment**

Antitoxins to three types of C. botulinum toxins have been prepared, reduced mortality rate from 65-25%.

**Prevention**

Strict regulation of commercial canning. Chief danger lies in home canned foods. Toxoid used for active immunization of cattle.

***C. perfringens***

It is implicated in gas gangrene. C. perfringens is indigenous members of the intestinal tract of humans and animals. Saprophytes found in soil. Large encapsulated, non-motile, spore former bacilli, produces eleven toxins. Alpha toxin is the most important (acts as lecithinase). Also they produce collagenase, hemolysin, proteinase, DNase, and some of them produce enterotoxin.

**Disease produced**

**1. Gas gangrene.**

An infection requires sites for germination of spores and multiplication of vegetative cells. The traumatized tissue offers an anaerobic environment. The organism does not invade healthy tissue. Most cases appear during war, surgeries or car accidents. Symptoms are included; Local pain in the area of the wound, swelling of the wound, skin rupture, revealing a necrotic, foul smelling wound. The most symptoms; delirium, apathy, disorientation, toxemia and death.

**2. Food poisoning**

Mostly caused by C. perfringens type A. When more than 101 are ingested, sporulated and produced enterotoxin will causes secretion of fluid and electrolytes. Symptoms are; abdominal pain, diarrhea, nausea and vomiting.

**3. Enteritis necroticans**

Food born disease caused by C. perfringens type C. producing hemorrhage and gangrene in the intestine due to beta toxin.

**4. Uterine infections**

Follow instrumental abortion and it occurs in 5% of women.

**Diagnostic lab tests**

1. Specimens: Wound materials, Pus, Tissue, Blood, Urine and Feces.

2. Smears: Presence of large Gram positive bacilli but the Spores not regularly found**.**

3. Culture: Blood agar, chopped meat-glucose medium, and thioglycolate medium, anaerobically. The growth transferred into milk agar will produce clot and gas. Colonies on blood ager are surrounded by an inner zone of clear hemolysis and an outer zone of incomplete hemolysis. Colonies on egg yolk agar will form a precipitate around the colonies.

**Treatment and prevention**

- ☐ Immediate debridement of the wound to remove all dead tissue.
- ☐ Application of anitserum.
- ☐ Antibiotic therapy (penicillin and tetracycline).
- ☐ Treating patients with high concentration of oxygen at elevated pressure.

In case of food poisoning, treatment does not require antibiotics since the disease is mild and self-limiting. It can be prevented by proper cooking,

**C. tetani**

**General characteristics**

Found in soil, human intestine (25%) and animal feces. Motile bacilli forming tennis racket shaped terminal spores. Obligate anaerobe but can stand certain concentration of oxygen. Can be distinguished by specific flagellar antigen. All share common somatic antigen. All produce the same antigenic type of toxin.

**Toxin produced**

**1- Tetanospasmine**

Responsible for tetanus. Heat labile protein. Neurotoxin released upon autolysis. It acts on CNS blocking the transmission of nerve impulses producing spastic.

**2- Tetanolysin**

A hemolysin can be reversibly inactivated by oxygen.

**Pathogenesis**

Tetanus results from small wounds which contaminated by C. tetani spore that germinate and produce toxin. The infection remains localized with minimal inflammatory damage. The toxin produced upon growth, sporulation and lysis of cells. The toxin migrates along neural paths from a local wound to sites of action in the CNS. Severe painful spasms and rigidity of voluntary muscles. The characteristic symptom of lock jaw involves spasms of masseter muscle, followed by progressive rigidity and violent spasms of trunk and limb muscles. Spasms of the pharyngeal muscles cause difficulty in swallowing. Death usually results from interference with mechanisms of respiration.

Symptoms appear after 4-6 days to 6 weeks incubation time depending on two factors:

1. The time of anaerobic condition to develop at the site of infection.

2. The time required for any toxin to reach CNS.

**Laboratory diagnosis**

- Clinical signs:

  Patients usually have a wound, exhibit trismus. Have a history of no immunization.

- **Culture:**

  Material from wound on blood agar or cooked meat medium heated to 80° C for 10-15 min to eliminate non spore former. 24 hrs later on blood agar swarming motility is examined.

- Spore stain.

**Treatment**

Tetanus antitoxin will neutralize any toxin in blood but already nerve fixed toxin. Penicillin used to destroy any cells thus preventing toxin production. Surgically removal of necrotic tissue which create anaerobiosis. Convulsions and spasms treated by barbiturate.