Pharmaceutical microbiology

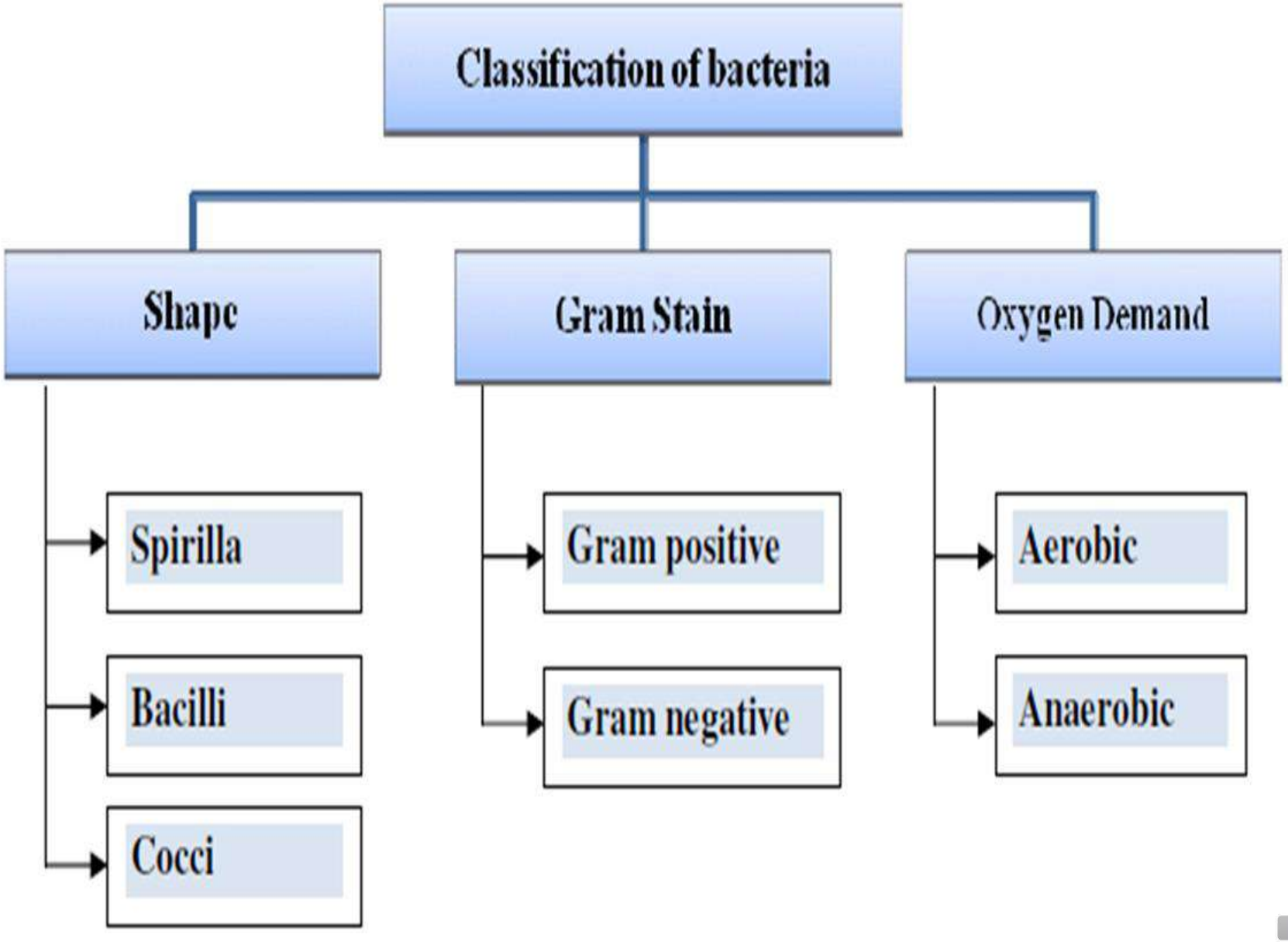
Chapter 3

Classification of bacteria

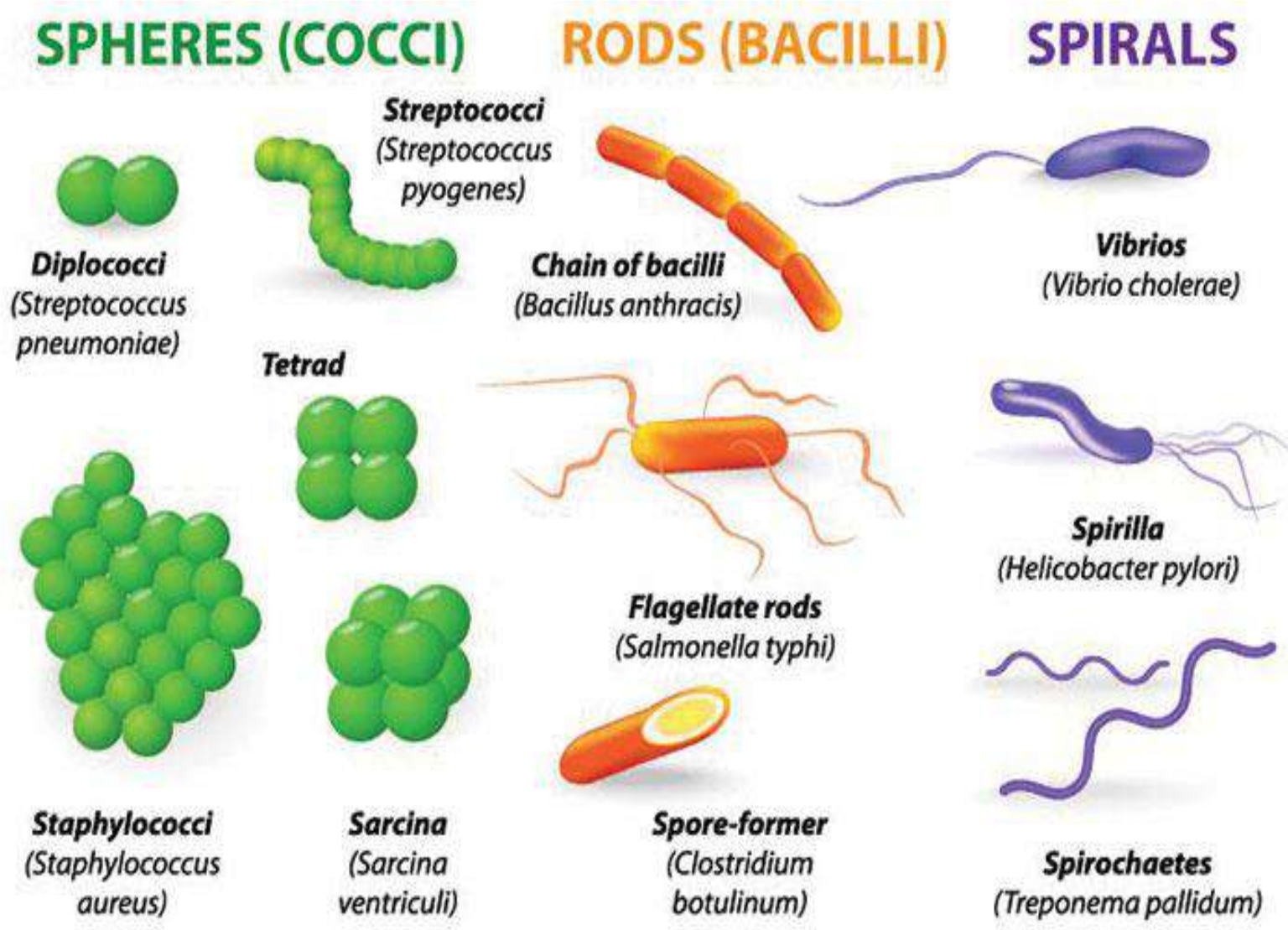
By Dr. Mohammed Hussein Taleb

Lecture 7

18/10/2020

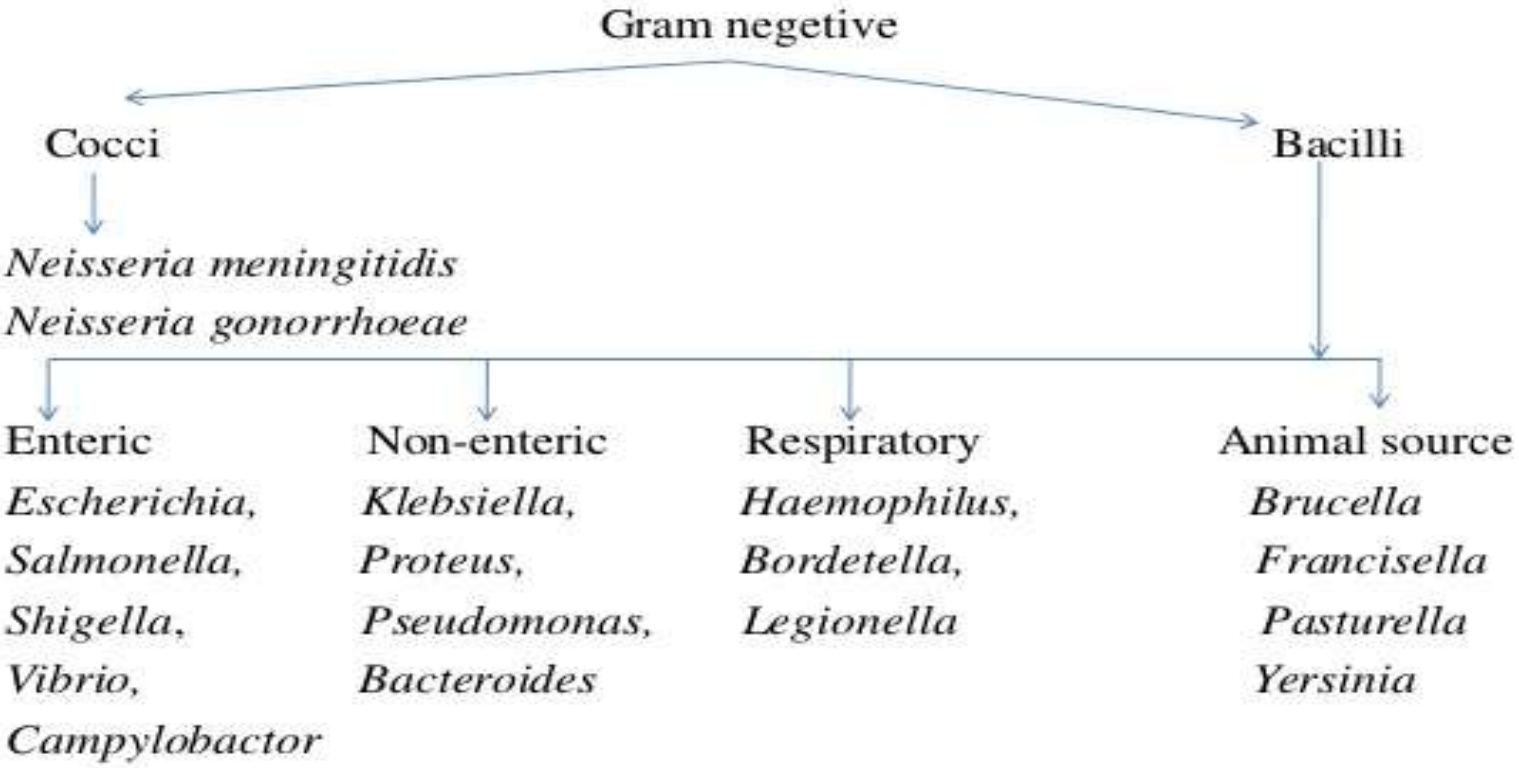

Classification of bacteria
Shape
Gram Stain
Oxygen Demand
Spirilla
Bacilli
Cocci
Gram positive
Gram negative
Aerobic
Anaerobic

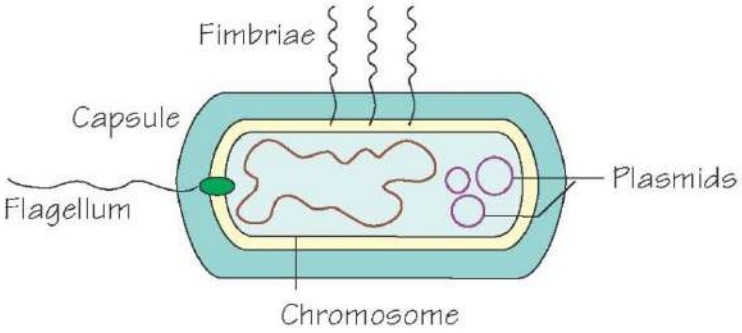
Shapes of bacteria



Gram negative bacteria

Example of Gram negetive :

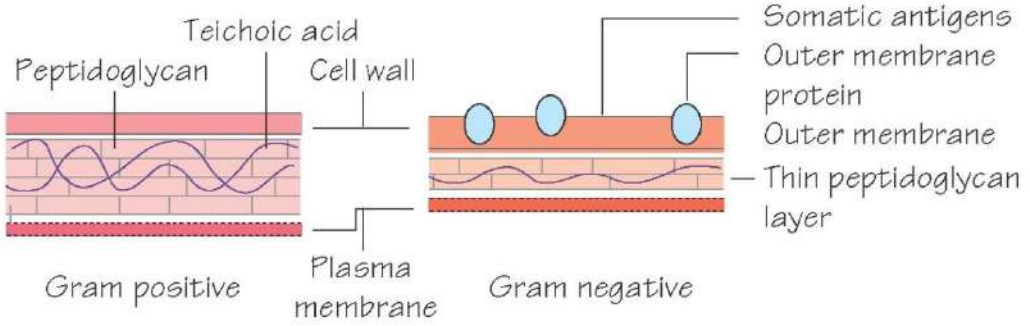




Bacterial classification

Shape
- Cocci
- Spiral
- Bacilli

Cocci are spherical; bacilli are long and thin, with coccobacilli in between; and there are also curved and spiral bacilli with different wavelengths



Gram reaction

Growth

Atmosphere

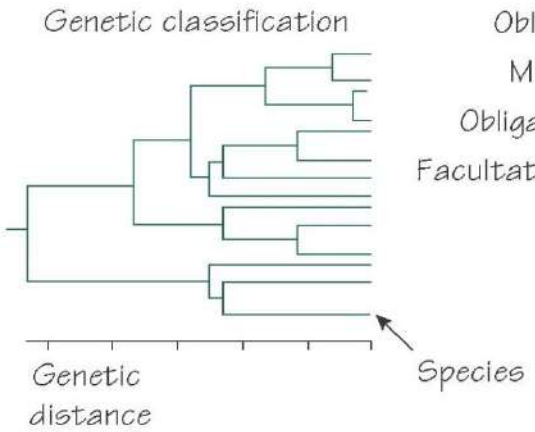
- Obligate aerobes → Require oxygen
- Microaerophiles → Require reduced oxygen
- Obligate anaerobes → Require no oxygen
- Facultative anaerobes → Anaerobic or aerobic
- Capnophiles → Require increased CO_2

Spores → Endospore

Present on *Clostridium* and *Bacillus* spp.

Biochemistry, Genetic classification → Batteries of reactions are needed for species identification

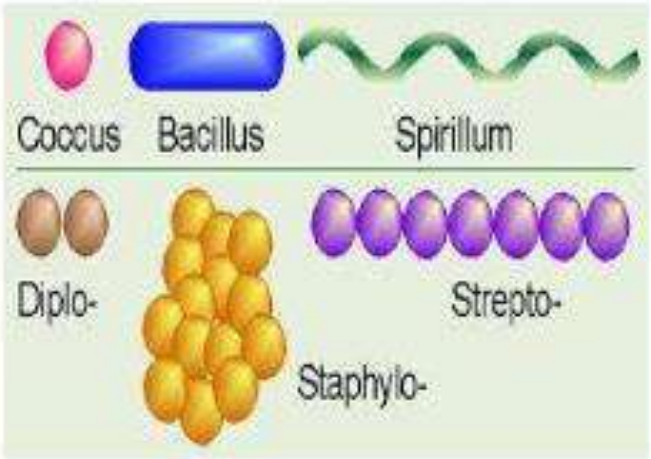
Serology typing



Classification on the basis of multifactors variation

On the basis of Shape

- **Cocci**
  - (a) Dipplococci e.g Neisseria Gonorrhorea
  - (b) Streptococci e.g S.mutans
  - (c) Sarcina e.g Sarcina rosea
  - (d) Tetrad e.g Micrococcus luteus
  - (e) Staphylococci e.g Staphylococcus aureus..
- **Baccilli**
  - (a) Dipplobacilli e.g Clostridium
  - (b) Coccobacilli e.g Bordtella pertusis
- **Vibrio** e.g Vibrio cholerae
- Spiral e.g Spirillus
- Spirochete e.g Treponema pallidum



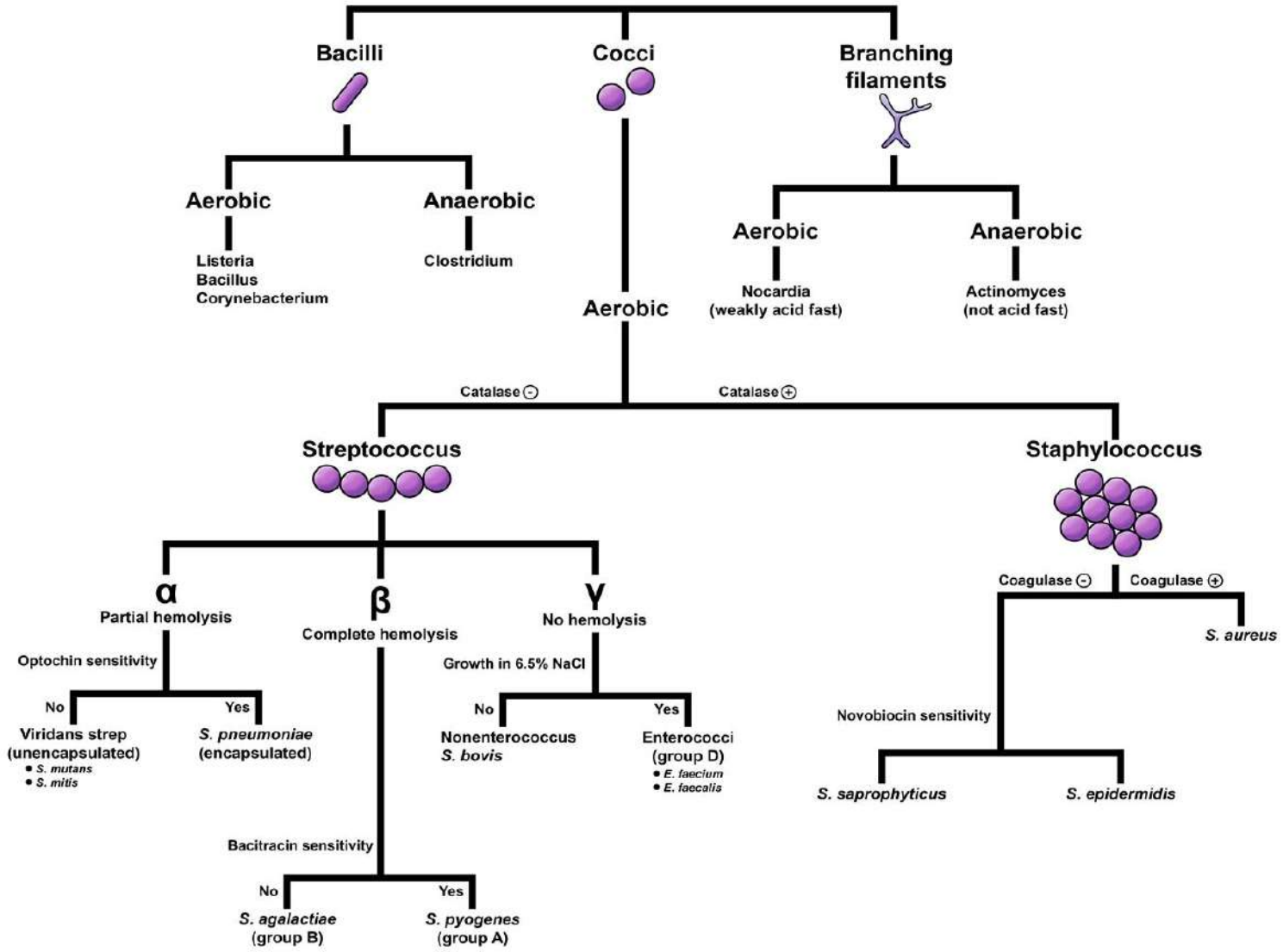
ON THE BASIS OF STAINING

- **Gram positive** e.g Streptococci aureus
- **Gram negative** e.g Neisseria Gonorrhorea

ON THE BASIS OF TEMPERATURE

- **Psychrophiles** e.g Bacillus psychrophilus
- **Psychrotrophs** e.g Listeria monocytogenes
- **Mesophiles** e.g E.coli
- **Thermophiles** e.g Bacillus stearothermophiles
- **Hyperthermophiles** e.g Sulpholobus

Gram-Positive Bacteria



Moises Dominguez

Pharmaceutical microbiology

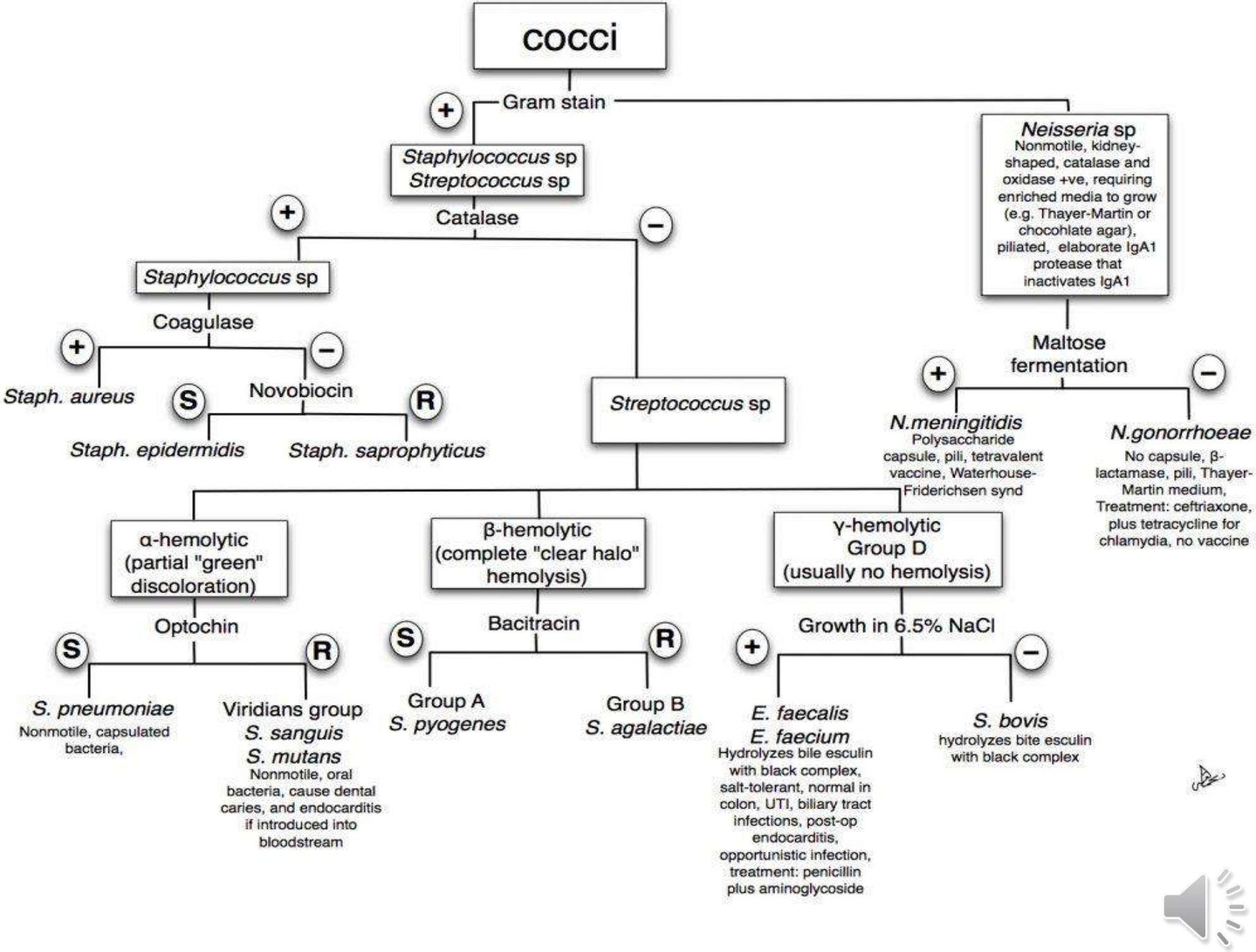
Chapter 3

Classification of bacteria

By Dr. Mohammed Hussein Taleb

Lecture 8

22/10/2020


cocci
Gram stain
+
Staphylococcus sp
Streptococcus sp
Catalase
+
−
Staphylococcus sp
Coagulase
+
−
Staph. aureus
S
Novobiocin
R
Staph. epidermidis
Staph. saprophyticus
Streptococcus sp
α-hemolytic (partial "green" discoloration)
Optochin
S
R
S. pneumoniae
Nonmotile, capsulated bacteria,
Viridians group
S. sanguis
S. mutans
Nonmotile, oral bacteria, cause dental caries, and endocarditis if introduced into bloodstream
β-hemolytic (complete "clear halo" hemolysis)
Bacitracin
S
R
Group A
S. pyogenes
Group B
S. agalactiae
γ-hemolytic Group D (usually no hemolysis)
Growth in 6.5% NaCl
+
−
E. faecalis
E. faecium
Hydrolyzes bile esculin with black complex, salt-tolerant, normal in colon, UTI, biliary tract infections, post-op endocarditis, opportunistic infection, treatment: penicillin plus aminoglycoside
S. bovis
hydrolyzes bite esculin with black complex
Neisseria sp
Nonmotile, kidney-shaped, catalase and oxidase +ve, requiring enriched media to grow (e.g. Thayer-Martin or chocohlate agar), piliated, elaborate IgA1 protease that inactivates IgA1
Maltose fermentation
+
−
N.meningitidis
Polysaccharide capsule, pili, tetravalent vaccine, Waterhouse-Friderichsen synd
N.gonorrhoeae
No capsule, β-lactamase, pili, Thayer-Martin medium, Treatment: ceftriaxone, plus tetracycline for chlamydia, no vaccine

G +ve Cocci

1-Staphylococcus

2-Streptococcus

1-Staphylococcus

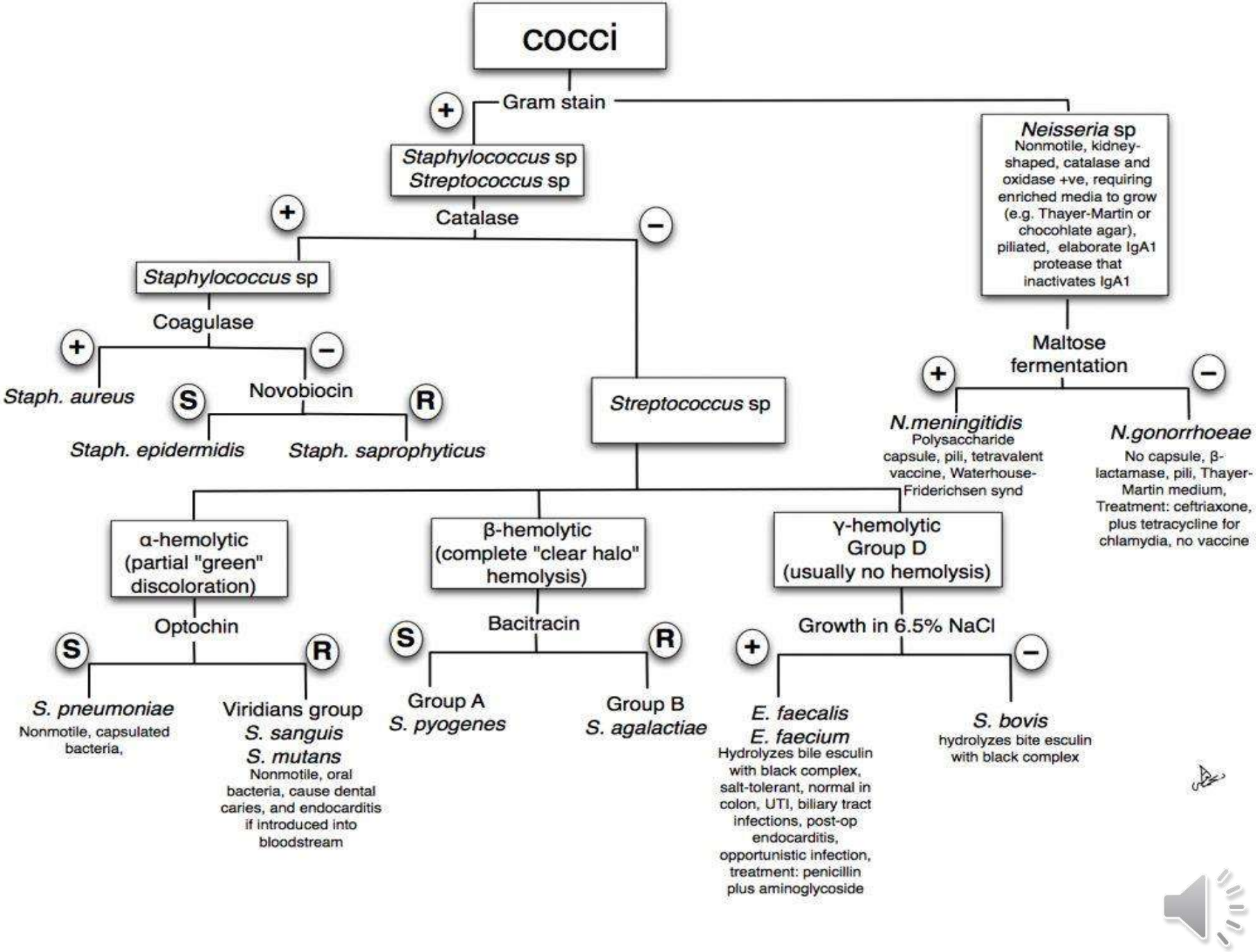
- The pathogenic staphylococci often hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins.
- The most common type of food poisoning is caused by a heat stable staphylococcal enterotoxin..
- Staphylococci rapidly develop resistance to many antimicrobial agents, which consequently presents difficult therapeutic problems.

Morphology and identification

A. Typical Organisms

- **Staphylococci** are **spherical cells about 1 µm in diameter arranged in irregular clusters** . **Single cocci, pairs, tetrads, and chains are also seen in liquid cultures.**

- **Staphylococci are nonmotile and do not form spores**. Under the **influence of drugs such as penicillin, staphylococci are lysed.**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No capsule, β-lactamase, pili, Thayer-Martin medium, Treatment: ceftriaxone, plus tetracycline for chlamydia, no vaccine

Staphylococcus

- **The staphylococci** are **gram-positive spherical cells**, usually arranged in **grapelike irregular clusters**. Some are members of the normal microbiota of the skin and mucous membranes of 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..
- Staphylococci rapidly develop resistance to many antimicrobial agents, which consequently presents difficult therapeutic problems.

S. aureus

C. growth Characteristics

- The **staphylococci produce catalase, which differentiates them from the streptococci.**
- Staphylococci are **variably susceptible to many antimicrobial drugs.** Resistance is caused by several mechanisms:
- 1. **β-Lactamase production** is common, is under **plasmid control**, and makes **the organisms resistant to many penicillins** (penicillin G, ampicillin, ticarcillin, piperacillin, and similar drugs).
- The plasmids are **transmitted by transduction and perhaps also by conjugation.**

- Most *S aureus* strains of clinical importance have polysaccharide capsules, which inhibit phagocytosis by polymorphonuclear leukocytes unless specific antibodies are present.
- At least 11 serotypes have been identified, with types 5 and 8 responsible for the majority of infections.
- These capsule types are targets for a conjugate vaccine.

Enzymes and Toxins

- **Staphylococci** can **produce disease** both through 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, although they may function as enzymes.**

- **Many of the toxins** are under the **genetic control of plasmids; some may be under both chromosomal and extrachromosomal control**; and for others, the mechanism of genetic control is not well defined.

A. Catalase

- Staphylococci produce **catalase**, **which converts hydrogen peroxide into water and oxygen.** The catalase test **differentiates the staphylococci, which are positive, from the streptococci, which are negative**.

B. 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.**

C. Exfoliative Toxins

- **Exfoliative toxin B is plasmid mediated and heat labile.**
- These **epidermolytic toxins yield the generalized desquamation of the staphylococcal scalded skin syndrome by dissolving the mucopolysaccharide matrix of the epidermis.**
- **The toxins are superantigens**

D. Toxic Shock Syndrome Toxin

- Most *S. aureus* strains isolated from patients with toxic shock syndrome produce a toxin called toxic shock syndrome toxin-1 (TSST-1), which is the same as enterotoxin F.
- The toxin is associated with fever, shock, and multisystem involvement, including a desquamative skin rash. The gene for TSST-1 is found in about 20% of *S aureus* isolates, including MRSA.

E. Enterotoxins

- **There are multiple (A–E, G–J, K–R and U, V) enterotoxins that, similar to TSST-1, are superantigens.**
- Approximately 50% of *S aureus* strains can produce one or more of them.
- **Important causes of food poisoning, enterotoxins are produced when *S aureus* grows in carbohydrate and protein foods.**
- **Ingestion of 25 µg of enterotoxin B results in vomiting and diarrhea.**
- **The emetic effect of enterotoxin is probably the result of central nervous system stimulation (vomiting center) after the toxin acts on neural receptors in the gut.**

Pharmaceutical microbiology

Chapter 3

Classification of bacteria

G+ve cocci

Dr. Mohammed Hussein Taleb

Lecture 9

25/10/2020

G+ve cocci

Staphyllococcus

Pathogenesis

- **Staphylococci**, particularly ***S epidermidis***, are members of the normal microbiota of the human skin and respiratory and gastrointestinal tracts. Nasal carriage of *S aureus* occurs in 20–50% of humans.
- *S. saprophyticus* causes urinary tract infections in young women.

- 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.
- At one end of the disease spectrum is staphylococcal food poisoning, attributable solely to the ingestion of preformed enterotoxin; at the other end are staphylococcal bacteremia and disseminated abscesses in all organs

Pathology

- The prototype of a staphylococcal lesion is the furuncle or other localized abscess. Groups of *S. aureus* established in a hair follicle lead to tissue necrosis (dermonecrotic factor).

- **Coagulase** is produced and coagulates fibrin around the lesion and within the lymphatics, resulting in formation of a wall that limits the process and is reinforced by the accumulation of inflammatory cells and, later, fibrous tissue.

- (abscess) Focal suppuration is typical of staphylococcal infection. From any one focus, organisms may spread via the lymphatics and bloodstream to other parts of the body.

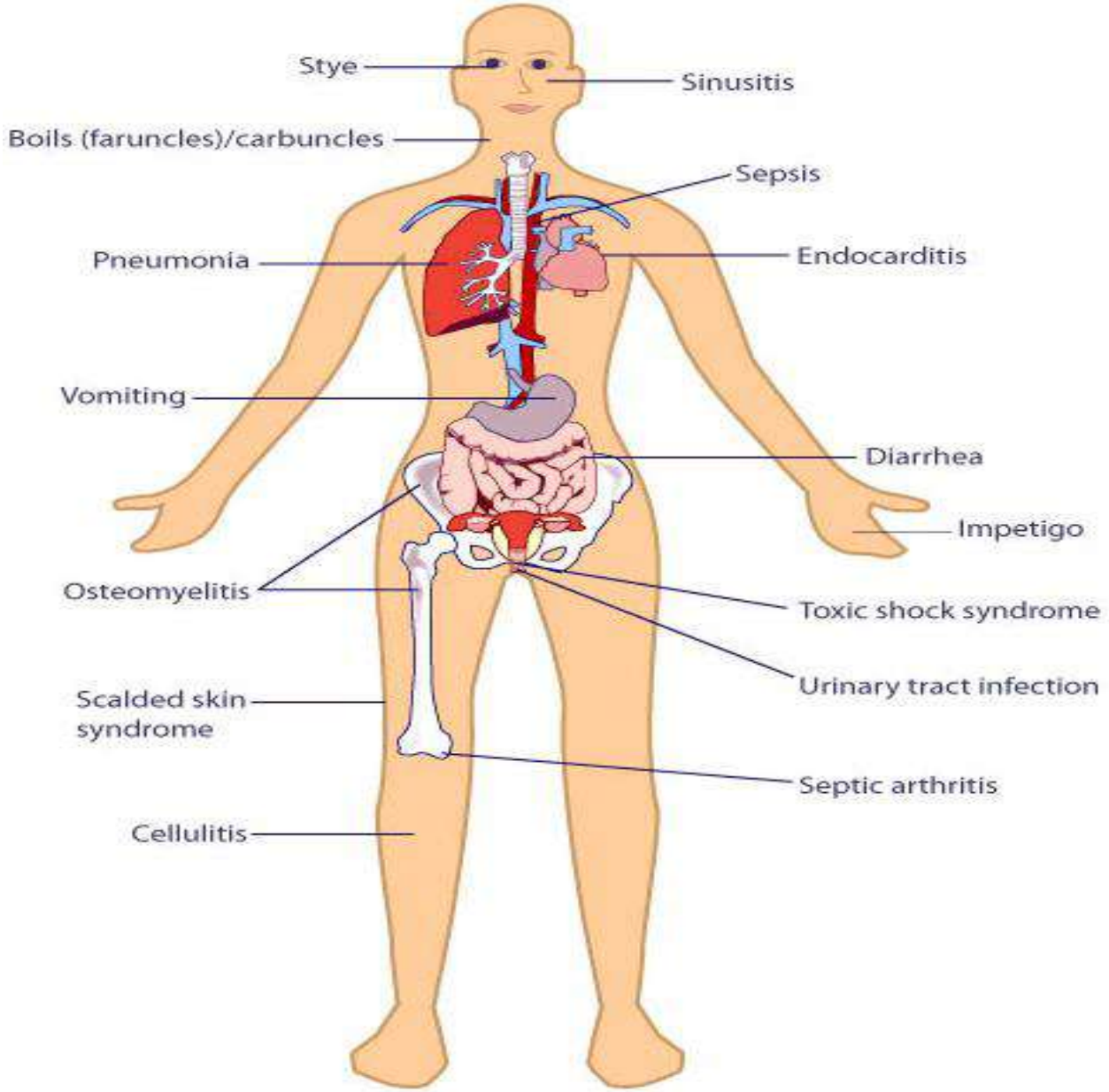
- In osteomyelitis, the primary focus of *S aureus* growth is typically in a terminal blood vessel of the metaphysis of a long bone, leading to necrosis of bone and chronic suppuration.

Boils, fruncle, and carbuncle

- Staphylococci also cause disease through the elaboration of toxins without apparent invasive infection. Bullous exfoliation, the scalded skin syndrome, is caused by the production of exfoliative toxins.
- Toxic shock syndrome is associated with TSST-1.

The scalded skin syndrome

tampon


Stye
Sinusitis
Boils (faruncles)/carbuncles
Sepsis
Pneumonia
Endocarditis
Vomiting
Diarrhea
Impetigo
Osteomyelitis
Toxic shock syndrome
Urinary tract infection
Scalded skin syndrome
Septic arthritis
Cellulitis

Bullous Impitigo

Clinical Findings

- A localized staphylococcal infection appears as a "pimple," hair follicle infection, or abscess. There is usually an intense, localized, painful inflammatory reaction that undergoes central suppuration and heals quickly when the pus is drained.
- *S. aureus* infection can also result from direct contamination of a wound, such as a postoperative staphylococcal wound infection or infection after trauma (chronic osteomyelitis subsequent to an open fracture, meningitis after skull fracture).

- If *S aureus* disseminates and bacteremia ensues, **endocarditis,** acute hematogenous osteomyelitis, meningitis, or pulmonary infection can result.
- Food poisoning caused by staphylococcal enterotoxin is characterized by a short incubation period (1–8 hours); violent nausea, vomiting, and diarrhea; and rapid convalescence. There is no fever.

- **Toxic shock syndrome** is manifested by an abrupt onset of high fever, vomiting, diarrhea, myalgias, a scarlatiniform rash, and hypotension with cardiac and renal failure in the most severe cases.

- It often occurs within 5 days after the onset of menses in young women who use high-absorbency tampons, but it also occurs in children and men with staphylococcal wound infections.

- **Toxic shock syndrome**—associated *S aureus* can be found in the vagina, on tampons, in wounds or other localized infections, or in the throat but virtually never in the bloodstream.

Treatment

- Most persons harbor staphylococci on the skin and in the nose or throat.
- Because pathogenic organisms are commonly spread from one lesion (eg, a furuncle) to other areas of the skin by fingers and clothing, scrupulous local antisepsis is important to control recurrent furunculosis.
- Serious multiple skin infections (acne, furunculosis) occur most often in adolescents.

Similar skin infections occur in patients receiving prolonged courses of corticosteroids.

- *S. epidermidis* infections are difficult to cure because they occur in prosthetic devices where the bacteria can sequester themselves in a biofilm.

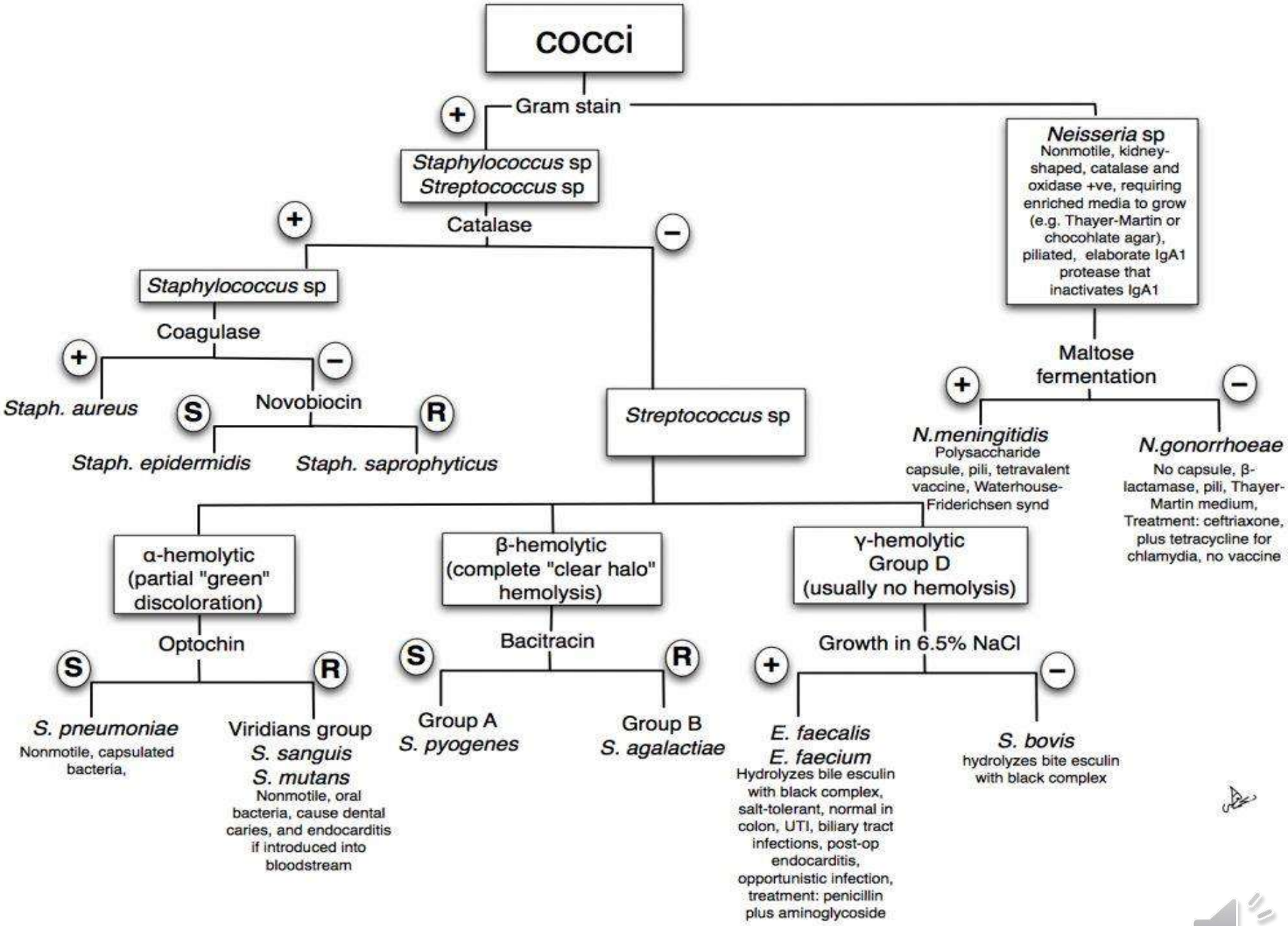
- *S. epidermidis* is more often resistant to antimicrobial drugs than is *S. aureus*; approximately 75% of S epidermidis strains are nafcillin resistant.

- Because of the **frequency of drug-resistant strains, meaningful staphylococcal isolates should be tested for antimicrobial susceptibility to help in the choice of systemic drugs.**

- **Resistance** to **drugs of the erythromycin group tends to emerge so rapidly that these drugs should not be used singly for treatment of chronic infection.**

- Drug resistance (to penicillins, tetracyclines, aminoglycosides, erythromycins, and so on) determined by plasmids can be transmitted among staphylococci by transduction and perhaps by conjugation.

- **Penicillin G–resistant *S. aureus* strains from clinical infections always produce penicillinase.** They constitute more than 95% of *S aureus* isolates in communities in the United States. They are **often susceptible to β-lactamase–resistant penicillins, cephalosporins, or vancomycin.**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No capsule, β-lactamase, pili, Thayer-Martin medium, Treatment: ceftriaxone, plus tetracycline for chlamydia, no vaccine
α-hemolytic (partial "green" discoloration)
β-hemolytic (complete "clear halo" hemolysis)
γ-hemolytic Group D (usually no hemolysis)
Optochin
S
R
S. pneumoniae
Nonmotile, capsulated bacteria,
Viridians group
S. sanguis
S. mutans
Nonmotile, oral bacteria, cause dental caries, and endocarditis if introduced into bloodstream
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Growth in 6.5% NaCl
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Hydrolyzes bile esculin with black complex, salt-tolerant, normal in colon, UTI, biliary tract infections, post-op endocarditis, opportunistic infection, treatment: penicillin plus aminoglycoside
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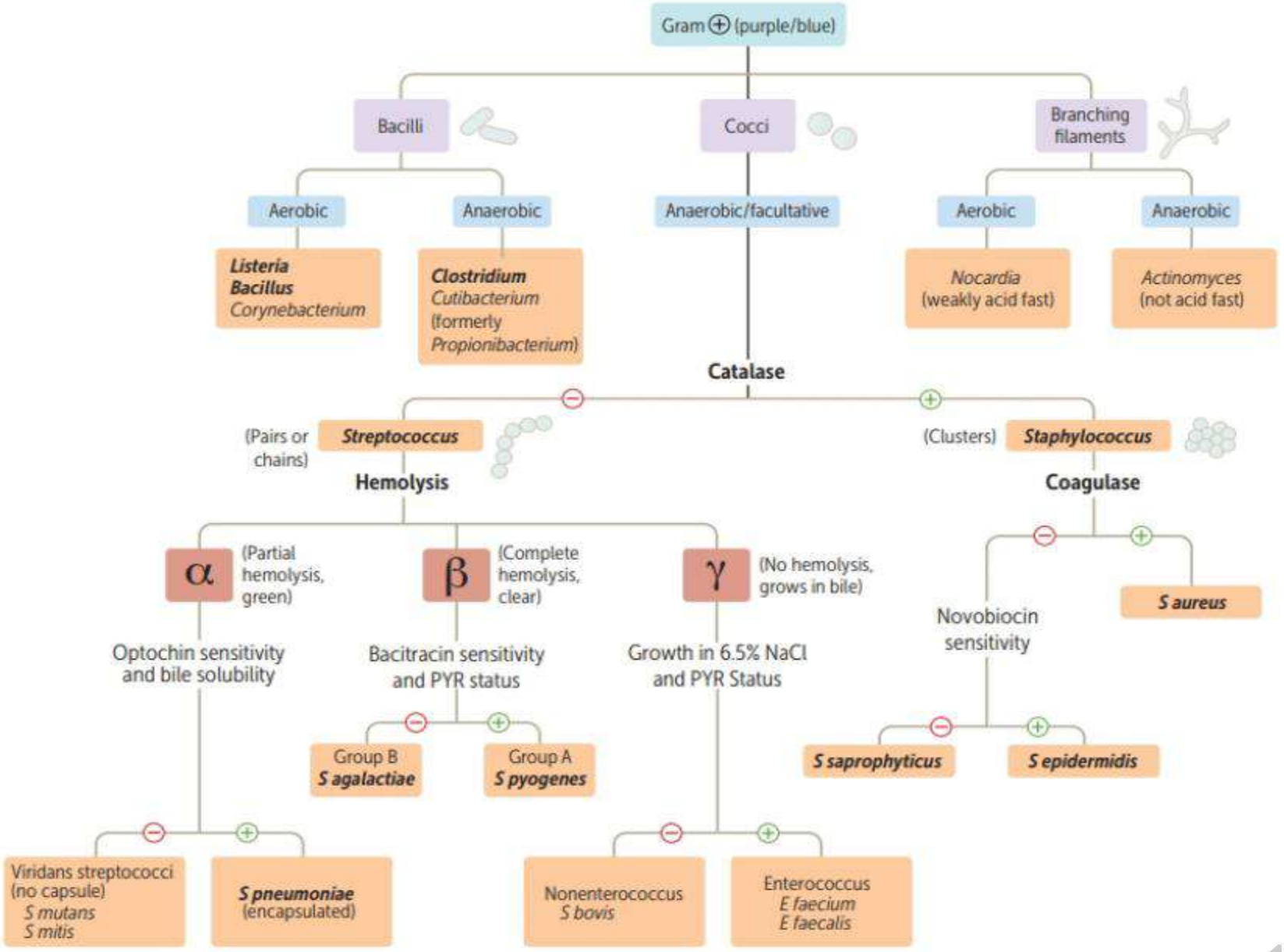
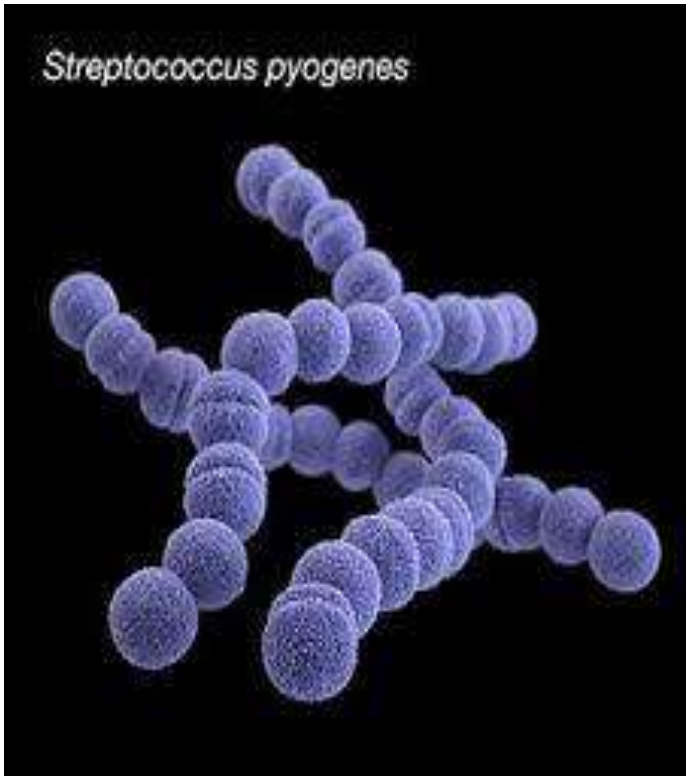
28/ 10/ 2020



Important **tests** are in **bold**. Important ***pathogens*** are in ***bold italics***.
Note: Enterococcus is either α- or γ-hemolytic.

- The streptococci are **gram-positive spherical bacteria that characteristically form pairs or chains during growth.**
- **Some are members of the normal human microbiota; others are associated with important human diseases attributable to the direct effects of infection by streptococci or in other cases to an immunologic response to them.**
- **Streptococci elaborate a variety of extracellular substances and enzymes.**

Streptococcus



Classification oF Streptococci

- The classification of streptococci into major categories has been based on a series of observations over many years:
- (1) Colony morphology and hemolytic reactions on blood agar,
- (2) Serologic specificity of the cell wall group-specific substance (Lancefield antigens) and other cell wall or capsular antigens,
- (3) Biochemical reactions and resistance to physical and chemical factors,
- (4) Ecologic features. Molecular genetics have also been used to study the streptococci.

A. Hemolysis

- Many streptococci are able to hemolyze RBCs in vitro in varying degrees.
- Complete disruption of erythrocytes with clearing of the blood around the bacterial growth is called **β - hemolysis.**
- Incomplete lysis of erythrocytes with reduction of hemoglobin and the formation of green pigment is called **α - hemolysis.**
- Other streptococci are nonhemolytic (sometimes called **γ-hemolysis).**

B. Group-Specific Substance (Lancefield Classification)

- This carbohydrate is contained in the cell wall of many streptococci and forms the basis of serologic grouping into **Lancefield groups A – H** and **K – U.** The serologic specificity of the group specific carbohydrate is determined by **an amino sugar.**

C. Capsular Polysaccharides

- The **antigenic specificity of the capsular polysaccharides** is used to classify ***Streptococcus pneumoniae*** **into more than 90 types** and to **type the group B streptococci (** ***Streptococcus agalactiae*** **).**

D. Biochemical Reactions

- Biochemical tests include sugar fermentation reactions, tests for the presence of enzymes, and tests for susceptibility or resistance to certain chemical agents.
- Biochemical tests are most often used to classify streptococci after the colony growth and hemolytic characteristics have been observed.
- Biochemical tests are used for species that typically do not react with the commonly used antibody preparations for the group-specific substances, groups A, B, C, F, and G. For example, the viridans streptococci are α-hemolytic

Streptococcus pyogenes

- Most streptococci that contain the group A antigen are *S pyogenes.* It is a prototypical human pathogen.

- *S. pyogenes* is the main human pathogen associated with local or systemic invasion and poststreptococcal immunologic disorders.

- *S. pyogenes* typically produces large (1 cm in diameter) zones of β hemolysis around colonies greater than 0.5 mm in diameter. They are usually are susceptible to bacitracin.

Morphology and Identification

- A. Typical Organisms
- Streptococci are gram positive; Most group A strains produce capsules composed of hyaluronic acid.
- The hyaluronic acid capsule likely plays a greater role in virulence than is generally appreciated and together with M protein is believed to be an important factor in the resurgence of rheumatic fever (RF).
- The capsule binds to hyaluronic-acid-binding protein, CD44, present on human epithelial cells

- Capsules of other streptococci (eg, *S agalactiae* and *S .pneumoniae*) are different.
- The *S. pyogenes* cell wall contains proteins (M, T, R antigens), carbohydrates (group specific), and peptidoglycans. Hair like pili project through the capsule of group A streptococci.

- The pili consist partly of M protein and are covered with lipoteichoic acid. The latter is important in the attachment of streptococci to epithelial cells.

Toxins and Enzymes

- **More than 20 extracellular products that are antigenic are elaborated by *S pyogenes*, including the following.**

A. 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, allowing the bacteria to escape from blood clots**. This process of digestion may be interfered with by nonspecific serum inhibitors and by a specific antibody, antistreptokinase. **Streptokinase has been given intravenously for treatment of pulmonary emboli, coronary artery, and venous thromboses.**

B. Deoxyribonucleases (DNAase)

- **Streptococcal deoxyribonucleases A, B, C, and D degrade DNA (DNases)** and similar to **streptokinase facilitate the spread of streptococci in tissue by liquefying pus.**
- Mixtures of streptokinase and DNases are used in **"enzymatic debridement."**
- **They help to liquefy exudates and facilitate removal of pus and necrotic tissue; antimicrobial drugs thus gain better access, and infected surfaces recover more quickly. An antibody to DNAse develops after streptococcal infections (normal limit, 100 units), especially after skin infections.**

C. Hyaluronidase

- **Hyaluronidase** splits hyaluronic acid, an important component of the ground substance of connective tissue.
- Thus, hyaluronidase aids in spreading infecting microorganisms (spreading factor). Hyaluronidases are antigenic and specific for each bacterial or tissue source.
- After infection with hyaluronidase-producing organisms, specific antibodies are found in the serum.

D. Pyrogenic Exotoxins

- **Pyrogenic exotoxins** are elaborated by *S. pyogenes*. There are 3 antigenically distinct **streptococcal pyrogenic exotoxins (Spe): A, B, and C.**
- **SpeA** has been most widely studied.
- It is produced by group A streptococci that carry a lysogenic phage. The **streptococcal pyrogenic exotoxins** have been associated with **streptococcal scarlet fever.**

E. Hemolysins

- The β-hemolytic group A *S pyogenes* **elaborates two hemolysins (streptolysins)** that not only lyse the membranes of erythrocytes but also damage a variety of other cell types.
- **Streptolysin O** is a protein .it combines quantitatively with **antistreptolysin O (ASO),** an antibody that appears in humans after infection with any streptococci that produce streptolysin O.

- This antibody blocks hemolysis by streptolysin O. This phenomenon forms **the basis of a quantitative test for the antibody.**
- **An ASO serum titer in excess of 160–200 units is considered abnormally high and suggests either recent infection with *S pyogenes* or persistently high antibody levels caused by an exaggerated immune response to an earlier exposure in a hypersensitive person**

- **Streptolysin S** is the agent responsible for the hemolytic zones around streptococcal colonies growing on the surface of blood agar plates.
- **It is not antigenic, but it may be inhibited by a nonspecific inhibitor that is frequently present in the sera of humans and animals and is independent of past experience with streptococci.**
- Most isolates of *S. pyogenes* produce both of these hemolysins. About 10% may produce only one.