GRAM-POSITIVE BACTERIA

Universiti Malaysia Kelantan
Faculty of Veterinary Medicine
Bacteriology and Mycology (DVT2064)

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GRAM-POSITIVE BACTERIA

- **Staphylococcus**
- **Streptococcus**
- **Corynebacterium**
- **Rhodococcus**

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The germ theory of disease

- Theory of disease according to Robert Koch (1843-1910), a German scientist

Koch's postulates

- A micro-organism can be accepted as a causative agent of an infectious disease only if the following conditions are satisfied.

The germ theory of disease

1. The micro-organism should be found in every case of the disease and under conditions which explain the pathological changes and clinical features.

2. It should be possible to isolate the causative agent in pure culture from the lesion.

3. When such pure culture is inoculated into appropriate laboratory animal, the lesion of the disease should be reproduced.

The germ theory of disease

4. The micro-organism should be found in every case of the disease and under conditions which explain the pathological changes and clinical features.

5. It should be possible to isolate the causative agent in pure culture from the lesion.

6. When such pure culture is inoculated into appropriate laboratory animal, the lesion of the disease should be reproduced.

7. It should be possible to reisolate the bacterium in pure culture from the lesion produced in the experimental animal.

Corollary to Koch's Postulate

- Specific antibody to the bacterium should be detectable in the serum during the course of the disease.

However, there are exceptions to Koch's postulate

Exceptions to Koch's postulates

1. Many healthy people and animals carry pathogens but do not exhibit symptoms of the disease.

2. Some microbes are very difficult or impossible to grow in vitro(in the laboratory) in artificial media. Eg. Treponema pallidum

3. Many species are species specific. Eg. *Brucella abortus* cause abortion in animals but no report in humans.

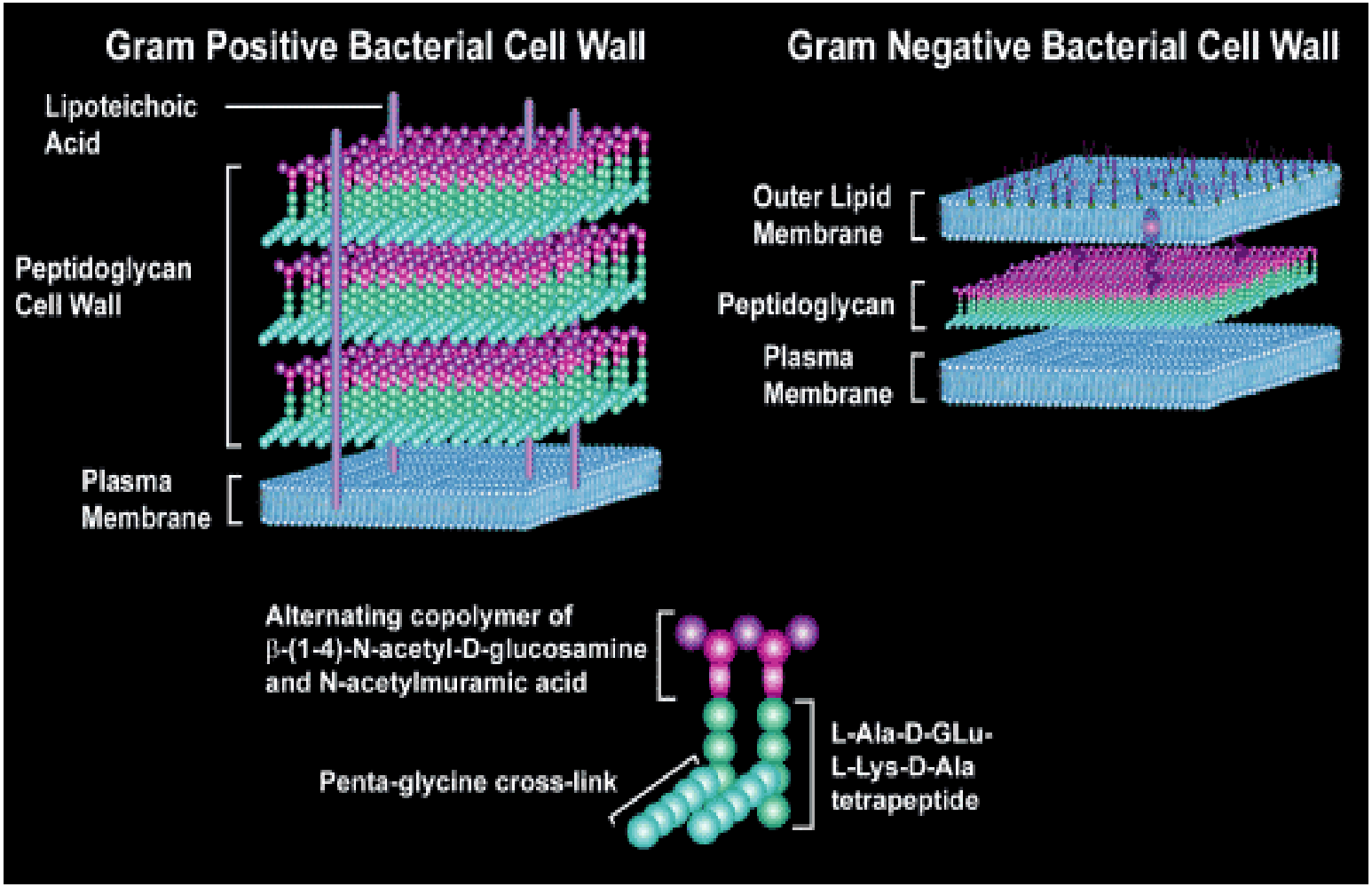
4. Certain diseases develop only when an opportunistic pathogen invades an immunocompromised host.

Gram-Positive Bacteria

- Have thick outer matrix composed of up to 90% of peptidoglycan layer
- The cell walls of gram-positive bacteria are made up of twenty times as much murein or peptidoglycan than gram-negative bacteria.
- The thick outer matrix of peptidoglycan, teichoic acid, polysaccharides, and other proteins serve the bacteria in many aspects

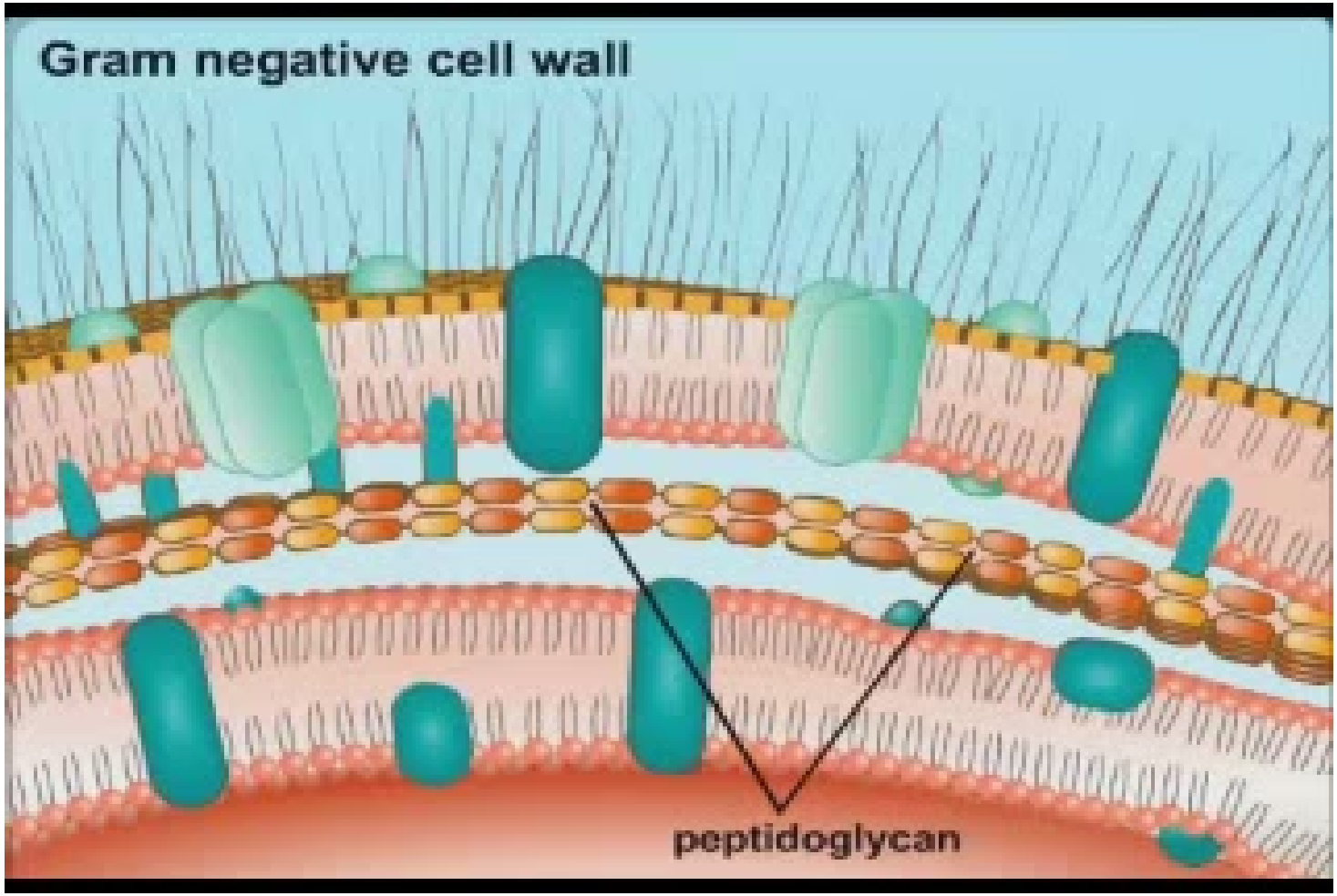
Gram-Positive Bacteria

- The Peptidoglycan Layer



Gram-Positive Bacteria

The Peptidoglycan Layer



Functions of Bacterial Cell Wall

- Provides shape to the bacterium
- Gives rigidity to the organism
- Protects the bacteria from environment
- Provides staining characteristics to the bacterium
- Contains receptor sites for phages/complements
- Site of action of antibody and colicin
- Contains toxic components to host

STAPHYLOCOCCUS

From Greek,

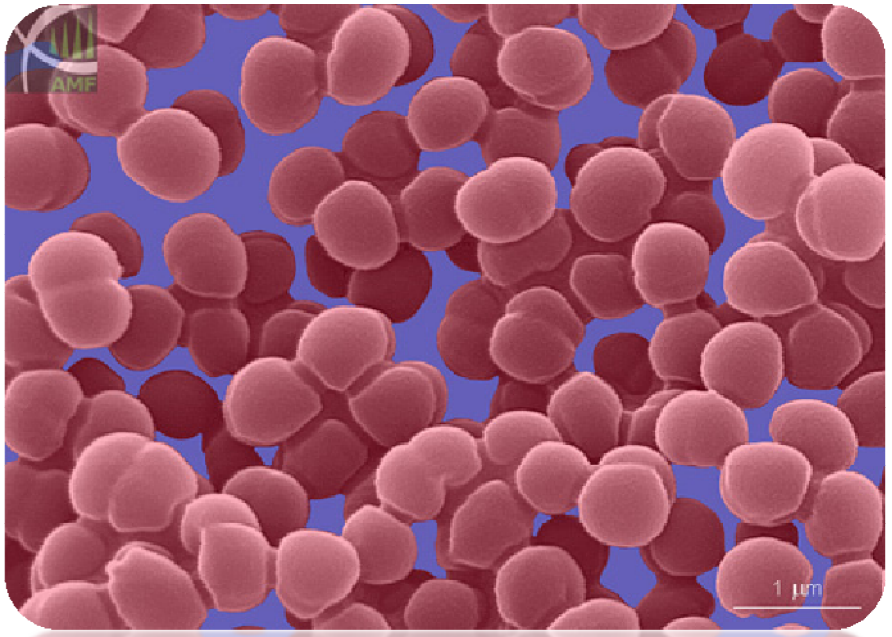
- *Staphyle*—grape

and

- *Kokkos*—granule

STAPHYLOCOCCUS

Characteristics



- Gram-positive bacteria
- About 1 µm in diameter
- Oxidase-negative
- Catalase-positive
- Non spore-forming
- Non-motile
- Facultative anaerobes
- Spherical cells usually arranged in grape-like clusters
- Single cocci , pairs, tetrads and chains are seen in liquid cultures

STAPHYLOCOCCUS

Characteristics

- Can readily grow in ordinary media under aerobic and microaerophilic conditions
- grow most rapidly at 37 ^{o}C but form pigment best at room temperature of 20-25 ^{o}C
- Colonies in solid media are round, smooth, raised and glistening.
- Relatively resistant to drying , heat, and 9% NaCI, but readily inhibited by 3 % hexachlorophene

STAPHYLOCOCCUS

Characteristics

- Common commensals on skin and mucous membrane
- Often cause pyogenic infections
- Pathogenic species often produce coagulase
- Important animal pathogens include *S. aureus*, *S. intermedius*, *S. hyicus*
- *S. aureus and S. intermedius are coagulase positive while S. hycus is coagulase variable*
- Coagulase negative staphylococcus are less virulent but may occasionally cause disease in animals and man

STAPHYLOCOCCUS

Antigenic structure:

- **Peptidoglycan(Mucopeptide):**
  - Is important in the pathogenesis of infection like eliciting production of cytokines and opsonic antibodies
  - Chemoattractant for polymorphs
  - Activate complement
- **Teichoic acid**: Polymer of glycerol or ribitol phosphate
- **Protein A**: Important in immunologic diagnostic test (coagglutination test).
- **Capsule**: Anti-phagositic property

STAPHYLOCOCCUS

Antigenic structure:

- **Enzymes**
  - ***Catalase***- Produced by staphylococci
    - Converts H_20_2 into H_20 and 0_2
  - ***Coagulase***
    - Clots oxidated or citrated plasma
    - Coagulase may deposit fibrin on the surface of organism and alter ingestion by phagocytic cells
  - ***Clumping factor***
    - A surface compound that is responsible for adherence of the organism to fibrinogen and fibrin

STAPHYLOCOCCUS

Antigenic structure:

- **Enzymes**
  - ***Hyaluronidase***
    - Spreading factor
  - ***Proteinases and lipases***
  - ***Staphylokinase***
    - Fibrinolysin
  - ***β-lactamase***
    - Provides resistance of staphylococcus to β-lactam antibiotic like penicillin.
  - ***Dnase***: Deoxyribonucleotidase
  - ***Nuclease***

STAPHYLOCOCCUS

Antigenic structure:

- ***Toxins***
  - Exotoxins(α, β, γ, δ)
    - Multiple soluble heat-stable, gut enzyme resistant toxins
    - Cause staphylococcal food poisoning
  - Toxic shock syndrome toxin (TSST)
    - Superantigen desquamative toxin Produced by *S.aureus* and Causes fever, shock, multiple-organ failure and skin rash.

STAPHYLOCOCCUS

Antigenic structure:

- Toxins
  - ***Exfoliative toxin***
    - Epidermolytic superantigen
    - Causes generalized desquamation of the skin (staphylococcal scalded skin syndrome).
  - ***Epidermolytic toxin A:***
    - Chromosomal gene product and heat stable
  - ***Epidermolytic toxin B***:
    - Plamid mediated and heat labile
  - ***Leukocidin:***
    - S aureus toxin which kills WBCs by forming pores and incresing cation permeability

STAPHYLOCOCCUS

Diseases in animals

- Exudative epidermitis in piglets (greasy-pig diseases): *S. hyicus*
- Tick pyaemia of lambs : *S. aureus*
- Bovine staphylococcal mastitis: *S. aureus*
- Botryomycosis (Scirrhous cord) (horse, pigs, cattle): *S. aureus*
- Wound infection (most animals): *S. aureus, S. hyicus, S. intermidus*
- Mastitis: *S. aureus, S. hyicus, S. intermedius*
- Bumble foot, omphalitis in poultry: *S. aureus*
- Pyoderma, otitis externa, cystitis, endometritis in dogs: *S. intermedius*

STAPHYLOCOCCUS

Laboratory Diagnosis

- ***Specimen***: Surface swabs, pus, blood, sputum, cerebrospinal fluid, exudates, milk
- ***Smear***: Gram positive cocci in clusters, singly or in pairs.
- ***Culture***: Grow well aerobically and in a CO_2 enriched ordinary media at an optimal temperature of 35^{o}C-37^{o}C.
- ***Media***: Non-enriched media (Nutrient agar, blood agar)
- ***Selective medium***: mannitol salt agar (contains 7-10% NaCl)

STAPHYLOCOCCUS

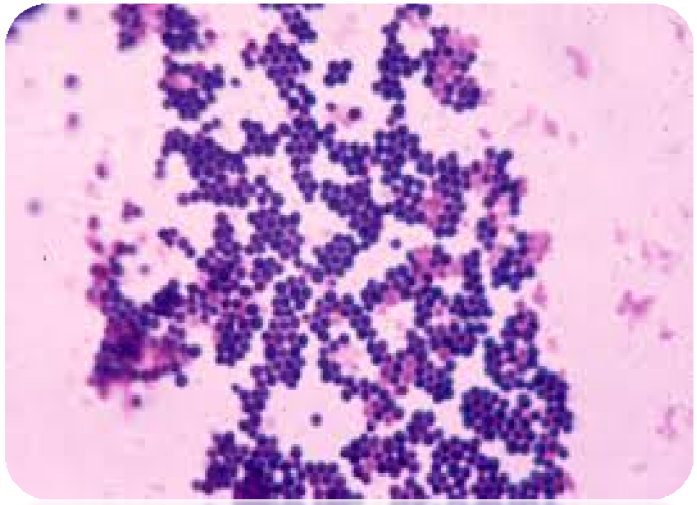
Laboratory Diagnosis

Colony Morphology

- Colour: usually white, opaque and up to 4mm in diameter.
  - Colonies of bovine and human strains of *S. aureus are golden yellow.*
  - *Saprophytic staphylococcus* may be pigmented
- Staphylococcus may produce haemolysis on sheep/ox blood agar. Types of haemolysis (α, β, γ, Δ)
- *S. aureus and S. intermedius produce double zones of narrow complete and wide* incomplete haemolysis on blood agar
- *S. hyicus is non-haemolytic*

S. aureus on BA

S. aureus on MSA



S. aureus on Gram-staining

MRSA on ORSAB

STAPHYLOCOCCUS

Laboratory Diagnosis

Biochemical Tests

Species	Colony colour	Haemolysis on sheep BA	Tube coagulase	Slide coagulase	Acetone production	Maltose utilization
S. aureus	Golden yellow	+	+	+	+	+
S. intermedius	White	+	+	V	-	±
S. hyicus	White	-	V	-	-	-

STAPHYLOCOCCUS

Laboratory Diagnosis

Biochemical Tests

Organism	Appearance of stained smear	Coagulase	Catalase	Oxidase	O-F test	Bacitracin test
Staphylococcus spp	Irregular cluster	±	+	-	F	Resistant
Micrococcus spp	Packets of four	-	+	+	O	Susceptible
Streptococcus and enterococcus spp	Chain	-	-	-	F	Resistant

STAPHYLOCOCCUS

Treatment

- Penicillin sensitive staphylococci……penicillin, ampicillin
- Penicillin resistant staphylococci……cloxacillin, Nafcillin
- Methicillin resistant staphylocicci…… Vancomycin

STREPTOCOCCI

General Characteristics

- Non-motile, non-sporulating, gram- positive facultative anaerobes
- Spherical or oval cells characteristically forming pairs or chains during growth
- Oxidase negative
- Catalase-negative
- Grow well on ordinary solid media enriched with blood, serum or glucose.
- Most streptococci grow in solid media as discoid colonies
- Capsular streptococcal strains give rise to mucoid colonies

STREPTOCOCCI

General Characteristics

- Streptococci are fragile and susceptible to desiccation
- Widely distributed in nature and are found in upper respiratory tract, gastrointestinal tract and genitourinary tract as normal microbial flora
- They are heterogeneous group of bacteria

STREPTOCOCCI

Classification

Classification of Streptocci based on:

1. Colony morphology and hemolytic reaction on blood agar
2. Serologic specificity of the cell wall group specific substance and other cellwall capsular antigens
3. Biochemical reactions and resistance to physical and chemical factors
4. Ecologic features

STREPTOCOCCI

Classification

Hemolytic reaction of streptococci:

Hemolysis	Appearance	Designation	Example
Complete	Clear, colourless, sharply defined zone	Beta(β)	S. pyogenes
Partial	Greenish discoloration (reduced hemoglobin)	Alpha(α)	Viridans streptococci
None	No change	Gamma(δ)	Enterococci

STREPTOCOCCI

Classification

Lancefield grouping of streptococci

- Streptococci produce group specific carbohydrates(C carbohydrates) identified using group specific antiserum.
- It is designated A-H and K-V. The clinically important streptococci are grouped under A,B,C,D,F and G.
- The main species and groups of medical importance are:
  - S. pyogenes…….. Lancefield group A
  - S. agalactiae…….. Lancefield group B
  - Enterococci……Lancefield group D

STREPTOCOCCI

Antigenic structure:

- ***Group-specific cell wall antigen***
  - Streptococcal cell wall carbohydrate is the basis for serologic grouping of streptococci (Lancefield groups A-H, K-U)
- ***M protein***
  - They are found in hair-like projections of the streptococcal surface and determine virulence
  - Major virulent factor for group A streptococci
  - There are two major structural classes of M protein(class I &class II)

STREPTOCOCCI

Antigenic structure:

- More than eighty serotypes of M protein of group A streptococci

- **T substance:**

  – Acid and heat labile unlike M protein, and has no relation to virulence of streptococci.

STREPTOCOCCI

Toxins and enzymes produced by Streptococcus pyogenes (Group A β-hemolytic streptococci)

S. Pyogenes

- The most pathogenic member of the genus
- It is present as a commensal in the nasopharynx in a variable proportion of healthy individuals.

Toxins and enzymes

- Streptokinase: Fibrinolysin
  - An active proteolytic enzyme which lyses fibrin by catalytic conversion of plasminogen to plasmin
  - For IV treatment of pulmonary edema and arterial and venous thrombosis

STREPTOCOCCI

Toxins and enzymes produced by Streptococcus pyogenes (Group A β-hemolytic streptococci)

Toxins and enzymes

- ***Streptodornase***: Streptococcal deoxyribonuclease
  - Mixtures of streptokinase and streptodornase are used in “enzymatic debridement”
- ***NADase***: Nicotinamide adenine dinucleotidase
- ***Hyaluronidase***: Spreading factor
  - It degrades the ground substance of connective tissue (hyaluronic acid)

STREPTOCOCCI

***Toxins and enzymes produced by Streptococcus pyogenes* (Group A β-hemolytic streptococci)**

Toxins and enzymes

- ***Hemolysins***:
  - Two types :Streptolysin O and Streptolysin S
- ***Erythrogenic toxin***: Pyrogenic exotoxins
  - It is responsible for the erythematous rash in scarlet fever.

STREPTOCOCCI

Pathogenic Streptococci

Bovine streptococcal mastitis

- *S. agalactiae*
- *S. dysgalactiae*
- *S. uberis*

} principal pathogens of mastitis

- *Enterococcus faecalis*
- *S. pyogenes*
- *S. zooepidemiccos*

} less associated with mastitis

STREPTOCOCCI

Pathogenic Streptococci

- Strangles in horses: *S. equi*
- Abscess and other suppurative conditions and septicaemia in many species of animals:
  - *S. pyogenes: humans*
  - *S. canis: dogs*
  - *S. suis: pigs*
  - *S. equisimilis : horses*

STREPTOCOCCI

Diagnosis

- History, clinical signs and pathology may be indicative of streptococcal infection

- Samples are collected and cultured promptly: streptococcal are highly susceptible to desiccation. Samples include pus and exudates

- Samples can be placed in transport medium

- Stained smear of clinical samples may reveal gram-positive cocci in chains

STREPTOCOCCI

Diagnosis

- Samples should be cultured on blood agar and MacConkey agar
- Incubate agar plates aerobically at 37^{o}C for 24-48 hours
- Streptococcal colonies are small, translucent and some may be mucoid
- Streptococci are differentiated based on:
  - Type of haemolysis
  - Lancefield grouping
  - Biochemical testing

STREPTOCOCCI

Diagnosis

A. Types of haemolysis on blood agar

- Beta-haemolysis: complete haemolysis of clear zones around colonies

- Alpha-haemolysis: partial, incomplete haemolysis, greenish or hazy zones around colonies

- Gamma-haemolysis: no observable changes in the blood agar around colonies

STREPTOCOCCI

Diagnosis

B. ***Lancefield grouping***: A serological method of classification based on the group-specific C substance Lancefield grouping. Includes:

- ***Ring specification test***
  - Extract C-substance by acid or heat from the *Streptococcus spp*
  - The extract (antigen) is layered over antisera of different specificities in narrow tubes placed in plasticine on slide
  - A positive reaction is indicated by the formation of a white ring of precipitate close to the interface of the two fluids within 30 minutes

STREPTOCOCCI

Diagnosis

B. ***Lancefield grouping***: A serological method of classification based on the group-specific C substance Lancefield grouping. Includes:

– ***Latex agglutination test***: latex-coated group-specific antibodies are commercially available for the test.

- Antigen is extracted enzymatically from the *streptococcus spp*
- Mix antiserum and antigen together on a slide
- Positive reaction is indicated by agglutination

STREPTOCOCCI

Diagnosis

C. Biochemical tests: oxidase, catalase, sugar fermentation tests. Biochemical tests are available commercially for rapid identification of streptococci

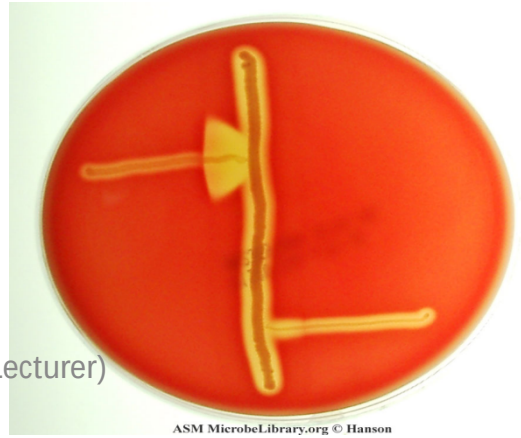
Species	Trehalose	Sorbitol	Lactose	Maltose
S. equi	-	-	-	+
S. zooepidemicus	-	+	+	(+)-
S. equisimilis	+	-	V	+

STREPTOCOCCI

Diagnosis

Differentiation of streptococci associated with mastitis:

- *S. pyogenes: Bacitracin sensitive (all group A)*
- *S. agalactiae: CAMP test positive with S. aureus and Corynebacterium pseudotuberculosis (all group B Streptococci)*



STREPTOCOCCI

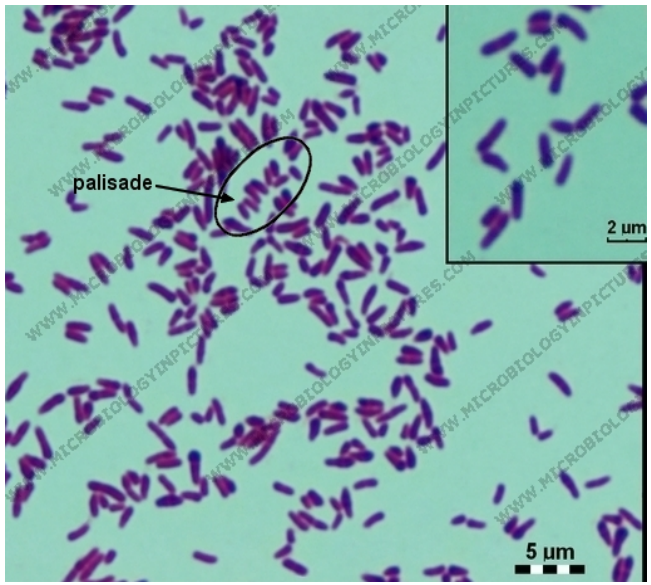
Diagnosis

Differentiation of streptococci associated with mastitis:

- *S. uberis: Aesculin hydrolysis (black brown zones of discolouration around dark coloured* colonies on Edward's medium).
- *S. pneumoniae: Capsule swelling test, bile solubility, Optorchin-sensitivity,*

CORYNEBACTERIUM

- Small Gram-positive pleomorphic (coccoid, club and rod forms) bacteria
- Stained smear reveals cells in palisades of parallel and angular clusters resembling Chinese letters
- Non-motile facultative anaerobes
- Catalase-positive, oxidase-negative
- Fastidious, require enrichment for growth
- Cause pyogenic infection
- Most pathogenic species are host specific
- Type species: *C. diptheriae, causes diphtheria in children*



CORYNEBACTERIUM

Diseases

- *Corynebacterium bovis*
  - Host (cattle): subclinical mastitis
- *C. kutscheri*
  - Host (laboratory rodents): superficial absceses, causes purulent foci in liver, lungs and lymph nodes
- *C. pseudotuberculosis (non-nitrate-reducing biotype)*
  - Host (Sheep and goats): caseous lymphadenitis
- *C. pseudotuberculosis (nitrate-reducing biotype)*
  - Host (horses, cattle): ulcerative lymphagitis, abscesses

CORYNEBACTERIUM

Diseases

- *C. renale (type I)*
  - Cattle: cystitis, pyelonephritis
  - Sheep and goats: ulcerative (enzootic) balanoposthitis
- *C. pilosum (renale type II)*
  - Cattle: cystitis, pyelonephritis
- *C. cystitides (renale type III)*
  - Cattle: severe cystitis, rarely pyelonephritis
- *C. ulcerans*
  - Cattle: mastitis

CORYNEBACTERIUM

Diagnosis

- Specimen: pus, exudates, tissue, sample, mid-stream urine
- Direct microscopy of Gram-stained smear may reveal coryneform bacteria
- Inoculate sample onto blood agar, selective media (McLeod's blood agar, Loeffler's medium) containing potassium tellurite, and MacConkey agar
- Incubate aerobically at 370C for 24 to 48 hours
- Identification: no growth on MacConkey agar

CORYNEBACTERIUM

Diagnosis

Colonial Characteristics:

- *C. bovis: a lipophilic bacterium. Small white, dry, non-haemolytic colonies*
- *C. kutscheri: whitish colonies, occasionally haemolytic*
- *C. pseudotuberculosis: small whitish colonies, surrounded by a narrow zone of* complete haemolysis evident after 72 hours of incubation. Colonies become dry, crumbly and cream-coloured with age
- Members of *C. renale group produce small, non-haemolytic colonies after 24* hours incubation. Produce pigment after 48 hours of incubation

CORYNEBACTERIUM

Diagnosis

Biochemical tests

- Nitrate reduction: *C. pseudotuberculosis biotype*
- All pathogenic corynebacteria are urease positive except *C. bovis*
- Enhanced haemolysis by *C. pseudotuberculosis when inoculated across a streak of Rhadococcus equi*

CORYNEBACTERIUM

Diagnosis

Differentiation of C. renale group

Feature	*C. renale* (type I)	*C. pilosum* (type II)	*C. cystidis* (type III)
Colour of colony	Pale yellow	Yellow	White
Growth in broth at pH 5.4	+	-	-
Nitrate reduction	-	+	-
Acid from xylose	-	-	+
Acid from starch	-	+	+
Casein digestion	+	-	-
Hydrolysis of Tween 80	-	-	+

RHODOCOCCUS

- Gram-positive aerobic bacteria
- Non-motile catalase-positive, oxidase-negative
- Weakly acid fast
- Grows on non-enriched media
- Rod or coccibacillus in shape
- Produces pigments, colonies are pink
- It forms capsule. Produces large, moist, viscid/mucoid colonies
- Important spp. R. equi

RHODOCOCCUS

Diseases

- Foals (1 - 4 months of age): suppurative bronchopneumonia and pulmonary abscessation
- Horse: superficial abscessation
- Pigs, Cattle: mild cervical lymphadenopathy
- Cats: subcutaneous abscesses, mediastinal granulomas

RHODOCOCCUS

- Specimens: tracheal aspirates, pus from lesion
- Inoculate blood and MacConkey agar
- Incubate aerobically at 37 oC for 24 to 48 hours
- No growth on MacConkey
- Does not ferment carbohydrate
- Does not haemolyse on blood agar. It is CAMP test positive. (enhanced haemolysis) with *S. aureus*
- Most strains are urease and H_2S positive