GRAM POSITIVE AND GRAM NEGATIVE COCCI

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Nomenclature:

- Naming of bacteria according to accepted international rule (International Code of Nomenclature of Bacteria1992).
- Only one official name comprising of two parts.

First part Generic name.

Second part Species name.

• Latin or Latinized

Italicized

- Generic name capital, species small letter
- Example: *Staphylococcus aureus*

Classification

- Rigid thick walled.
- Flexible thin walled.
 Spirochetes Treponema, Borrelia & Leptospira
- Wall less (Mycoplasma).

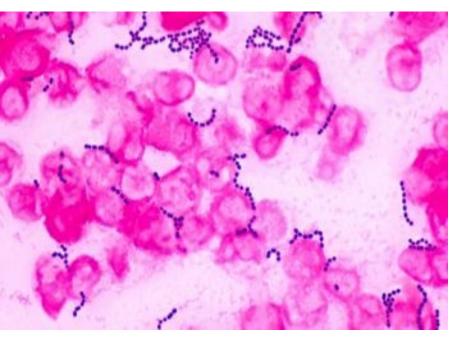
Rigid thick walled

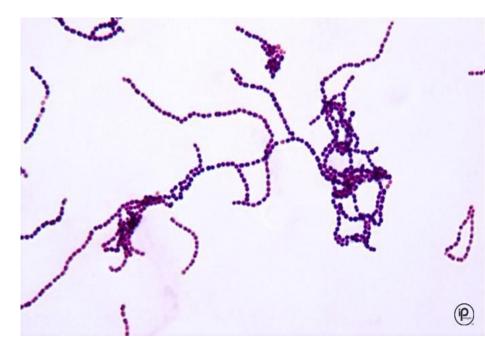
 <u>Cocci</u>: Gram Positive (Staphycococcus & Streptococcus) Gram Negative (Neisseria) (Meningitidis &

Staphylococci: aureus & epidermidis

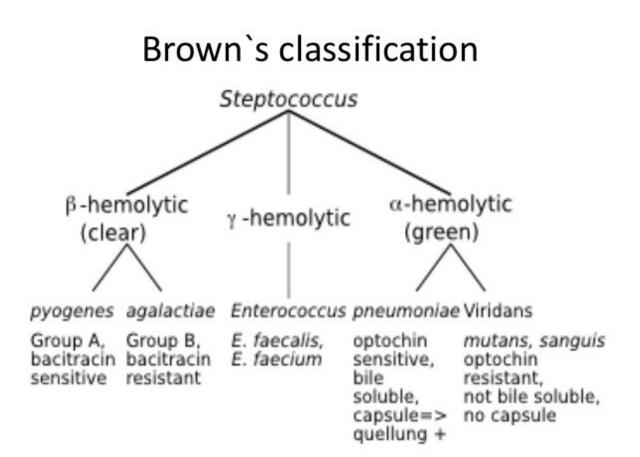


Streptococci (GPC in chains)



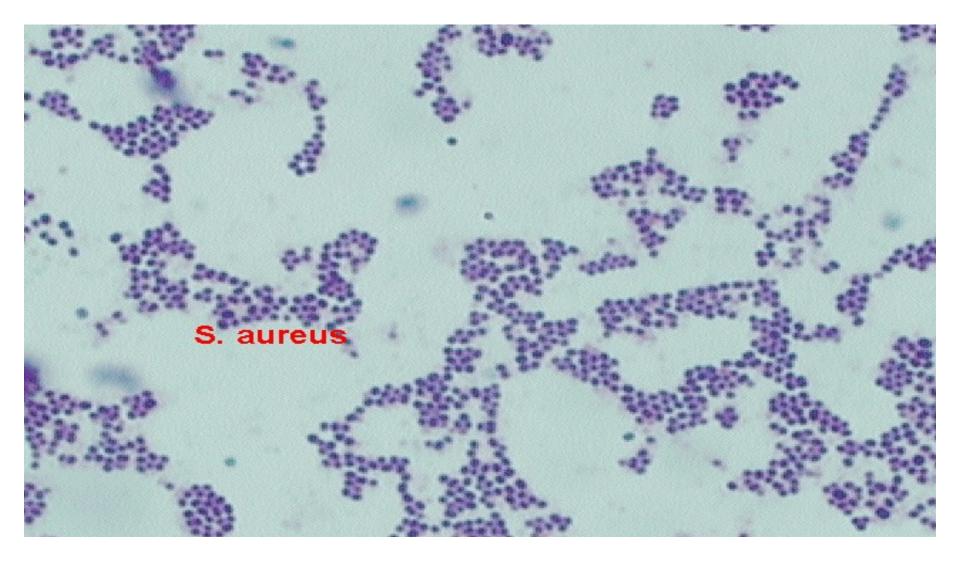


Streptococci



Staphylococcus

- Family: Staphylococcaceae
- Genus: Staphylococcus
- GRAM+VE
- Non-motile, Non sporing
- Arranged in irregular grape like clusters
- Catalase + ve



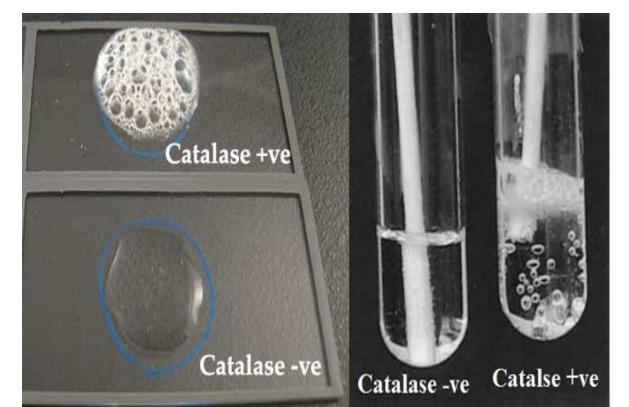
Catalase Enzyme test

- Staphylococci are catalase positive
- Streptococci are catalase negative



Catalase Enzyme test





DNase Test

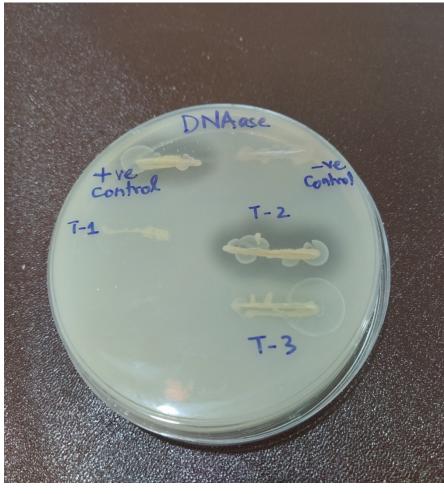
Deoxyribonuclease (DNase) Test: Principle:

DNA	DNase enzyme	Nucleotides
Insoluble		soluble
In acid		In acid

Procedure:

- 1. Inoculate DNase agar plate with the test organism.
- 2. Incubate the plate at 35oC for 24 hrs.
- 3. Flood the plate with 1M HCl.



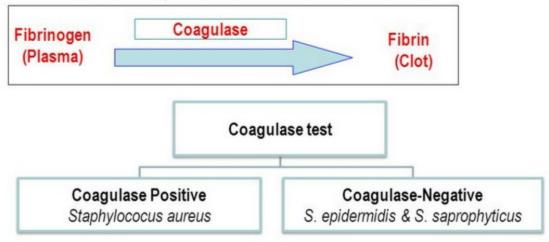


Coagulase enzyme test

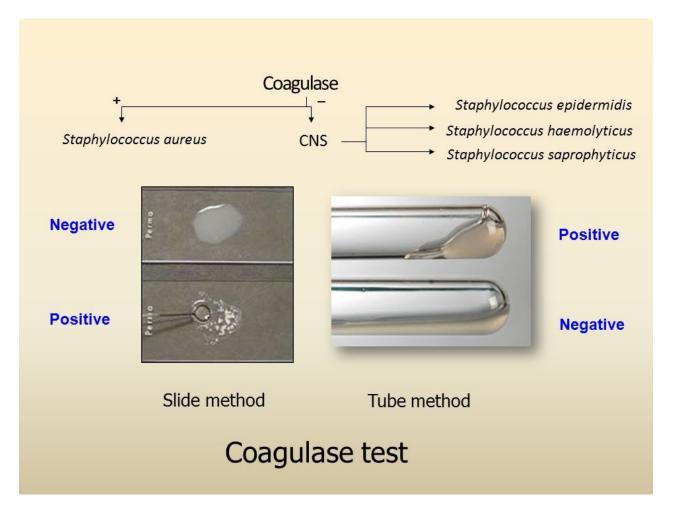
Coagulase Test

Principle:

- This test is used to differentiate between S. aureus (CPS) & other Staphylococcus species (CNS)
- · This test is done by tube method or slide method



Coagulase enzyme test



Coagulase enzyme test

Coagulase Test

This test is used to identify the *Staphylococcus aureus*, which produces the enzyme coagulase

- Principle
 - Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *Staphylococcus aureus*
 - Free coagulase which converts fibrinogen to fibrin by activating a coagulase reacting factor present in plasma. Free coagulase is detected by clotting in the tube test
 - Bound coagulase (clumping factor) which converts fibrinogen directly to fibrin without requiring a coagulase reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test

• Coagulase Negative

White colonies Usually non pathogenic Divided into 2 groups

Staph. epidermidis group

Staph epidermidis, S. hominis

S. capitis, S. auricularis

S. haemolyticus, S. simulans

• Staph. Saprophyticus U.T.I in young females

Uncomplicated cystitis

Novobiocin resistant

Novobiocin susceptibility

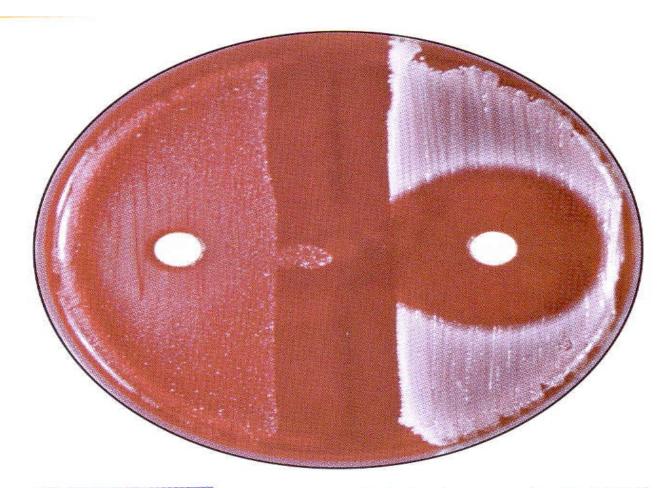


FIGURE 5-62 Novobiocin disk test on a sheep blood agar plate. *Staphylococcus saprophyticus* (R) is on the left: *Staphylococcus epidermidis* (S) is on the right.

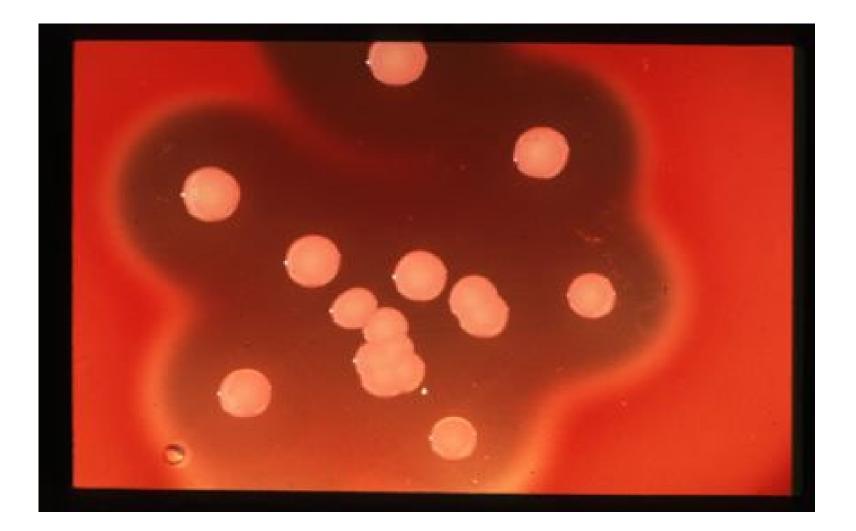
Staphylococcus aureus

- <u>Culture:</u>
- Can grow easily on ordinary media, not fastidious
- Optimum temp 35C° (15—40C°)
- Facultative anaerobe, good growth under aerobic conditions.

Media:

 Nutrient agar, Blood agar, glucose broth.
 Colonies: round 1—2 mm raised, convex, opaque, Smooth shiny surface, Soft, Golden yellow pigment
 Beta haemolysis on blood agar

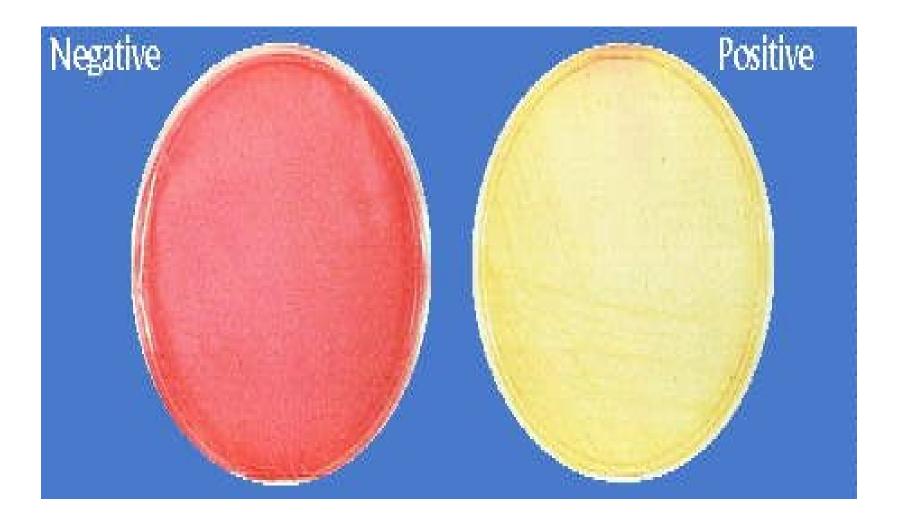
Beta haemolysis on blood agar



• Mannitol salt agar

Used as selective medium to isolate from samples having mixed flora

Mannitol Salts Agar (MSA)



Biochemical Reactions

- Catalase +ve
- Oxidase -ve
- coagulase+ve
- Liquify gelatin
- Nitrates [] Nitrites

<u>Viability</u>

- Remain alive for months on agar media at room temp
- Resistant to usual antiseptics & antibiotics
- Methicillin resistant staphylococcus aureus (MRSA)

<u>Toxins:</u>

- 1. Haemolysins. (alpha delta) (α ß, γ , δ)
- 2. Leucocidin
- 3. Epidermolytic toxin
- 4. Toxic shock syndrome toxin -1 (TSST-1)
- 5. Enterotoxin

Enzymes:

1. Coagulase

2. Hyaluronidase

3. Staphylokinase

4. Geletinase

- 5. Protease
- 6. Lipase
- 7. Penicillinase
- 8. DNase
- 9. Catalase

Toxins:

- **. Haemolysins**. (Alph- delta) (α ß, γ , δ)
- **α haemolysin** produces pores in cell membrane of red cells and lyses them.
- It is responsible for zone of beta haemolysis seen around S. aureus colonies
- It is dermo necrotic on subcutaneous injection
 It is also potent neurotoxin

- **B haemolysin** is a sphingomyeilnase which acts on sphingomyeiln and causes lyses of RBCs.
- Y, δ also cause lyses of RBCs acting as surfactants

• Leucocidin

- This toxin forms pores in cell membrane which alter cellular permeability
- It causes swelling and lyses of polymorphs having direct toxic effect
- \succ it also causes degranulation of cytoplasm

- Epidermolytic toxin (Exfoliative toxin)
- This toxin has proteolytic activity and dissolves mucopolysaccharide matrix of epidermis resulting in intra epithelial splitting of cellular links in stratum granulosum
- It results in blistering and generalized desquamation Painful rash appears and areas if skin slough off in small children

- Toxic shock syndrome toxin -1 (TSST-1)
- Same as enterotoxin F.
- ➢ It is a superantigen stimulating release of large amounts of IL-1,IL-2 and TNF
- It causes extensive rash, shock and

multisystem involvement

• Enterotoxin

There are six types A---F

➢ Food poisoning

 \succ It is a superantigen.

Heat stable and resists action of gut enzymes

➢ Vomiting and diarrhoea without fever occur as a result of ingestion of preformed toxin

Enzymes:

<u>Coagulase</u>

- Indicator of pathogenicity
- Lays down fibrin barrier which is impermeable to leucocytes
- Gives the organisms protection from phagocytosis
- Fibrin layer gives protection even if ingested by leucocytes.

- <u>Hyaluronidase (spreading factor)</u>
- Brings about destruction of hyaluronic acid, the ground substance matrix of connective tissue.
- It spreads the infection to adjacent tissues

- Staphylokinase
- This enzyme activates plasminogen to plasmin
- It is fibrinolytic and fibrin clot is broken down allowing spread of infection

• <u>Geletinase</u>

Brings about liquefaction of gelatin

Protease

- It has proteolytic properties
- It produces rapid necrosis of tissues including bone

• <u>Lipase</u>

- It is lipolytic activity
- It helps spread the organisms in cutaneous and subcutaneous tissues

<u>.Penicillinase (Beta lactamase)</u>

It acts by opening up Beta lactam ring of Penicillins causing resistance to these antibiotics

- <u>DNase (deoxyribonuclease)</u>
- Indicator of pathogenicity
- Destroys DNA

<u>Catalase</u>

- It inactivates toxic hydrogen peroxide and free radicals
- It provides protection to the organisms after ingestion by polymorphs

Antigenic structure

- 1. Capsule
- 2. Peptidoglycan
- 3. Teichoic acid
- 4. Protein A

- <u>Capsule</u>
- It prevents ingestion of organisms by polymorphs
- It may also promote adherence to the host cells and prosthetic devices

• <u>Peptidoglycan</u>

- It acts like endotoxin
- It is chemotactant for neutrophils
- It stimulates complement & coagulation cascade

.Teichoic acids

- Unique to staphylococci
- Helps adherence to mucosal surfaces

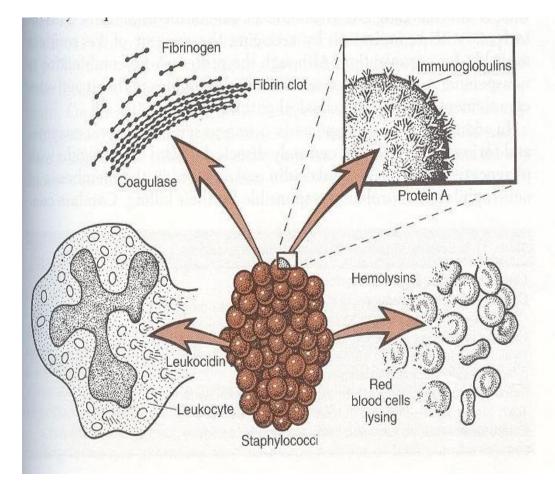
• <u>.Protein A.</u>

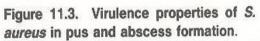
▶ Major protein in the cell wall

\succ It binds to the FC portion of IgG at the

complement binding site, thereby preventing the activation of complement

Phagocytosis is greatly reduced





Transmission:

- Direct, Hand Contact
- Air borne

Endogenous

Pathogenesis:

- Nose- Natural home of staph aureus-40 to 50% normal human nasal carriers
- 5-10% persons skin carriers especially axilla & perinium
- Found on clothing, bed linen & furniture
- Also found as normal flora of Resp and Gastrointestinal tract.

- Invasion after adhesion
- Multiplication
- Toxins & enzymes
- Inflammation Acute
- Repair

<u>Pathology</u>

- Localised Abscess:
- Hair follicles involved
- Tissue necrosis
- Fibrin around lesion
- Accumulation of inflammatory cells-mostly neutrophils
- Fibrous tissue

- Liquifaction of lesion at centre-Abscess
- Pointing in direction of least resistance
- Filling of cavity with granulation tissue
- Spread through lymphatics/ blood
- Healing

Infections

- Skin lesions
- Abscess
- Pustules
- Boils
- Carbuncles
- Impetigo

- Folliculitis
- Paronychia
- Styes
- Blephritis

Metastatic Infections

- Bacteremia
- Osteomyelitis disease of growing bone
- Pulmonary and cardiovascular infection

<u>Diagnosis</u>

- <u>Specimens:</u>
- Pus, Sputum, Faeces/ Vomitus, Blood, Urine, CSF, Aspirate
- Culture, Nutrient/ Blood Agar
- Staining Gram+ve
- Catalase+ve
- Mannitol+ve

- Coagulase+ve
- DNase+ve
- SEROLOGY—No particular value
- Phage typing- For epidemiology
- Nasal/ Perineal Swabs For carriers

Coagulase Negative staphyloccoci (CONS)

- A large group of related species of staphylococci
- Colonies are non pigmented, white in colour
- Coagulase negative
- DNase negative

Common contaminant of clinical specimens

Opportunistic pathogens

Produce no toxins

Secrete an exopolysaccharide slime called extra cellular slime substance (ESS)

- ESS forms a biofilm on plastic surface and bind to fibronectin of plastic catheters and canulae
- The biofilm protects organisms from antimicrobical agents and other immune mechanisms
- Adherent bacteria persist on foreign bodies and evade host defenses

Cause Infections of

1. Indwelling devices

2. Intravenous catheters

3. Haemodialysis shunts

4. CSF shunts

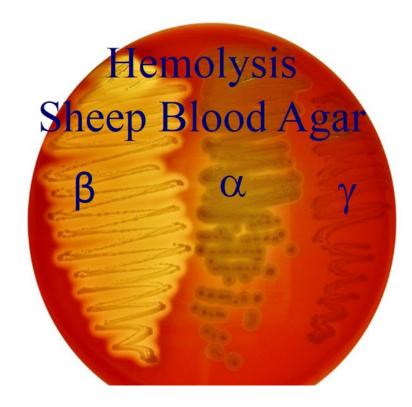
5. Peritoneal dialysis catheters

6. Pace maker wires/electrodes

7. Prosthetic joints, cardiac valves

8. Vascular grafts

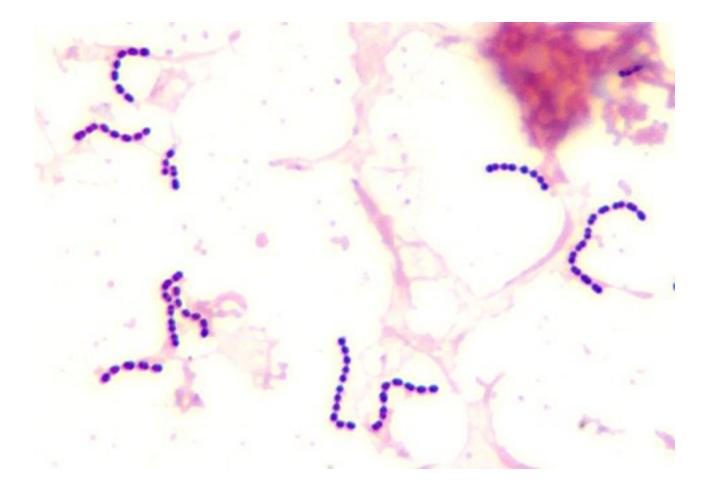
9. Breast implants



<u>Streptococcus</u>

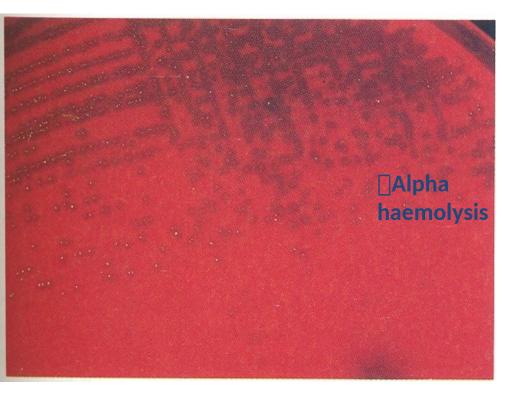
- Family: Streptococcaceae
- Genus: 1) Streptococcus
- 2) Enterococcus
- Wide distribution in nature
- Found in milk, dairy products, water, dust.
- In man & animals found in throat, mouth, intestines, genital tract.

- Spherical to ovoid
- .5—1µ in size
- Occur in chains
- Catalase—ve
- Fastidious in growth (Blood)
- Facultative anaerobe/ Anaerobe

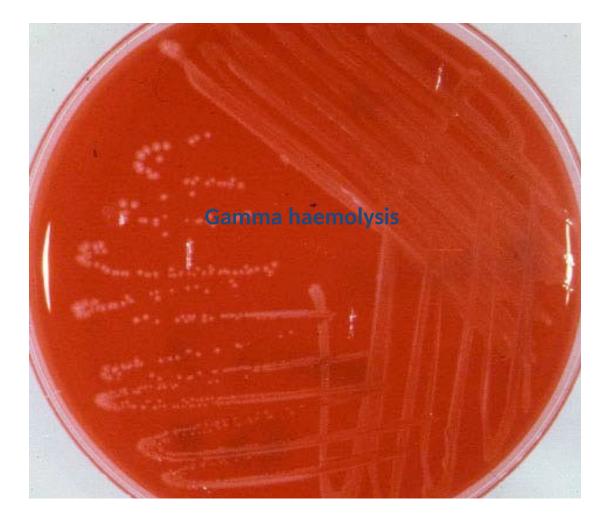


Classification

- 1. By Haemolysis
- 2. By Serology
- **By Haemolysis:**
- β heamolytic
- α haemolytic
- y haemolytic (Non haemolytic)







By Serology

1. Lancefield groups A—H & K—U

Specific C.hydrates. (C. substance)

2. Griffith types of group 'A' (M. proteins)

M. Protein

- The most important virulence factor
- Determines the serotype of S. pyogenes
- It protrudes from the outer surface of the cell and interferes with ingestion by phagocytes
- There are approximately 80 serotypes based on the M protein

<u>1.Haemolytic group.(β haemolysis)</u>

- Strept. pyogenes A
- Strept. agalactiae B
- Strept. equi C
- Strept. canis G
- Strept. porcinus E
- Group L spp L

<u>Viridans Group (α / γ)</u>

- Strept. sanguis H
- Strept. milleri F (C G)
- Strept. salivarius K
- Strept. mitior O (K,M)
- Strept. lactis N

Enterococci

- E. feacalis D
- E. faecium D
- E. durans D

Lancefield grouping: Streptococci



Classification - Lancefield

Lancefield realized that all species in each "group" generally (and conveniently) shared clinically significant properties such as type of hemolysis, normal host, body system or tissue where indigenous, etc. For example:

- Group A S. pyogenes: human upper respiratory
- Group B S. agalactiae: human urogenital
- Group C S. zooepidemicus: from animal products
- Group D S. faecalis: bile-resistant, fecal origin

Streptoccus pyogenese

- Most important pathogen
- Isolated only form man
- 20% carried in throat of normal children
- Lower rate in adults.

- Spherical, 0.5 1 μ in diameter
- Gram positive
- Occur typically in chains (sometimes singly or in pairs)
- Non sporing, non-motile
- Capsulated (Hyaluronic acid)

Culture:

• Facultative anaerobe

- Optimum temp 37C°(22—42 C°)
- Fastidious, grow poorly on ordinary media
- Improved on addition of blood, tissue fluid, serum & glucose

- On blood agar small, translucent, less than 1mm, round smooth edge & slightly convex colonies
- Small droplets of fluid like

- Surrounded by area of clear β haemolysis.
- Bacitracin sensitive

<u>Toxins</u>

. Streptolysin O&S <u>Streptolysin O</u>

- Oxygen labile exotoxin
- Readily undergoes oxidation to an inactive form.
- Protein in nature (MWT 60,000)
- Produces haemolysis seen when growth deep in the medium.

- Strongly antigenic and antibodies against it are formed
- Quantitative estimation of antibodies called Antistreptolysin O titre (ASOT)
- ASOT more than 200 units abnormal

- <u>Streptolysin S</u>
- Responsible for haemolytic zone around streptococcal colonies on the surface of blood agar plates
- Protein in nature (MWT 20,000)
- Not antigenic

- Erythrogenic (Pyrogenic toxin / Dick toxin)
- Three types A,B & C.
- Protein in nature
- Heat stable
- Produced by some strains
- Causes scarlet fever
- Fever with extensive rash
- Antigenic

- Pyrogenic exotoxin A is responsible for some cases of streptococcal Toxic Shock Syndrome (TSST)
- Being superantigen stimulates huge amounts of cytokines like IL-1, IL-2 & TNF
- Produces shock and multiorgan involvement
- Pyrogenic exototoxin B is a protease rapidly destroying tissues and causes necrotizing fasciitis

Enzymes

- <u>Streptokinase</u>
- Converts plasminogen to plasmin
- Active fibrinolytic agent
- Can be used to treat venous thrombosis, pulmonary emboli and coronary artery thrombosis

- <u>2.Deoxyribnuclease (Streptodornase)</u>
- Breaks down DNA

 Responsible for serous character of streptococcal pus and spreading character of streptococcal infections

- <u>Hyaluronidase</u>
- _Splits hyaluronic acid an important component of ground substance of connective tissue

- Aids in spreading infection
- Antigenic

• <u>Proteinase</u>

_Produced in early sages of infection Destroys many proteins Helps in recovery

INFECTIONS OF STREPTOCOCCUS PYOGENES

. ACUTE SORE THROAT

2. SCARLET FEVER

3.SUPERFICIAL INFECTIONS

4. IMPETIGO

5. ERYSIPELAS

- 6. Septicemia
- 7. Meningitis
- 8. Brain abscess
- 9. Ac.arthritis
- 10. Acute endocarditis

DIAGNOSIS:

- Throat Swab
- Deep nasal swab
- Pus
- Blood
- Serum
- High vaginal swab

- Smears- Gram stain
- Culture -Bacitracin sensitive
- Antigen detection test
- Serology
- ASOT, Anti DNase, Anti M, Antistreptokinase

INFECTIONS OF GROUP B STREPTOCOCCI (S. AGALACTIAE)

- Some women become colonized with this organism in vagina and rectum
- Colonization of vagina results to infect the new born during delivery or inutero when membranes rupture prematurely
- New born may also get infected after birth

Neonatal Infections:

- Disease in new born is of two types
- 1. Early onset disease
- 2. Late onset disease
- **<u>1 Early onset disease (within first 5 days)</u></u>**
- Septicemia
- Pneumonia
- Meningitis

- <u>2. Late onset disease (7days to 3 months)</u>
- Meningitis.
- Post partum infection in women
- (Endometritis, Bacteremia)

Group C & G Infections

- Pharyngitis
- Pneumonia
- Cellulitis
- Soft tissue infections
- Bacteremia, Endocarditis

Enterococci (Group D)

- **1. Enterococcus faecalis**
- 2. Enterococcus faecium
- Occur as commensals in the intestine of man and animals
- Diseases caused by enterococci
- UTI
- Bacteremia
- Wound infection

- Puerperal sepsis
- Liver abscess
- Perinephric abscess
- Endocarditis
- Neonatal meningitis

<u>Viridans Group (α heamolytic/oral)</u>

- 1. Streptococcus mutans
- 2. Streptococcus sanguis
- 3. Streptococcus milleri
- 4. Streptococcus anginosus

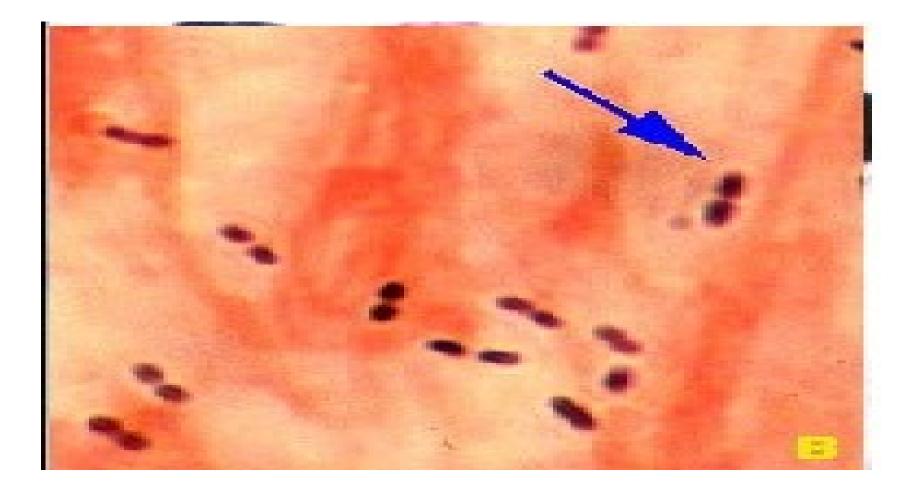
Most constitute part of normal mouth and upper respiratory tract flora

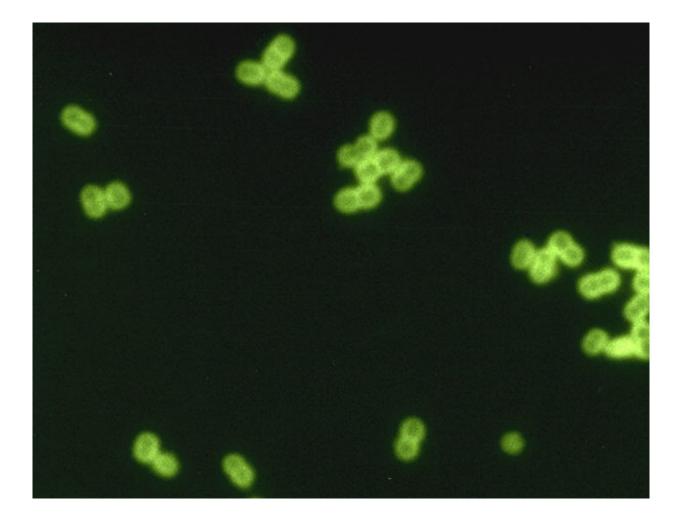
Diseases caused by viridans streptococci

- Subacute bacterial endocarditis (SBE)
- Dental caries
- Sinusitis
- Brain abscess
- Liver abscess
- Meningitis

STREPTOCOCCUS PNUMONIAE (PNEUMOCOCCUS)

- <u>Morphology:</u>
- Diplococci, broader ends apposed, distal ends narrowed
- Also single or short chains
- Gram positive





- On prolonged incubation G –ve due to autolysis
- Size 1 µ (.25---1.25 µ)
- Non motile, Non sporing
- Capsulate

Culture:

- Optimum temp 37 C° (25—40 C°)
- PH 7.6 ---7.8
- Facultative anaerobe
- 5---10 % CO2 improves growth

- Poor growth on simple media
- Requires blood, serum or glucose
- Blood agar– best medium
- Colonies small, round, some what

flattened, transparent

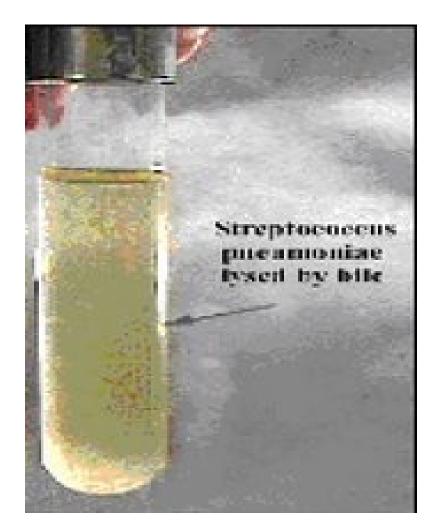
- After 48 hrs margins raised
- Alpha haemolysis around colonies
- Some types produce large mucoid colonies

Autolysis increased by surface active agents

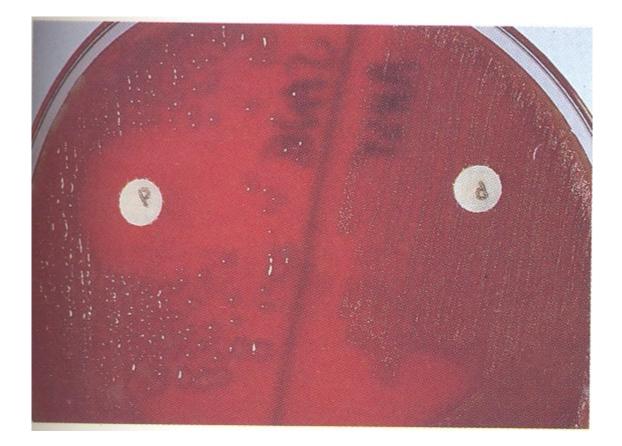
 (bile salts)

- Bile solubility test
- Optochin sensitivity test
- Turbidity in broth
- Virulent for mice

Bile solubility test







Antigenic Structure:

- Capsular polysaccharide
- M. Proteins
- C. Polysaccharide
- Metabolites: Pnumolysin, Leucocidin IgA protease

Pathogenicity:

- None of metabolites toxic
- Capsule retards phagocytosis
- Ability to multiply in tissues
- Inflammation –out pouring fibrinous, proteinaceous material into alveoli
- Red cells+ WBC's

Infections:

- . Lobar pneumonia
- **2.** Sinusitis
- 3. Otitis media
- 4. Osteomyelitis
- 5. Arthritis

Meningitis

- 7. Endocarditis
- 8. Peritonitis
- 9. Conjunctivitis

Lobar pneumonia

- Pneumococcus is the major cause of community
- acquired bacterial pneumonia of lobar type in humans
- Infection is endogenous, bacteria are aspirated form

throat of normal children and adults

Diagnosis:

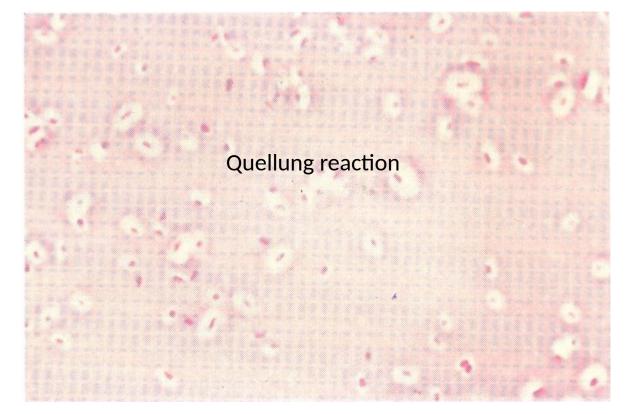
- <u>Specimens:</u>
- Sputum
- Blood
- CSF
- Aspirates

<u>TESTS</u>

• Stained Smear

• Culture

- Capsule swelling test (Quellung Reaction)
- Animal inoculation



<u>NEISSERIA</u>

Family: Neisseriaceae Genus: Neisseria SPECIES:

N. meningitidis (Meningococcus)

N. Gonorrhoeae (Gonococcus)

General Characters- Neisseria

Morphology:
Gram negative

*Diplococci

*.8 µm in diameter (.8-1)

Kidney shaped (bean)

*****Flat/ concave sides adjacent

Pathogenic species found within polymorphs mostly.

*Non-pathogenic outside

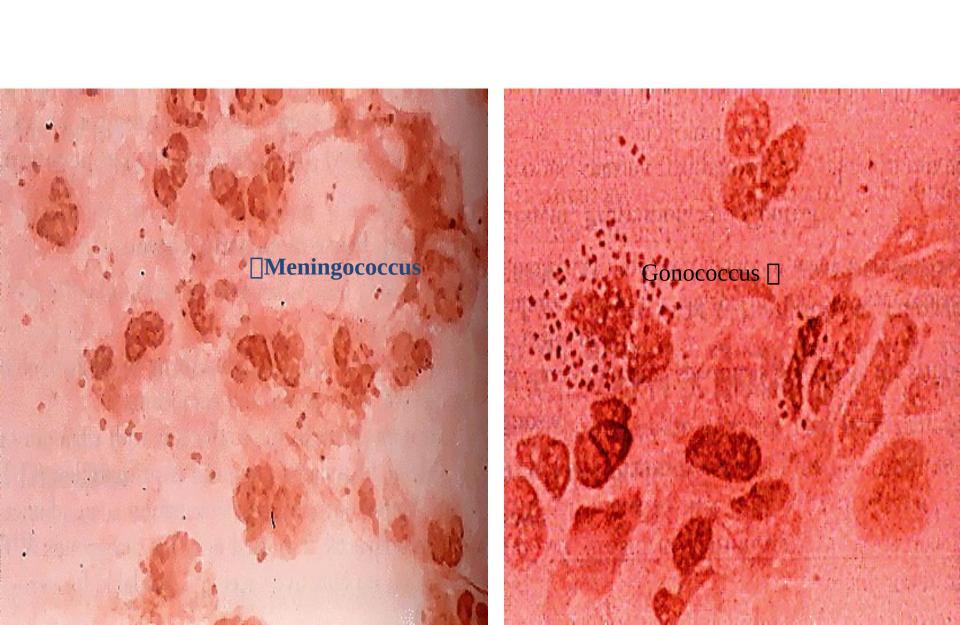


* Fimbriated

*Non sporing

Meningococcus capsulated

Gonococcus non capsulated



Culture:

Strict aerobe

Growth improved in 5-10% Co₂

*****Optimum temp 37 C° PH 7.4–7.6

*Moisture improves growth

*No growth on ordinary media

Enriched media required

Blood, serum, tissue fluids

Chocolate agar most suitable medium

*Made by heating blood agar to 80 C°

Selective Media:

Mueller- Hinton medium

Modified New York City (MNYC) medium

*Modified Thayer – Martin medium

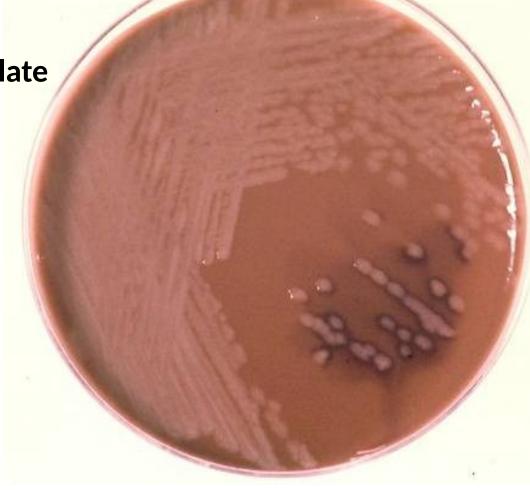
Gonococcus more delicate with exacting requirements

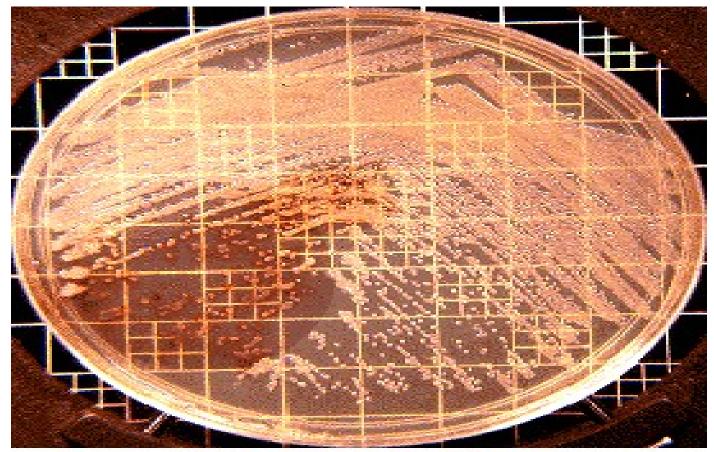
Some strains require particular amino acids, bases or vitamins ✤In 48 hrs colonies Round, convex, grayish white, glistening, raised, 1—2 mm in diameter of Meningococcus

Smaller 0.5—1mm of Gonococci

Non pathogenic [] opaque, brittle & wrinkled colonies, some pigmented

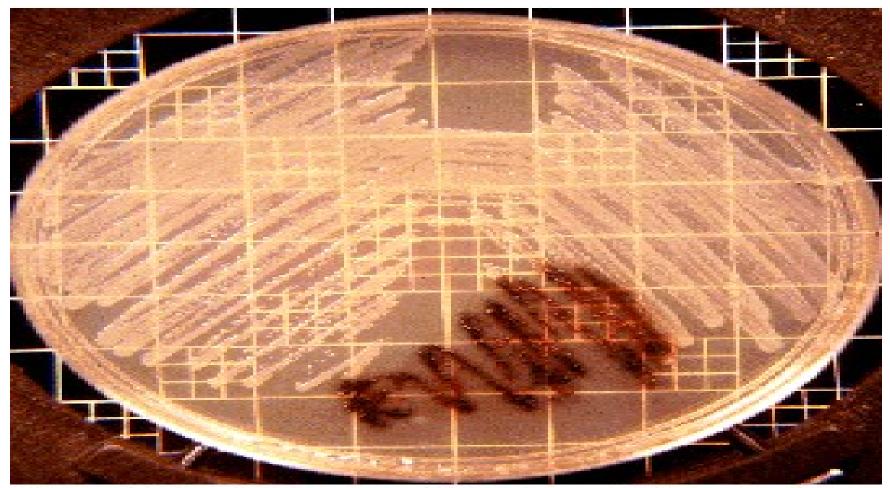
Meningococcus – chocolate agar





Neisseria meningitidis NewYork City (NYC) agar,(antibiotics+) /CO2

Neisseria gonorrhoeae, oxidase+ New York City (NYC) agar,(antibiotics+)/CO2



Biochemical Reactions

Oxidase +ve (strong)









Maltose +ve (Meningococcus)

Maltose -ve (Gonococcus)



<u>Viability</u>



Die quickly by drying

Moist heat at 55 C° kills in less than 5 min

***** Very sensitive to usual antiseptics

Antigenic Structure Gonococci

Pili (fimbriae) Helps in adherence to host cells
 Outer membrane proteins include

1. Porin protein - Por (protein I) form pores for entry and exit of small molecules

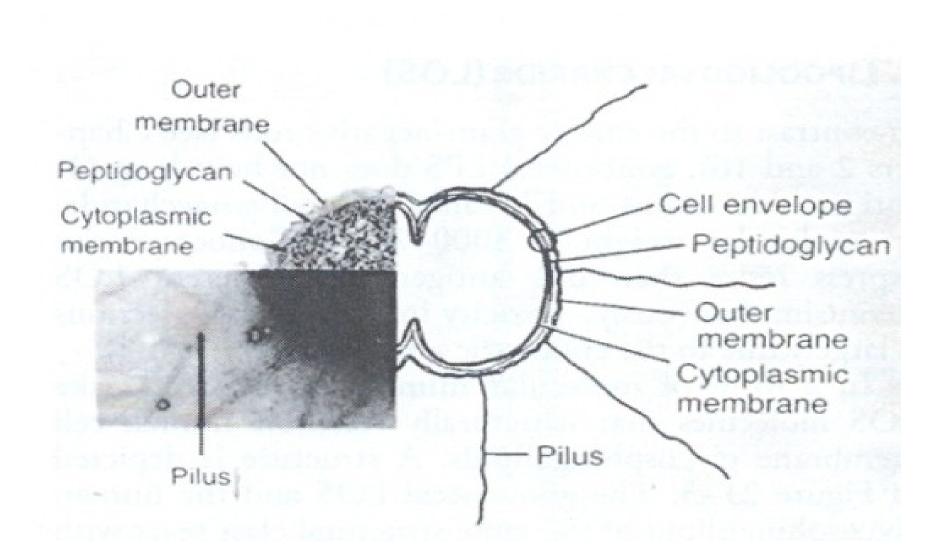
2. Opacity protein- Opa (protein II) helps in adhesion to host cekk.

. Reduction modifiable protein – Rmp (Protein III) forms pores Lipooligosaccharide (LOS) contains lipid A and core polysaccharide without repeat units, acts as endotoxin Ig A Protease This enzyme destroys IgA the local antibody • <u>Antigenically heterogeneous</u> Gonococcus is heterogeneous antigenically to avoid

host defenses. It is capable to change its surface

antigens (fimbriae, Opa & LOS) by frequently

switching from one antigenic form to other. This is done by gene rearrangement

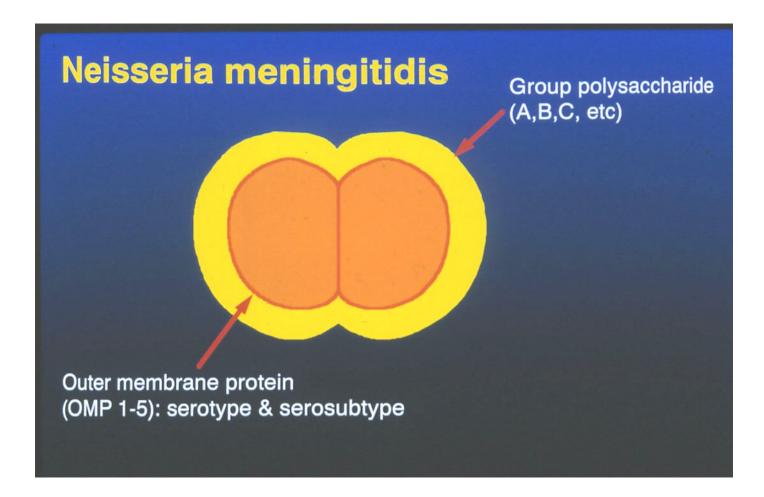


<u>Meningococci</u>

Capsular poly saccharide (13 groups)

Outer membrane proteins like gonococci

Ig A protease
 Lipopolysaccharide (LPS)



Cell wall structure – Meningococcus

Diseases By Gonococci

- Gonorrhoea
- A. In males
- Acute urethritis leading to
- 1. Epididymitis
- 2. Epididymo-orchitis

3. Prostatitis

- 4. Periurethral abscess
- 5. Oropharyngeal infections
- 6. Rectal infections

- <u>B. In females:</u>
- _Cervicitis leading to
- 1. Urethritis
- 2. Salpingitis
- 3. Infertility

4.Vulvovaginitis in girls

OPHTHALMIA NEONATORUM

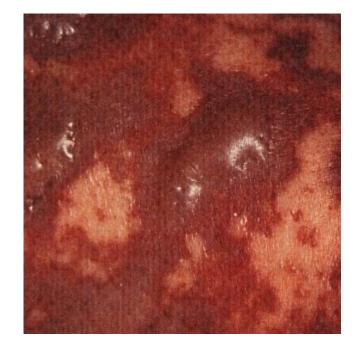






Bacteremia leading to Disseminated gonococcal infection (DGI)







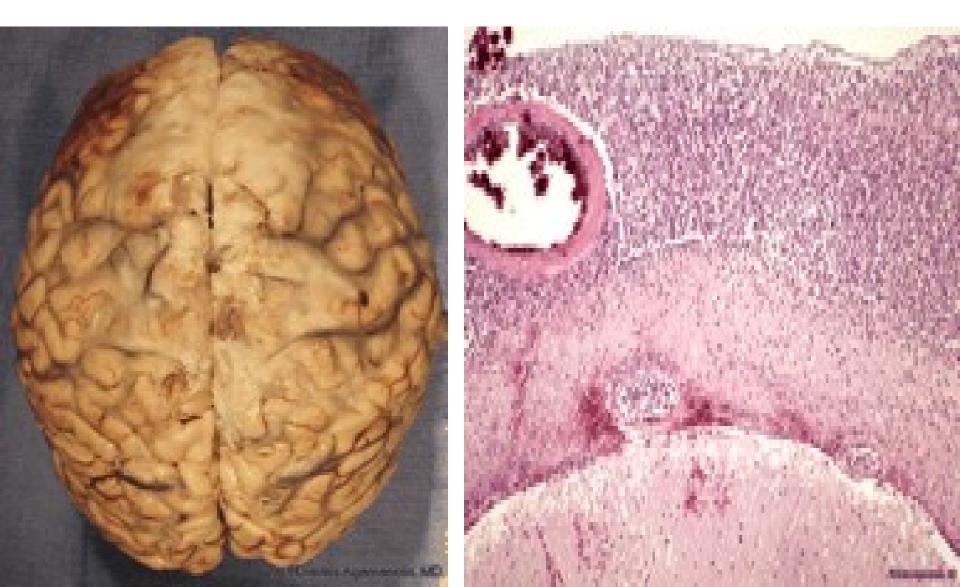
ArthritisMeningitis

* Endocarditi

S



Meningococcus 1. Meningitis [] (A,B,C,Y,w-135)



2. Meningococcemia

Small haemorrhagic skin lesions called 'petechiae' appearing on trunk and extremities

These may coalesce and form areas of cutaneous hemorrhagic necrosis

Petechial haemorrhages



<u>3. Fulminent meningococcemia</u> (Waterhouse- Friderichsen Syndrome)

- Acute haemorrhage and necrosis of adrenal glands
 Fever, extensive rash, adrenal insufficiency, shock and death
- 4. Osteomyelitis
- 5. Arthritis
- 6. Pericarditis

7. Endophthalmitis



Diagnosis: (Meningococcus)

<u>Specimens:</u> CSF for ☆ Microscopy

*Chemistry

Culture.

Blood for culture

Aspirate from skin lesion for microscopy & culture

Pus from infected joint for culture

Throat/ nasopharyngeal swabs for carriers

Culture

Moist, Co₂ enriched incubation

*Chocolate agar

*Modified Thayer Martin

Mueller Hinton Modified New York city

Biochemical Reactions

Glucose +ve

Oxidase +ve

Maltose +ve

<u>Serology</u>

*Antigen detection in CSF

Serotying of isolate

Diagnosis (Gonococci)

- <u>Specimens:</u>
- Urethral swab
- Cervical swab
- * Rectal Swab
- *Eye swab
- Throat swab when required

For Microscopy & Culture

In DG1



Swab of skin lesions

* Aspirate of joint

Culture:

- *Direct inoculation
- *Amies transport medium
- Selective media (MNYC)Oxidase + ve
- ✤ Glucose + ve
- Maltose ve

Serology:

*Direct FAT on colonies

*Co-agglutination





