

Lecture 4: Gram Positive & Negative Cocci

Gram Positive Cocci

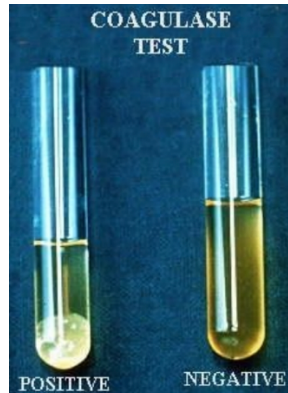
STAPHYLOCOCCI

Gram-positive cocci - 1 um diameter growing in grape-like clusters

- *Staphylococcus epidermidis* – normal flora of skin and mucosae; occasionally pathogenic
- *Staphylococcus aureus* – pathogenic; often found as normal flora
- *Staphylococcus saprophyticus* – in the environment and on skin; can cause urinary infections

Staphylococcus aureus

- “Staphule” – grape in Greek
- Grows well on simple media (agar, blood-agar) producing 2-3 mm colonies in 24 h (37 C)
- Toxins are problems
 - Cytotoxins α , β , δ , γ , P-V leucocidin
 - Leukocytes, erythrocytes, macrophages, platelets, and fibroblasts
 - Haemolysins
 - Dermonecrotic, lethal
 - Enterotoxin (A-E, G-I)
 - Superantigens
 - Important cause of food poisoning – nausea, vomiting, cramps, diarrhoeas
 - Exfoliative toxins (ETA, ETB)
 - “scalded skin” – syndrome in infants
 - **Toxic shock syndrome toxin 1** – pyrogenic exotoxin C and enterotoxin F
 - Cause of toxic shock syndrome
- Enzymes
 - Coagulase – coagulation of fibrin
 - Made by almost all pathogenic staphylococci
 - Used in laboratory test to differentiate from *S. epidermidis*, *S. capitis* and *S. saprophyticus*
 - *In vitro* coagulase test used as main test for identification of *Staphylococcus aureus*
 - Beta-lactamase (penicillinase)
 - Destroys penicillin
 - Other enzymes are also produced
 - Hyaluronidase, staphylokinase, etc



- Many *S. aureus* strains are found in normal pop (~15%)
- Carried in anterior nares, axilla, perineum and hands
 - Problem
 - 85-90% of strains isolated in hospital are penicillin resistant

Clinical Findings

- *S. aureus* tends to produce localized purulent infections
 - Pustules, boils, styes, conjunctivitis, otitis, etc
- Complications can be
 - Pneumonia, osteomyelitis, septicaemia, endocarditis
 - Food poisoning, toxic shock syndrome, scalded skin syndrome
- **Important cause of hospital acquired nosocomial** infections from stitch abscesses, infected wounds, or generalized infection

Epidemiology

- *S. aureus* strains are lysed by number of different bacterial viruses – bacteriophages
- Cells originating from same parental cell are lysed by same bacteriophage
 - Makes it possible to use “bacteriophage types” as identity markers in tracing sources of infection
- Preventative measures include
 - Aseptic technique in ER and OR, wound precaution
 - Education of health personnel
 - Handwashing

Staphylococcus epidermidis

STREPTOCOCCI

- Part of normal skin/mucous membrane flora
- Non-pathogenic, except in compromised patients where can cause post-operative infections
 - Brain
 - Open heart
 - Endocarditis
 - Shunt infections

- Considered an opportunistic pathogen

Streptococci

- “streptos” – Greek for twisted
- Arranged in pairs or forming chains
- Gram-positive cocci usually arranged in pairs or forming chains
 - Fastidious in nutritional requirements – grow on blood agar – are subdivided according to
 - Haemolytic properties (alpha/beta-haemolysis)
 - Greenish brown zone of partial RBC destruction
 - Clear zone of complete RBC destruction around colonies
 - Carbohydrate C antigen
 - Extracted from cell wall, subdivides streptococci in grps A-T
 - (Lancefield classification)
 - M-protein
 - Divides beta-haemolytic streptococci into 70+ serotypes
 - Mostly group A
 - Important **virulence factor**: antiphagocytic + degrades complement C3b

Streptococcus pyogenes

- Group A, beta-hemolytic, *S. pyogenes* causes:
 - **Acute tonsillitis** (strep throat) – can lead to rheumatic heart disease
 - Impetigo, cellulitis, etc (**wound/burn skin infections**)
 - Puerperal fever – **sepsis** – post-partum or post abortion
 - **Septicaemia**
- Complications following streptococcal infections
 - Acute glomerulonephritis
 - Rheumatic fever – involves joints and heart valves
 - *S. pyogenes* produces toxins and enzymes related to virulence
- Caused by **toxins**
 - **Streptolysins** (O and S) – beta-haemolytic
 - Highly toxic for neutrophils and macrophages
 - Anti-streptolysin-O (ASO) antibody reaches high titers after recent infections – except in skin infections
 - **Streptococcal pyrogenic exotoxins** (Spe)
 - Superantigens SpeA, SpeB, SpeC
 - Scarlet fever rash
- Enzymes
 - Hyaluronidase
 - Splits hyaluronic acid (helps spreading of bacteria)
- Virtually all are penicillin G *sensitive* (vs. *S. aureus*)
- *Streptococcus pyogenes* found in 5-10% of healthy individuals

- All strains are sensitive to penicillin G and so it's the antibiotic of choice
 - Note penicillin sensitivities

Prevention

- Education of health personnel
- Aseptic obstetric procedures
- Early detection and treatment

Flesh-eating disease – Necrotizing fasciitis

- *Streptococcus pyogenes* culprit
- Does not actually “eat” anything
- Toxin is responsible for damage
- Research indicates that
 - Hijacking human plasminogen from blood, attach to surface and activate it into protease – good for spreading
 - Bacteriophage has gene encoding for enzyme allowing bacteria to escapes entrapment and killing by neutrophils (WBC)

STREPTOCOCCUS GROUP B

Streptococcus agalactiae

- Group B
- Found in vagina – can cause neonatal infections
 - **Early septicaemia**
 - Respiratory distress or shock at birth within 24 hours
 - High fatality rate – serious
 - **Delayed meningitic form**
 - 1-12 weeks post-partum
 - Neurologic/mental abnormalities may result
 - Sequelae

STREPTOCOCCUS GROUP D

Other Streptococci

- ***Streptococcus faecalis***
 - Group D, - Enterococcus
 - Part of normal flora of GI-tract (gastro-intestinal tract)
 - Can cause infections when introduced into tissues
 - Prey on compromised individuals
- ***Viridens streptococci***
 - Includes group of different species of streptococci – alpha-hemolytic and non-hemolytic
 - Found in oral cavity of health individuals
 - **Can cause endocarditis** in individuals w damaged heart valves

Streptococcus pneumoniae

- A.K.A pneumococcus
- Diplococci - pairs
- Polysaccharide capsule has antiphagocytic properties
 - ~90 distinct capsular serotypes
- Found in naso-pharynx of healthy individuals
- Can cause
 - Lobar pneumonia
 - Meningitis
 - Lobar pneumonia – most often an auto-infection – carrier epidemic
 - More frequent in infancy, old age, and alcoholics
- Prevention strategies
 - Elderly
 - Alcoholics
 - Avoiding crowded living
 - Vaccinations

Gram Negative Cocci

- *Neisseria meningitidis*
- *N. meningitidis*
 - Found in naso-pharynx of healthy individuals – carrier state – lasts a few days-months
 - Gram negative diplococci
 - Grow best on enriched media (heated blood-agar) chocolate agar, 5-10% CO₂, 37
 - Use selective media (i.e Thayer-Martin) when isolating from nasopharynx
 - Frequently found in the naso-pharynx of healthy individuals
 - **Antiphagocytic** polysaccharide capsule
 - **13 different serogroups** – divided by their polysaccharide capsule
 - **A, B, C, X, Y W135** most prevalent
 - Carriers can occasionally develop infection or pass organism to non-immune individuals who develop infections
 - Only infection **humans**
 - Usually **children** or those living in crowded living in crowded living quarters
 - **Occasional epidemics**
 - Infection can result in
 - **Meningitis** – purulent, high fatality rate if not treated promptly
 - **Septicaemia** (meningococcaemia) – high fever, starts as skin rash
 - **Waterhouse-Friderichsen Syndrome** – bilateral adrenal cortical haemorrhage w fulminating collapse/death in less than 24 hour
 - **Complication of septicaemia** – more severe form septicaemia by *N. meningitidis*



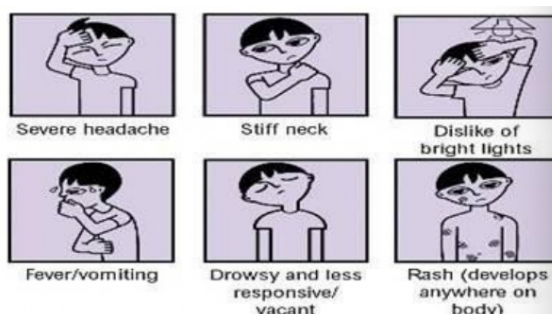
- First described in 1894 by Arthur Francis Voelcker – 1861-1946
- Then in 1901 by the British dermatologist Ernest Gordon Graham Little (1867-1950)
- It was first reported as an entity by Waterhouse in 1911, and subject was comprehensively reviewed in 1918 by Danish paediatrician Carl Friderichsen
 - Waterhouse-Friderichsen syndrome

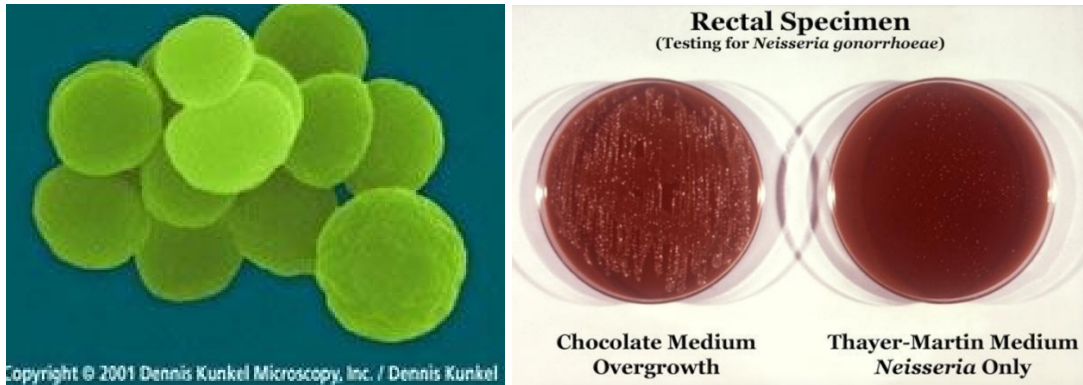
Prevention and Treatment

- Penicillin is primary antibiotic used, chloramphenicol or 3rd generation cephalosporins
- **Vaccination** is recommended for children (11-12 years) teenagers and college/university students living in dormitories
 - Conjugated vaccine for serogroups A, C, Y and W135
 - Now have meningococcus vaccine for infants at 2-5 months – serogroup C

Neisseria gonorrhoeae – *N. gonorrhoeae*

- Gram negative diplococci, 0.6-1 um in diameter
- In a clinical lab, grow on **Thayer-Martin plates**, in damp environment w CO₂
 - Very sensitive to drying and changes in temperature
- Causative agent of **STD gonorrhea**
- In US, second highest reported STD, after chlamydia
 - >350 000 cases/year reported in US (2001)
 - Number of cases in now decreasing every year

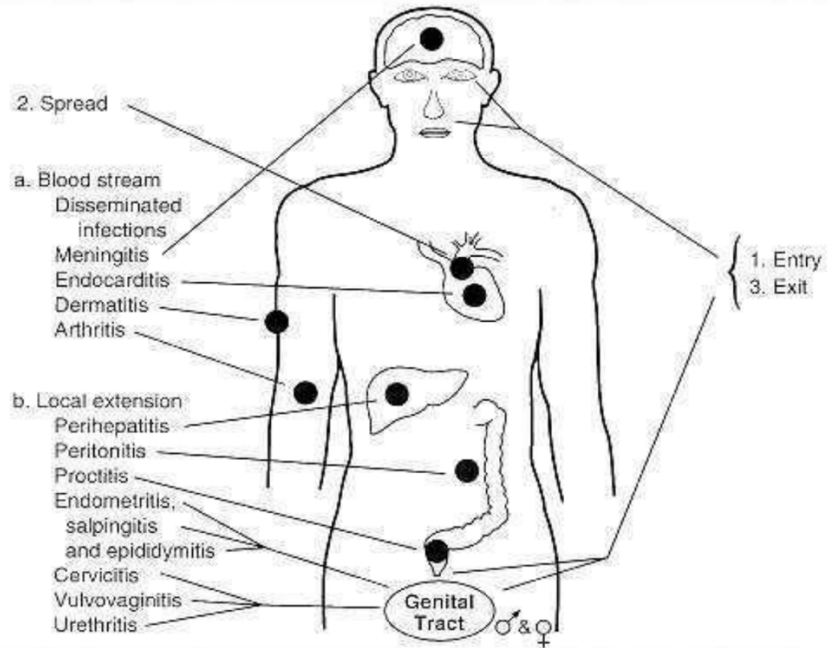




- Clinical **gonorrhea**
 - Men
 - Causes **acute infection of urethra** – purulent urethritis (90-95%)
 - If untreated, may cause epididymitis, prostatitis, etc
 - Women
 - 50% are **ASYMPTOMATIC**
 - Cervicitis – infecting urethra and rectum
 - If untreated, can cause PID – pelvic inflammatory disease
 - Salpingitis, pelvic peritonitis, adnexal abscesses etc, resulting in sterility
 - Both sexes
 - Rectal gonorrhoea/pharyngeal infection are occasionally present
- **Disseminated Gonococcal Infection (DGI)**
 - Gonococcal bacteraemia
 - 1-3% cases, usually women
 - Fever, cutaneous (skin) infection, arthritis
- Neonatal infections
 - Rare, but newborns can acquire infection from mother during birth
 - Causes **gonococcal ophthalmia** neonatorum – **acute purulent conjunctivitis**

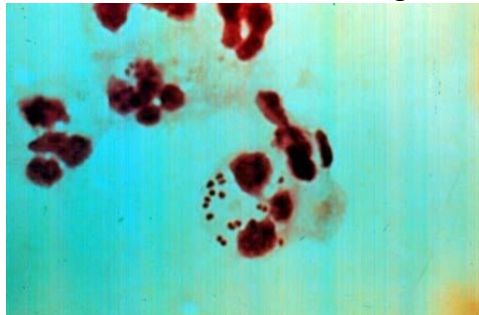
Clinical manifestations – laboratory diagnosis – of *N. gonorrhoeae*

- Grows well only on special media
- Sensitive to extremes of temperature + drying



- Diagnosis
 - Men
 - Use **microscopy** to directly observe swabs of urethral discharge
 - Show intracellular gram-negative diplococci
 - For practical purposes diagnostic
 - Women
 - **Culture** is necessary from endocervical, urethral and anal swabs

Urethral smear w intracellular Gram negative diplococci



- Plates, Thayer-Martin or N.Y.C agars ideally inoculate immediately, kept in atmosphere containing CO₂ (i.e candle jar)
 - Different transport media have been devised
 - Stuart, Amies
- Epidemiology: Prevention and treatment
 - Men – acute, 90-95%
 - Women – 50% asymptomatic
 - Penicillin **resistance is emerging**
 - South-East Asia

- West Africa
- Canada
- US
 - Plasma-mediated resistance
- Treat using **ceftriaxone, cefixime, ciprofloxacin or ofloxacin** combined w **doxycycline/azithromycin**
- Resistance to ciprofloxacin – quinolones – emerging
- Simultaneous treatment of partners is essential
- No vaccine available

